LIFE HISTORY FITNESS OF F1 HYBRIDS OF

TEX & PA21 POPULATIONS OF

DAPHNIA PULEX

by

OHITHA REDDY SANA

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 2024

ACKNOWLEDGMENTS

I would like to acknowledge and thank Dr. Sen Xu for giving me an opportunity in his lab and mentoring me during my research at The University of Texas at Arlington. I will always be grateful to Dr. Xu for considering and giving me an excellent opportunity. I would also like to thank Mr. Thinh Pham, my Ph.D. mentor, for assisting me with the research and guiding me throughout the experiment. He trained me in the usage of laboratory equipment and prepared me for this experiment. I could have not undertaken this journey without Mr. Pham, thank you for being patient and teaching me all the necessary information to succeed in this experiment, and training me for four semesters to understand the dynamics of projects in the laboratory.

Moreover, I would like to thank the Department of Biology for providing me with a space to work. Lastly, I would like to extend my sincere thanks to my parents, friends, and peers who supported me morally throughout the duration of the lab.

April 21, 2023

ABSTRACT

LIFE HISTORY FITNESS OF F1 HYBRIDS OF TEX & PA21 POPULATIONS OF *DAPHNIA PULEX*

Ohitha Reddy Sana, B.S. Biology

The University of Texas at Arlington, 2023

Faculty Mentor: Sen Xu

Evolutionary theory suggests that fitness decreases with hybridization as a result of negative epistatic interactions between alleles that rose independently in genetic backgrounds making hybrids incompatible with parents' species. However, heterosis can occur as a result of the deleterious effects of negative epistatic interactions making hybrids fitter than their parents. To examine this, the fitness of first-generation (F1) hybrids of Tex and PA populations of *Daphnia pulex* was determined using body size as a life history trait. F1 hybrids obtained from crossing between seven Tex parental isolates and PA parental isolates were taken from stock and grown under standard conditions (18°C under 18:6 light: dark photoperiod; *Scenedesmus obliquus* concentration of 500,000 cells per ml) to measure their body size. The body size was measured using a Leica M125 microscope. The body size of F1 hybrids was significantly different from the parents in 37 of the 50 Tex hybrids

while others performed equally well as the parents. The body size of F1 hybrids of the PA21 population was significantly different in 20 of the 29 hybrids examined. Variations were also found among F1 siblings and parental isolates. This experiment would be an addition to current research on hybridization to understand hybrid effects and predict genetic outcomes in hybrids in the field of agriculture and animal breeding.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Background

Hybridization is an interesting field of study. Evolutionary biologists constantly study the roles of fitness effects in hybrid zone evolution as hybrid fitness plays an important role in agriculture and animal breeding. They observe the underlying genetic mechanisms that play a role in hybrid fitness to better understand hybridization and predict hybridization effects on species. Life history traits are used to determine fitness. Life history traits of an organism are characteristics that explain its survival and reproduction patterns (Fox & Messina, 2013). Examples of life-history traits are body size, age at sexual maturity, size at sexual maturity, fecundity, brood size, brood number, clutch size, and patterns of reproduction.

Research conducted on hybridization led to the development of various theories and models to explain hybrids and gene interactions. For example, evolutionary theory suggests that fitness decreases with hybridization. This is explained as a result of negative epistatic interactions between alleles that rose independently in genetic backgrounds making hybrids incompatible with parents' species (Ebert, 2005). However, heterosis can occur as a result of the deleterious effects of negative epistatic interactions making hybrids fitter than their parents (Bernardes et al., 2017).

These two opposing theories raise the question: will hybrids present lower fitness or higher fitness than their parents? To answer this, the fitness of first-generation hybrids

of Tex and PA21 populations of *D. pulex* was studied by observing life-history traits (body size). This experiment would be an addition to current research on hybridization and evolution.

CHAPTER 2

LITERATURE REVIEW

2.1 Hybridization

Hybridization is the crossing between two individuals of the same or different species (Gabaldón, 2020). Examples of hybrids are ligers, mules, zebroids, wholphins, killer bees, camas, etc. There are two types: interspecific hybridization and intraspecific hybridization. Interspecific hybridization is the cross between individuals of different species whereas intraspecific hybridization is the cross between different individuals of the same species. When two different genetic backgrounds interact with each other during hybridization, the hybrid can present either hybrid depression (lower fitness than parents) or heterosis (higher fitness than parents).

2.1.1 Hybrid Depression

Evolutionary theory suggests that speciation leads to the formation of reproductive barriers between the species. Consequently, it leads to the evolution of behaviors/genes that reduce the fitness of hybrids when hybridization occurs (Coyne, 1992). The Dobzhansky-Muller model predicts that negative epistatic interactions (recessive) between alleles that rose independently in genetic backgrounds are the reason for the reduction of fitness in hybrids (Orr, 1995). The model states that the substitution of mutations in one species can have harmful effects when combined with those of other species leading to incompatibilities (Presgraves, 2003). Examples of some harmful effects are sterility and inviability. Natural selection against hybrids and hybridization load also drives towards

reduced fitness in hybrids (Moran et al., 2021). Although many empirical studies have supported the Dobzhansky-Muller model, some limitations challenge this model paving the way for opposing theories/predictions.

2.1.1.1 Limitations of the Dobzhansky-Muller Model

The Dobzhansky-Muller model considers only the deleterious gene interactions. It considers the effects of gene interactions between two different species but ignores the disrupting beneficial gene interactions that evolved independently in the parents' species. Lastly, the model does not explain the mechanisms behind mutations that get fixed when species diverge (Dagilis et al., 2019). To address these limitations, Fisher's Geometric Model has been developed. The model suggests that heterosis occurs due to uniparentally inherited traits or low fitness in parents (Fraisse et al., 2016).

2.1.2 Heterosis

Heterosis refers to the phenomenon where the hybrids of two diverse species exhibit higher fitness than their parents (Birchler et al., 2010). Taking Fisher's Geometric model into account, recent theories and studies suggest that heterosis can occur as a result of deleterious effects of negative epistatic interactions in parent populations making the hybrids fitter than both or either of their parents. To evaluate these two theories, *Daphnia pulex* are chosen as subjects for this experiment.

2.2 Daphnia

Daphnia are small planktonic species that belong to Branchiopoda, also known as water fleas. They feed on bacteria, protists, and algae in the water. They usually inhabit freshwater habitats such as vernal, rock, and ephemeral pools. They are extremely sensitive to changing environments, making them an important indicator of environmental changes.

They exhibit diel vertical migration where they tend to migrate toward the surface of the water at night and migrate downward during the daytime to avoid predators (Ebert, 2005). *Daphnia* males are usually smaller than females and are distinguished from females based on their larger antennules and modified post-abdomen. In addition, females possess a brood chamber to carry their eggs. There are more than 100 species in the genus *Daphnia*. This experiment focuses on *D. pulex* (Figure 2.1).

Figure 2.1: Female *Daphnia pulex* Prepared for Measurement

2.2.1 Life Cycle and Reproduction

Daphnia can exhibit both cyclic parthenogenesis (CP) and obligate parthenogenesis (OP) depending on the clones they are (Xu et al., 2013). Throughout their life cycles, CP clones can switch back and forth between asexual reproduction via ovulating embryos and sexual reproduction via ephippia. Meanwhile, OP clones can only reproduce asexually regardless of producing embryos in the brood pouch or the ephippia (Ebert, 2005). With asexual reproduction, the female produces and develops parthenogenetic eggs in the brood chamber for about a day after reaching sexual maturity (5-10 post-birth.) After the female produces eggs in the brood chamber, the embryos hatch and remain in the brood chamber

for about three days for further development (Ebert, 2005). The babies are released by the mother through ventral flexion of the post-abdomen. An adult female can produce eggs every 3-4 days if in favorable environmental conditions. Asexual reproduction produces daughters with the same genetic blueprint; however, sexual reproduction can introduce genetic variation and diversity into the population.

Daphnia undergoes sexual reproduction when environmental conditions are unfavorable such as low food and water, high population density, high predation, and high competition (Ebert, 2005). Sexual reproduction produces haploid eggs that need to be fertilized by males which develop into resting embryos. These resting eggs/embryos are covered in a protective shell called ephippia/ephippium which later hatches into young *Daphnia* (Ebert, 2005). Ephippia can float in water and survive harsh environmental conditions and hatch when external conditions change such as appropriate photoperiod, food availability, and temperature.

Daphnia's ability to exhibit both cyclic parthenogenesis (CP) and obligate parthenogenesis (OP) makes them an excellent subject for genetic studies. They are typically used to study population divergence, phylogenetic relationships between species, migration, inbreeding, and hybridization. Hence, *Daphnia* is the perfect subject to study hybridization as they can produce hybrids when crossing experiments are performed in the laboratory as well as produce genetically identical animals via asexual reproduction to make comparisons between the two.

CHAPTER 3

METHODOLOGY

3.1 Crossing Experiments

Parental isolates (Tex & PA21 population of *D. pulex*) were sampled from 42°12, -83°12 Textiles Road, Michigan, and grown in 250ml beakers at 18ºC under 12:12 (light: dark) photoperiod in artificial lake water COMBO (Kilham et al., 1998). Crossing experiments were performed with 20 males and 20 females placed together in a beaker. Females were stressed with low food and water to induce sexual reproduction. The first-generation (F1) hybrids from these crossing experiments between Tex23, Tex65, Tex20, Tex36, Tex53, Tex58, and Tex85 as well as the F1 hybrids from the crossing experiments between PA21 086, PA21 017, PA21 064, PA21 069, PA21 074, PA21 081, and PA21 108 were obtained and grown. The F1 hybrids were named as shown in Table 3.1 & Table 3.2.

Table 3.1: F1 Hybrids of Paternal and Maternal Tex Population

Paternal	Maternal	F1 Hybrids
Tex 23 (P1)	Tex 20 (M1)	H1, H4, H5, H6, H11
	Tex 36 (M2)	H1, H2, H4, H5, H6
	Tex 53 (M3)	H1, H2, H11, H12, H13
	Tex 58 (M4)	H1, H2, H6, H7, H8
	Tex 85 (M5)	H1, H2, H3, H4, H6
Tex 65 (P2)	Tex 20 (M1)	H1, H2, H4, H5, H18
	Tex 36 (M2)	H2, H5, H6, H9, H10
	Tex 53 (M3)	H1, H4, H5, H7
	Tex 58 (M4)	H1, H4, H5, H10, H11
	Tex 85 (M5)	H1, H2, H3, H4, H5, H6

Table 3.2: F1 Hybrids of Paternal and Maternal PA21 Population

Paternal	Maternal	F1 Hybrids
PA21 086 (P3)	PA21 062 (M6)	H1, H2
	PA21 069 (M7)	H2, H3, H4
	PA21 074 (M8)	H1
	PA21 081 (M9)	H1, H2
	PA21 108 (M10)	H1, H2, H3, H4, H5, H7
PA21 017 (P4)	PA21 062 (M6)	H1, H2, H4, H5
	PA21 069 (M7)	H2, H3, H4, H5
	PA21 074 (M8)	H1, H3
	PA21 108 (M10)	H1, H2, H3, H4, H5

3.2 Life History Assay

The F1 hybrids/genotypes and parental genotypes obtained were grown for two generations in 250ml beakers under an 18:6 (light: dark) photoperiod in artificial lake water COMBO at 18^{o}C to avoid maternal effects. The third-generation females were grown in wells under the same standard conditions to eliminate the effects of environmental factors (temperature, photoperiod, amount of food, etc.) on life history traits and ensure consistency. Animals were fed algae *Scenedesmus obliquus* with a concentration of 500,000 cells per ml (Moy et al., 2021). The artificial lake water COMBO was changed and animals were fed every 1-2 days.

After the third-generation females reached maturity and started reproducing asexually, the first batch of babies was discarded. The second and consecutive batch of babies (neonates) were collected and grown for 0-2 days under the same standard conditions mentioned above.

3.2.1 Measuring Body Size

Ten to thirty neonates per clone/genotype were chosen at random and their body size was measured. The body size was measured using the Leica M125 microscope (pre-calibrated to 8X magnification) in the computer software Leica Application Suite V4 (cite software). A ruler at 8X magnification was used to measure the size based on the distance between the top of the head and the base of the tail of the *Daphnia*, the tail was not included in the measurement (Figure 3.1).

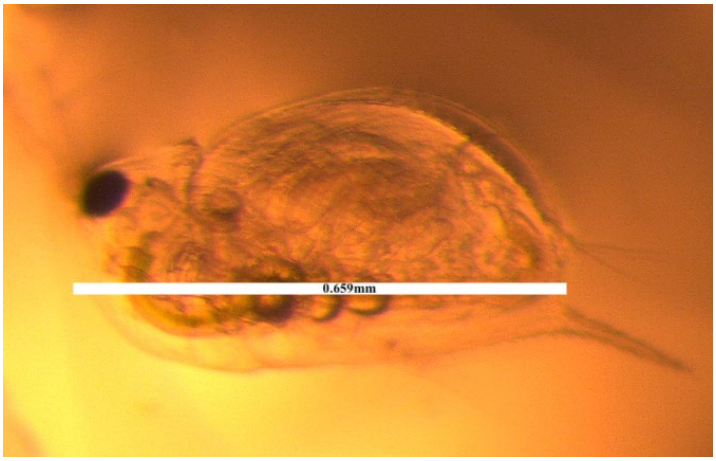

Figure 3.1: Hybrid P2M5H6 From Tex65 and Tex85 Cross

3.3 Data Analysis

3.3.1 One-Way ANOVA & Two-Way ANOVA

After the data were obtained, one-way ANOVA & two-way ANOVA tests were performed using statistical software, R and R Studio version 2023.03.0+386. In addition to the required automatic packages, afex, dplyr, broom, and reshape2 packages were also used to construct ANOVA tables to make comparisons and study interactions between parent isolates and F1 hybrids.

3.3.2 Tukey Test

Tukey tests were performed using the same software & packages used for one-way ANOVA and two-way ANOVA tests. This test showed significant differences between parent isolates, F1 hybrids, and F1 siblings.

3.3.3 Box Plots

Box plots were constructed for each cross using Microsoft Excel to get a visual representation of the raw data and make comparisons between paternal, maternal, and F1 hybrids.

CHAPTER 4

RESULTS

4.1 Parental Isolates of Tex

Two-way ANOVA tests revealed significant differences between the parents. The parental isolates Tex23, Tex65, Tex20, Tex36, Tex53, Tex58, and Tex85 were significantly different from each other. Among the parental isolates, Tex65 (paternal) had a larger body size (0.832 ± 0.030mm) than all the maternal genotypes Tex20 (0.621 ± 0.077mm), Tex36 (0.663 ± 0.045mm), Tex53 (0.682 ± 0.027mm), Tex58 (0.687 ± 0.026mm), and Tex85 (0.707 ± 0.033mm) with p-values <0.001, <0.001, <0.05, <0.001, and <0.001 respectively. In addition, Tex23 (paternal) was also significantly larger (0.726 ± 0.048mm) than all the maternal genotypes Tex20 (0.621 ± 0.077mm), Tex36 (0.663 ± 0.045mm), Tex53 (0.682 ± 0.027mm), Tex58 (0.687 ± 0.026mm), and Tex85 (0.707 ± 0.033mm) with p-values <0.001, <0.01, <0.05, <0.01, and <0.001 respectively.

4.2 F1 Hybrids of Tex

4.2.1 Tex23

One-way & Two-way ANOVA tests revealed significant differences in 37 of the 50 hybrids examined. The neonates of all crosses were either significantly smaller than one or both of their parents or performed equally well as the parental isolates. However, some F1 hybrids were significantly larger than either of their parents. Two of the five F1 hybrids of Tex23 and Tex20 were significantly smaller than the paternal genotype Tex23 ($p<0.001$)

as shown in Figure 4.1. However, all hybrids of Tex23 and Tex20 were significantly larger than the maternal genotype Tex20 as shown in Figure 4.1. Two of the five F1 hybrids (P1M2H1 and P1M2H2) of Tex23 and Tex36 were significantly larger than the paternal genotype Tex23 ($p < 0.001$). One of the five hybrids (P1M2H6) of Tex23 and Tex36 was significantly smaller than the maternal genotype Tex36 ($p < 0.001$). No significant differences were found among P1M2H4 and P1M2H5. No significant differences were found among hybrids of Tex23 and Tex53. One of the five F1 hybrids (P1M4H7) of Tex23 and Tex58 was significantly larger than both parents ($p < 0.001$). No significant differences were found among P1M4H1, P1M4H2, P1M4H6, P1M4H8, Tex58, and Tex23. The F1 hybrids of Tex23 and Tex85 were significantly smaller than the paternal genotype Tex23 ($p < 0.001$) as shown in Figure 4.2. However, no significant differences were found in P1M5H3 and P1M5H4. No significant differences were found between F1 hybrids and maternal genotypes.

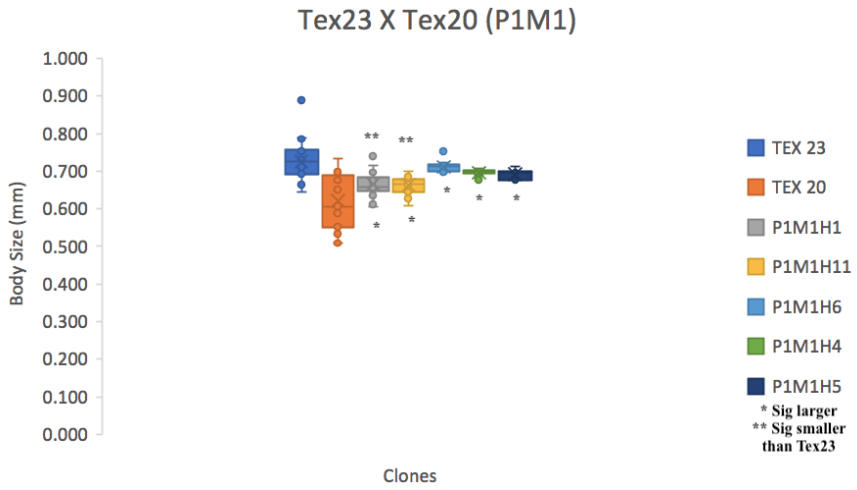

Figure 4.1: The Size Comparison Between F1s and Their Parents (Tex23 and Tex20). All hybrids are significantly larger than the mother (Tex20) and two of the five hybrids are significantly smaller than the father (Tex23).

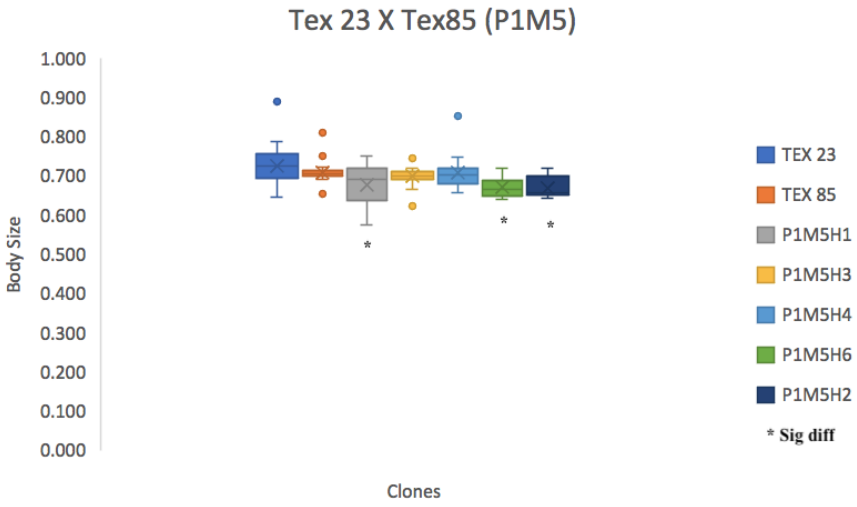

Figure 4.2: The Size Comparison Between F1s and Their Parents (Tex23 and Tex85). Three of the five hybrids are significantly different from the parents.

4.2.2 Tex65

The F1 hybrids of Tex65 and Tex20 were significantly smaller than the paternal genotype Tex65 ($p < 0.001$) as shown in Figure 4.3. However, all hybrids of Tex65 and Tex20 were significantly larger than the maternal genotype Tex20 ($p < 0.001$). Similarly, all F1 hybrids of Tex65 and Tex36 ($p < 0.001$) were significantly smaller than the paternal genotype as shown in Figure 4.4. All hybrids of Tex65 and Tex85 ($p < 0.001$) were also significantly smaller than the paternal genotype. All F1 hybrids of Tex65 and Tex58 ($p < 0.001$) were significantly smaller than the paternal genotype Tex65. Lastly, F1 hybrids of Tex65 and Tex53 ($p < 0.001$) were significantly smaller than the paternal genotype Tex65. No significant differences were found between F1 hybrids and the maternal genotype.

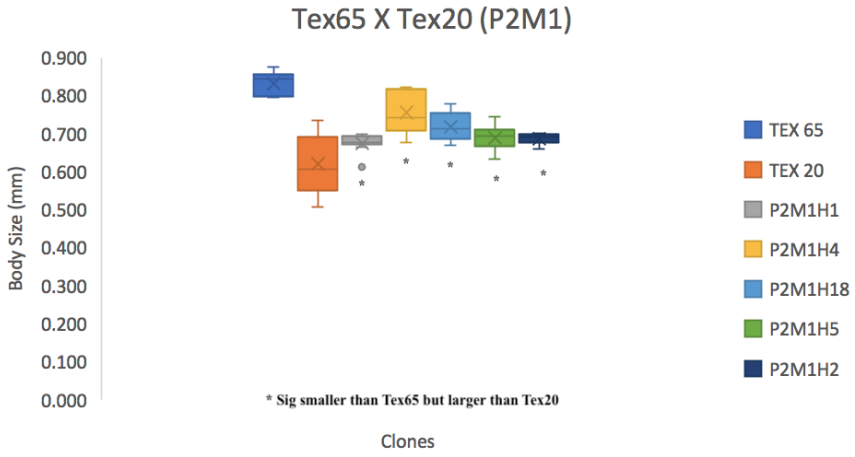

Figure 4.3: The Size Comparison Between F1s and Their Parents (Tex65 and Tex20). All hybrids are significantly smaller than the father (Tex65) but larger than the mother (Tex20).

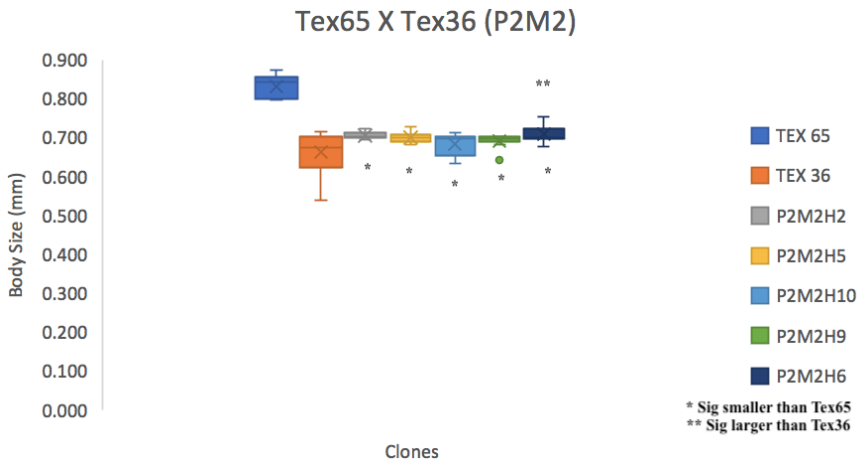

Figure 4.4: The Size Comparison Between F1s and Their Parents (Tex65 and Tex36). All hybrids are significantly smaller than the father (Tex65). One of the five hybrids is significantly larger than the mother (Tex36).

4.3 Parental Isolates of PA21

Two-way ANOVA tests revealed significant differences between the parents. The parental isolates PA21 017, PA21 086, PA21 062, PA21 074, PA21 069, PA21 081, and PA21 108 were significantly different from each other. Among the parental isolates, PA21 074 (maternal) had a larger body size (0.799 ± 0.007mm) than the paternal genotypes PA21 017 (0.681 ± 0.010mm) and PA21 086 (0.642 ± 0.009mm) with p values <0.001 for all. PA21 017 and PA21 086 (paternal genotypes) were significantly larger than PA21 062 (0.583 ± 0.009mm), maternal genotype ($p < 0.001$). In addition, PA21 069 (0.683 ± 0.009mm), PA21 081 (0.686 ± 0.011mm), and PA21 108 (0.713 ± 0.020mm) were significantly larger than the paternal genotype PA21 086 ($p < 0.001$).

4.4 F1 Hybrids of PA21

4.4.1 PA21-086

One-way & Two-way ANOVA tests revealed significant differences in 20 of the 29 hybrids examined. The neonates of all crosses were significantly larger in most cases while some were statistically insignificant. Five of the six hybrids of PA21 086 and PA21 108 were significantly larger than the paternal genotype PA21 086 ($p < 0.05$) as shown in Figure 4.5. However, P3M10H3 was significantly smaller than the maternal genotype PA21 108 ($p < 0.001$). All hybrids of PA21 086 and PA21 062 were significantly bigger than the maternal genotype PA21 062 ($p < 0.001$). Two of the three hybrids of PA21 086 and PA21 069 were significantly bigger than the paternal genotype PA21 086 ($p < 0.001$). However, P3M7H2 was significantly bigger than both parents ($p < 0.001$). No significant difference was found in P3M7H4. All hybrids of PA21 086 and PA21 081 were significantly larger than the paternal genotype PA21 086 ($p < 0.05$). The hybrid of PA21 086 and PA21 074 was significantly larger than the paternal genotype PA21 086, however, it was significantly smaller than the maternal genotype PA21 074 ($p < 0.001$). Signs of heterosis were observed in crosses between PA21 086 and PA21 062, PA21 069, PA21 081, and PA21 108 respectively.

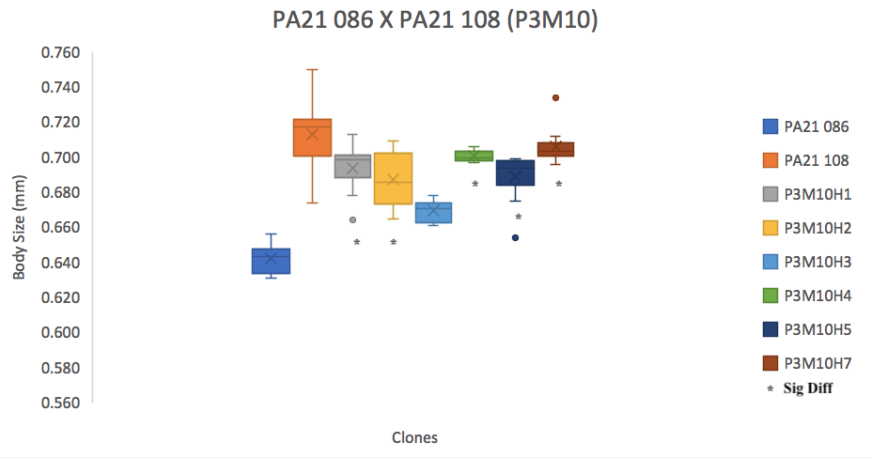

Figure 4.5: The Size Comparison Between F1s and Their Parents (PA21 086 and PA21 108). Five of the six hybrids are significantly different from the parents.

4.4.2 PA21-017

The F1 hybrids of PA21 017 and PA21 062 were significantly larger than the maternal genotype PA21 062 ($p < 0.001$). Two of the four hybrids were significantly larger than both parents ($p < 0.05$). All hybrids of PA21 017 and PA21 074 were significantly larger than the paternal genotype PA21 017 ($p < 0.001$). No significant differences were found between F1 hybrids and the maternal genotype. One of the four hybrids of PA21 017 and PA21 069 (P4M7H4) was significantly larger than both parents ($p < 0.001$). No significant differences were observed between P4M7H2, P4M7H3, P4M7H5, and the parents. One of the five hybrids of PA21 017 and PA21 108 was significantly larger than both parents ($p < 0.001$). No significant differences were observed between P4M10H1, P4M10H2, P4M10H4, P4M10H5, and the parents. Signs of heterosis were observed in crosses between PA21 017 and PA21 062 and PA21 074 respectively.

4.5 Variations Among F1 Siblings (Tex)

Variations were found among siblings (hybrids that share one of the same parents) of F1 Tex hybrids. The hybrids of Tex65 and Tex20 (P2M1) were significantly larger than the hybrids of Tex23 and Tex20 (P1M1) by 0.026mm ($p < 0.01$). The hybrids of Tex23 and Tex36 were significantly larger than the hybrids of Tex23 and Tex20 by 0.029mm ($p < 0.01$). The hybrids of Tex23 and Tex58, Tex23 and Tex85, and Tex23 and Tex58 were also significantly different from each other ($p < 0.001$). In addition, the hybrids of Tex65 and Tex85 were significantly smaller than Tex65 and Tex20 ($p < 0.001$). The hybrids of Tex65 and Tex85 were also significantly different from hybrids of Tex65 and Tex36, Tex65 and Tex53, and Tex65 and Tex58 ($p < 0.001$).

4.6 Variations Among F1 Siblings (PA21)

Variations were found among F1 siblings of PA21. The hybrids of PA21 086 and PA21 062 were significantly larger than PA21 086 and PA21 108 ($p < 0.001$). The hybrids of PA21 069 were significantly larger than PA21 086 and PA21 108 ($p < 0.001$). Similarly, the hybrids of PA21 086 and PA21 081 were significantly larger than PA21 086 and PA21 108 ($p < 0.001$). Variations were found among all hybrids of PA21 017 and respective maternal genotypes.

CHAPTER 5

DISCUSSION

In this experiment, the fitness of intraspecific hybrids was determined by examining the body size (life-history trait). The purpose was to determine if hybrids present hybrid depression (lower fitness than parents) or heterosis (higher fitness than parents). To maintain consistency and avoid environmental effects on the hybrids that might alter the results, standard conditions were maintained throughout the experiment. In addition, the females were grown for two generations before using the third-generation females to avoid previous stock background interference. These two practices eliminated the possible external effects acting on the hybrids' survival and behavior.

5.1 Results

The hybrids of Tex and PA21 populations differed from each other. Significant differences were found in 37 of the 50 Tex hybrids examined. While most of the Tex hybrids presented hybrid depression in comparison to paternal genotypes, a few hybrids were significantly larger than the maternal genotypes. Thirteen out of the fifty hybrids examined showed no significant differences suggesting that they performed equally well as both or either of their parents. Hence, no conclusions can be made about Tex hybrids. Meanwhile, significant differences were found in 20 of 29 PA21 hybrids examined. The majority of the PA21 hybrids showed signs of heterosis. No signs of hybrid depression were found in PA21 hybrids as most of the hybrids were significantly larger than both or

either of the parents while others performed equally well as the parents (insignificant differences). Variations were found among both Tex and PA21 parental isolates suggesting that the body size of parental isolates was significantly different from each other. Lastly, variations were also found among F1 siblings suggesting possible strong maternal or paternal effects. The hybrids of Tex23 and Tex20 significantly differed from Tex65 and Tex20 despite sharing the same mother. In addition, significant differences were found among PA21 hybrids despite sharing the same father (see Chapter 4) suggesting that maternal and paternal genes equally play a role in the hybrids.

While hybrids are difficult to sample and observe in a natural environment, laboratory experiments do produce hybrids for different genotypes and populations of Daphnia. As F1s show great variation in hybrid fitness (hybrid depression, heterosis, and equal fitness) relative to parents, we can hypothesize that hybrids do occur and can survive in nature.

5.2 Future Implications

Hybridization increases diversity and genetic variation giving rise to different species. Over the years, hybridization is studied to better understand the genetic mechanisms as well as predict the fitness consequences. The pursuit of heterosis is important as higher fitness is necessary for hybrids to survive and reproduce to pass their genes to the next generation thus maintaining the new variations in the population. In addition, heterosis plays an important role in agriculture and animal breeding (Suzanne, 1999). For example, studying hybridization can help produce high-yielding crops with better quality and resistance to several diseases (Birchler et al., 2010). In addition,

hybridization in a cow can give rise to individuals that can produce more milk with better quality (Puppel et al., 2018). Hence, hybridization has become an important aspect of the economic industry.

Previous studies show heterosis for life-history traits such as brood size and days to maturity (Moy et al., 2021) in interspecific hybrids of D. *pulex* and *Daphnia pulicaria*. However, reduced fitness was observed for body size. Hybridization was studied in various other organisms using different life-history traits. For example, a study examined the intraspecific hybrids of *Saccharomyces paradoxus,* and yeast, as well as interspecific hybrids of *S. paradoxus* and *S. cerevisiae* by observing competitive growth values (Bernardes et al., 2017). The results show that heterosis is strong in interspecific hybrids than in intraspecific hybrids. A possible explanation is that the complementation of low-fitness alleles in S. *cerevisiae* by high-fitness alleles in S. *paradoxus* resulted in heterosis.

This study focused on intraspecific hybrids by examining body size. In the future, examining other life-history traits as well as observing interspecific hybrids might help draw more conclusions. In addition, observing hybrids' genetic makeup and comparing it to the parents will help understand hybridization better. This experiment would be a great addition to current research in hybridization to help make predictions about hybrid fitness as well as provide a deeper insight into evolution.

CHAPTER 6

CONCLUSION

The F1 hybrids of Tex and PA21 genotypes of *D. pulex* showed variations in fitness. PA21 population showed signs of heterosis suggesting no harmful barriers between the species that will prevent hybrids' survival and reproduction. This experiment focused on intraspecific hybrids. Despite this, important observations were made that can help predict hybrid fitness patterns in intraspecific crosses. Future research can be conducted by examining other life-history traits as well as observing interspecific hybrids. In addition, this experiment observed first-generation hybrids. Future studies can include observations on second and consecutive generations of hybrids as well as backcrosses to get a deeper insight into genetic mechanisms driving hybrids.

APPENDIX A

SUPPLEMENTARY DATA TABLES (TEX)

Table A.1: Mean & SD of Tex23, Maternal Clones, and Their F1 Hybrids

Clone	Mean (mm)	Standard Deviation (mm)
Tex23	0.726	0.048
Tex20	0.621	0.077
P1M1H1	0.666	0.034
P1M1H4	0.696	0.008
P1M1H5	0.692	0.012
P1M1H6	0.712	0.017
P1M1H11	0.663	0.023
Tex36	0.663	0.045
P1M2H1	0.687	0.022
P1M2H2	0.691	0.009
P1M2H4	0.698	0.010
P1M2H5	0.683	0.015
P1M2H6	0.745	0.040
Tex53	0.682	0.027
P1M3H1	0.707	0.009
P1M3H2	0.701	0.020
P1M3H11	0.712	0.017
P1M3H12	0.680	0.037
P1M3H13	0.716	0.022
Tex58	0.687	0.026
P1M4H1	0.707	0.039
P1M4H2	0.693	0.014
P1M4H6	0.691	0.014
P1M4H7	0.798	0.059
P1M4H8	0.716	0.028
Tex85	0.707	0.033
P1M5H1	0.677	0.051
P1M5H2	0.670	0.026
P1M5H3	0.699	0.030
P1M5H4	0.707	0.044
P1M5H6	0.671	0.022

Table A.2: Mean & SD of Tex65 and Its F1 Hybrids

Clone	Mean (mm)	Standard Deviation (mm)
Tex65	0.832	0.030
P2M1H1	0.675	0.024
P2M1H2	0.686	0.013
P2M1H4	0.756	0.056
P2M1H5	0.688	0.029
P2M1H18	0.718	0.034
P2M2H2	0.706	0.045
P2M2H5	0.700	0.012
P2M2H6	0.709	0.021
P2M2H9	0.691	0.017
P2M2H10	0.683	0.025
P2M3H1	0.707	0.060
P2M3H4	0.711	0.028
P2M3H5	0.728	0.026
P2M3H7	0.709	0.013
P2M4H1	0.700	0.014
P2M4H4	0.711	0.015
P2M4H5	0.711	0.018
P2M4H10	0.715	0.031
P2M4H11	0.741	0.039
P2M5H1	0.644	0.030
P2M5H2	0.688	0.023
P2M5H3	0.677	0.038
P2M5H4	0.661	0.020
P2M5H5	0.681	0.012
P2M5H6	0.656	0.010

APPENDIX B

SUPPLEMENTARY DATA TABLES (PA21)

Table B.1: Mean & SD of PA21 Parentals and Their F1 Hybrids

Clone	Mean (mm)	Standard Deviation (mm)
PA21 086	0.642	0.009
PA21 062	0.583	0.009
P3M6H1	0.794	0.013
P3M6H2	0.671	0.035
PA21 069	0.683	0.009
P3M7H2	0.772	0.016
P3M7H3	0.687	0.014
P3M7H4	0.668	0.010
PA21 074	0.799	0.007
P3M8H1	0.681	0.012
PA21 081	0.686	0.011
P3M9H1	0.684	0.006
P3M9H2	0.707	0.060
PA21 108	0.713	0.020
P3M10H1	0.694	0.014
P3M10H2	0.687	0.017
P3M10H3	0.669	0.006
P3M10H4	0.701	0.003
P3M10H5	0.689	0.014
P3M10H7	0.706	0.011
PA21 017	0.681	0.010
P4M6H1	0.774	0.011
P4M6H2	0.718	0.019
P4M6H4	0.712	0.016
P4M6H5	0.712	0.010
P4M7H2	0.714	0.013
P4M7H3	0.684	0.016
P4M7H4	0.739	0.019
P4M7H5	0.702	0.005
P4M8H1	0.689	0.007
P4M8H3	0.697	0.009
P4M10H1	0.681	0.012
P4M10H2	0.685	0.010
P4M10H3	0.776	0.041
P4M10H4	0.706	0.051
P4M10H5	0.688	0.005

REFERENCES

Bernardes JP, Stelkens RB, Greig D. 2017. Heterosis in hybrids within and between yeast species. Journal of Evolutionary Biology. 30(3):538–548.

Birchler, J. A., Yao, H., Chudalayandi, S., Vaiman, D., & Veitia, R. A. (2010). Heterosis. *The Plant Cell*, *22*(7), 2105–2112.

Coyne, J. Genetics and speciation. *Nature* 355, 511–515 (1992).

Dagilis, A. J., Kirkpatrick, M., & Bolnick, D. I. (2019). The evolution of hybrid fitness during speciation. *PLOS Genetics*, *15*(5), e1008125.

Ebert D. 2005. Ecology, Epidemiology, and Evolution of Parasitism in Daphnia.

Bethesda (MD): National Center for Biotechnology Information (US). 118.

Ebert, D. (2005). *Introduction to Daphnia Biology*. Nih.gov; National Center for Biotechnology Information (US).

Fox, C. W., & Messina, F. J. (2013). Life History. *Oxford Bibliographies Online Datasets*.

Fraïsse, C., Gunnarsson, P. A., Roze, D., Bierne, N., & Welch, J. J. (2016). The genetics of speciation: Insights from Fisher's geometric model. *Evolution*, *70*(7), 1450-1464.

Gabaldón, T. (2020). Hybridization and the origin of new yeast lineages. *FEMS Yeast Research*, *20*(5).

Kilham SS, Kreeger DA, Lynn SG, Goulden CE, & Herrera L. 1998. COMBO: a defined freshwater culture medium for algae and zooplankton Hydrobiologia 377(1/3):147-159.

Moran, B. M., Payne, C., Langdon, Q., Powell, D. L., Brandvain, Y., & Schumer, M. (2021). The genomic consequences of hybridization. *ELife*, *10*, e69016.

Moy I, Green M, Pham TP, Luu DH, Xu S. 2021. The life history fitness of F 1 hybrids of the microcrustaceans Daphnia pulex and Daphnia pulicaria (Crustacea, Anomopoda). Invertebrate Biology. 140(2): e12333.

Orr, H. A. (1995). The population genetics of speciation: the evolution of hybrid incompatibilities. *Genetics*, *139*(4), 1805–1813.

Presgraves, D. C. (2003). A Fine-Scale Genetic Analysis of Hybrid Incompatibilities in Drosophila. *Genetics*, *163*(3), 955–972.

Suzanne Edmands, Heterosis And Outbreeding Depression In Interpopulation Crosses Spanning A Wide Range Of Divergence, *Evolution*, Volume 53, Issue 6, 1 October 1999, Pages 1757–1768.

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), 4549-4561.

BIOGRAPHICAL INFORMATION

Ohitha Reddy Sana is an undergraduate student at The University of Texas at Arlington pursuing an Honors Bachelor of Science in Biology and Psychology. She joined the Honors College as a freshman and continued to progress toward the completion of the honors degree. She served as Vice-President for UTA Volunteers and was involved in various student organizations such as Freshmen Leaders on Campus, Leadership Honors Program, and Minority Association of Pre-Medical Students at UT Arlington. While exploring different careers in the field of biology, she joined as an undergraduate research assistant in Dr. Xu's lab. During research with Dr. Xu, Ohitha developed a new perspective toward research. She plans to pursue a master's in biology and continue to develop and assist with research projects specifically in genetics. She will always be grateful to UTA for providing her with various opportunities and helping her explore to decide her career.