UNIVERSITY OF CALIFORNIA, SAN DIEGO

General Reproductive Biology and the Effects of Long-Term Parthenogenesis in Drosophila mercatorum

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

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2013
The Thesis of Hiroto Kameyama is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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University of California, San Diego

2013
# TABLE OF CONTENTS

Signature Page........................................................................................................ iii

Table of Contents........................................................................................................ iv

List of Tables.............................................................................................................. v

List of Figures............................................................................................................. vi

Acknowledgements ................................................................................................... vii

Abstract..................................................................................................................... viii

Introduction................................................................................................................ 1

Materials and Methods............................................................................................... 4

Results......................................................................................................................... 9

Discussion.................................................................................................................... 14

Tables and Figures.................................................................................................... 24

References.................................................................................................................... 34


**LIST OF TABLES**

**Table 1.1** Age at which sexual and parthenogenetic *D. mercatorum* strains become sexually mature.

**Table 1.2** The mean latency period before courtship was initiated.

**Table 2.1** Mean progeny produced by singly mated *D. mercatorum* females from sexual or parthenogenetic strains, and the percent of progeny that were female.

**Table 2.2 (a-c)** Results from the ANOVA testing the effect of several variables on progeny number from a single mating.

**Table 2.3** Results from the ANOVA testing the effect of several variables on female percentage of total progeny from a single mating.

**Table 3:** Number of progeny produced by twice-mated females (S.39).

**Table 4.** Total progeny production by 4-day-old females (S.39 and P.05) kept with two 4-day-old males (S.39) for a lifetime.

**Table 5.1** Average number of eggs laid in 20 days by virgin P.05 and S.39 females.

**Table 5.2** ANOVA results of the effect of reproductive type and strain on egg laying.

**Table 6:** Uncorrected COI distances of *D. mercatorum* generated in PAUP.
LIST OF FIGURES

Figure 1. Box plot of the progeny separated by reproductive type and replicate ................................................................. 25

Figure 2. Summary of mean number of progeny (with standard error bars) produced by females in various conditions ................................................................. 27

Figure 3. (a-d) Number of eggs laid daily, expressed by individual females....... 28

Figure 4. Average number of eggs laid per day by strain, with standard error bars .................................................................................................................................. 30

Figure 5. Daily egg laying of sexual Hawaiian flies taken from Templeton (1975) ....................................................................................................................................... 31

Figure 5. Daily egg laying of parthenogenetic (P.03) Hawaiian flies taken from Templeton (1975) ....................................................................................................................... 32
ACKNOWLEDGEMENTS

I would like to thank Dr. Therese Markow for her mentorship and support throughout my project. She has opened my eyes to scientific research and the skills I learned under her are invaluable and will constructively influence the rest of my life.

I would also like to thank Dr. Maxi Polihronakis Richmond and Sarah Johnson for their constant support in answering any of my questions and giving me feedback through the project. I would have been completely lost and lonely in the lab without both of their guidance.

I would like to thank Dr. Ryan Shultzaberg for his assistance in the statistical analysis of the project.

I would like to thank Dr. Elsa Cleland and Dr. Carolyn Kurle for being members of my committee.

Lastly, I would like to thank my friends and family who have encouraged and supported me in all of my endeavors.
ABSTRACT OF THE THESIS

General Reproductive Biology and the Effects of Long-Term Parthenogenesis in *Drosophila mercatorum*

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Master of Science in Biology

University of California, San Diego, 2013

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Purely parthenogenetic strains of *Drosophila mercatorum* were selected for by Dr. Alan Templeton in 1974 and have been kept in the UC San Diego *Drosophila* Species Stock Center for close to four decades. The distinct clonal reproduction of parthenogenesis in *D. mercatorum* is expected to negate sexual selection, select for superior asexual reproduction, and also allow neutral mutations. I investigated this
hypothesis by studying the general reproductive biology of sexual and parthenogenetic D. mercatorum. This study showed that 38 years of purely parthenogenetic reproduction have significantly diverged these strains from the sexually reproducing strains in mating propensity, progeny production, sex ratio of the progeny, and virgin egg-laying. Such results suggest parthenogenesis to be an intense divergent force in evolution.
**Introduction**

Parthenogenesis is a type of asexual reproduction in which an unfertilized egg becomes a viable offspring without fertilization by a male’s gamete. It is a relatively common form of reproduction in phylogenetically disparate taxa; many insects such as ants (Himler, 2009) and honeybees (Tucker, 1958) reproduce parthenogenetically but it is also observed in many reptiles such as lizards, geckos (Cuéllar, 1972) and boa constrictors (Booth, 2010). The majority of parthenogenetic species are facultatively parthenogenetic, meaning that they regularly reproduce sexually but some females have the ability to produce parthenogenetic offspring, or have haplodiploidy sex-determination; unfertilized haploid eggs become males and the females are diploid. Facultatively parthenogenetic species are believed to be in the early evolutionary stages of becoming obligate parthenogenetic species. Studying facultatively parthenogenetic species can provide insight into the evolutionary transition from a sexually reproducing bisexual species to obligatory parthenogenetic species.

In *Drosophila*, 40 species have been screened for parthenogenesis (Markow, 2013). Of the 40 species, 25 showed some early parthenogenetic development, such as development of an embryo or development to the larval stage. Currently, completely parthenogenetic strains have been successfully created in four species and parthenogenetic strains of *Drosophila mercatorum* have been created on a number of occasions. Carson (1967) created the first parthenogenetic *D.*
*mercatorum* strain through artificial selection using bridge cycles of sexual and parthenogenetic generations. Later, Templeton (1976) established a parthenogenetic strain directly from wild-caught females in Hawaii without the use of artificial selection or bridge cycles. He placed wild-caught females in separate vials and the F1 progeny were collected. Twenty three percent of the F1 virgin females produced parthenogenetic offspring and seventy percent of the parthenogenetic F1 had offspring that were also able to produce parthenogenetically. These strains are still maintained in the UCSD Drosophila Species Stock Center.

All known parthenogenesis in *Drosophila* is an automictic process where diploidy of the embryo is restored either by replication of the gamete’s own chromosomes or fusion of two haploid cells. The earliest stages of parthenogenesis in *D. mercatorum* at the cellular level are well understood due to a detailed cytological study (Eisman, 2007). In the case of *D. mercatorum*, diploidy in the gamete is restored by fusion of the pro-nucleus to its own polar body and therefore homozygosity of the progeny is obligatory. The process nullifies any homologous recombination in formation of the gamete and results in a success rate of egg to adult viability of only 3%. However, before all of the molecular processes of parthenogenesis, it is essential for unmated females to have the ability to lay unfertilized eggs.

A single parthenogenetic generation can create an intense bottleneck effect and cause a great loss of genetic diversity. Subsequent parthenogenetic generations
are expected to select for mothers producing eggs with a higher rate of automictic success and therefore more parthenogenetic progeny. Furthermore, sexual selection is expected to be relaxed, as females no longer need to attract mates or efficiently use sperm. Therefore, genes can accumulate selectively neutral mutations (Muller, 1949) or mutations that are advantageous to clonal reproduction (Pikls et al. 1996). In consequence, the females may have become less attractive to males and/or have inefficient sexual reproduction. Long-term parthenogenetic strains are ideal to study divergence of reproductive fitness due to the isolating effects of parthenogenesis.

Two of the parthenogenetic strains of *D. mercatorum* established by Templeton in January 1974 have been kept in the Drosophila Species Stock Center at University of California San Diego and were used for this study. These parthenogenetic strains have been completely segregated from male flies for 38 years and have never been observed to produce any male progeny. My study aimed to investigate the basic sexual reproduction of sexual and parthenogenetic strains of *D. mercatorum* in order to ask about the effects on reproductive fitness by long-term parthenogenesis. This study investigated the divergence in (1) sexual maturity, courtship latency, and copulation duration, (2) progeny of a single mating, (3) progeny in a lifetime, and the (4) daily egg-laying of virgin females.
Materials and Methods

Stocks of flies and culturing methods

Two sexual strains and two parthenogenetic strains of *Drosophila mercatorum* supplied by the UC San Diego Drosophila Stock Center were used for this study. One sexual strain was established with flies from Kamuela, Hawaii (UCSD stock number: 15082-1521.22 is referred to as S.22) and the other from Tucson, Arizona (UCSD stock number: 15082-1521.39 is referred to as S.39). The two parthenogenetic strains used were established in 1974 from natural populations at Kamuela, Hawaii (Templeton, 1976) (UCSD stock number: 15082-1525.03 is referred to as P.03 and 15082-1525.05 is referred to as P.05).

All strains were cultured in quarter-pint glass milk bottles with scored and yeasted banana medium at 23°C. Adult flies were changed to fresh food bottles every week to start new cultures.

Determining the age at sexual maturity, courtship latency and copulation duration

Newly eclosed adult flies from S.39 and P.05 were collected daily from the milk-bottles and sexed. Separate sexes were kept at a maximum density of ten per vial in yeasted banana medium to ensure virginity until the mating experiments. A day before being used in a mating experiment, the flies were individually placed into
new yeasted vials. The following day, virgin flies of each test age were exposed for an hour to two fully mature (8-11 days old) virgin flies of the opposite sex. Time until courtship, the duration of courtship, the occurrence of copulation, and copulation durations were all monitored and recorded. Trials were conducted with 9-19 flies for each test age of one to five days.

Initially, no parthenogenetic females of any age mated in the allotted hour. Therefore, in order to determine if parthenogenetic females would mate if allowed more time, P.05 exposure to S.39 males was extended to 24 hours. Evidence of mating was then determined by looking for sperm in the seminal receptacle of the females. Dissections were performed in phosphate-buffered solution (PBS) by pulling the most posterior tip of the abdomen with ultra-fine forceps. All the female reproductive organs, including the vagina, reaction mass, ovaries, and sperm receptacle were placed in the PBS and covered with a cover slip. The ventral seminal receptacles were located and examined for sperm under 40x magnification.

**Progeny of a single mating**

Virgin flies of all stocks were collected and kept in the aforementioned method. Individual 5-day-old females were placed into fresh vials and the next day, at 6 days of age, exposed to two mature virgin males. S.22 and S.39 were mated with their own respective strain while the parthenogenetic females, P.03 and P. 05, were mated with S.39 males. For the sexual strains, males were removed from the vials immediately after mating was observed, usually within twenty minutes. As
copulation required more time for the parthenogenetic females, the males were kept with the females for 24 hours before separation. Sample sizes of six to nine females were used in each replicate. At least two replicates were completed for each strain.

Female flies were transferred into new, yeasted, and scored vials every subsequent day to reduce the potential influence of larval overcrowding on the development of adult progeny. Emerging adult flies were sexed and counted daily.

Progeny of double matings of the sexual stain

Virgin S.39 were collected and mated at 6 days old, as described in “Progeny of a single mating”, and males were removed immediately after copulation. Females were then transferred into new vials daily. Simultaneously, two new mature virgin males were placed in the vials for an hour the day after the initial mating and monitored for copulation. Males were removed either in the event of copulation or after an hour if copulation was not successful. This step was repeated daily until each of the females had mated twice. Emerging adult flies were sexed and counted daily. Two replicates of six and eight samples were completed for this experiment.

Wild-caught female sperm load

*D. mercatorum* sperm load data was provided from an unpublished study by Dr. Therese A. Markow. Wild-caught females were immediately isolated without anesthetization into glass food vials with live yeast. The number of progeny
produced per female was used as a proxy for sperm load, rather than number of eggs laid as some oviposited eggs may not be fertilized (Markow, 2012)

Progeny in a lifetime

Virgin females of S.39 and P.05 were individually placed into vials at two days old and exposed to two mature S.39 males that were three days old. All three flies were transferred together into new vials every other day. The flies were continuously transferred into new vials until female mortality, the females stopped depositing eggs, or when the experiment was terminated at 54 days. Since females were kept in vials to lay eggs for two days, a paste of live yeast and deionized water was added to all vials containing 50+ third-instar larvae to eliminate any limitations to food. Emerging adult flies were sexed and counted daily. Nine females were used for the S.39 strain and seven females were used for the P.05 strain.

Daily egg laying of unmated females

Newly eclosed virgin females flies of S.39 and P.05 were kept individually in scored yeasted vials and transferred daily to new vials. The numbers of eggs oviposited was counted every 24 hours for 20 days.
Mercatorum sperm length

Virgin S.39 males of 6+ days were placed in 70% ethanol and their testes were dissected out into PBS on a glass slide. The proximal end containing the most mature sperm bundles was slit using a needle and the individual sperm bundles were gently pulled apart from the testis. Images of the sperm bundles were taken using a Nikon Eclipse E800 compound microscope with a Diagnostic RT Monochrome camera. The measurement of the sperm bundle was then acquired using the NIH image-processing program (Image J). The average of the four longest sperm bundles of the 14 imaged was used to estimate sperm length.

Statistical analysis

All statistical analyses were performed in Excel, JMP, or R software using the student’s t-test and single/multiple variable ANOVA.
Results

The age at sexual maturity, courtship latency and copulation duration

Experiments to determine age at sexual maturity were conducted with one sexual and one parthenogenetic strain. Following standard protocols (Markow and O’Grady, 2005) to determine age at sexual maturity in *Drosophila*, males and females were left together for one hour. Sexual strain females continuously increased in mating percentage from 0% at one day of age to 100% at five days old (Table 1.1). Males of the same sexual strain also showed a steady increase from one day of age, but appeared to mature earlier than the females. Nonparametric Kolmogorov-Smirnov test showed no significant difference between the sexes (P value: 0.8186)

When this protocol was employed for the parthenogenetic strain, no females mated during the allotted hour, even at four days of age. In additional mating experiments with five-day-old, seven-day-old, and eleven-day-old parthenogenetic females, only one seven-day-old female mated out of a total of seventeen samples. Assuming that the seven-day-old parthenogenetic females were most likely mature, more matings were expected. Therefore, I used a different approach when testing the age of reproductive maturity of the parthenogenetic females versus the sexual females. Mating pairs were housed together in a vial for 24 hours and the females were dissected to determine if they were inseminated. When dissected after exposure to mature males for 24 hours, the frequency of mating was variable for 2-6 day old females (Table 1.1.).
To test the prediction that the females of parthenogenetic strains have become less attractive to males, I measured the latency at which sexual and parthenogenetic females were courted. The mean courtship latency between sexual and parthenogenetic females was not significantly different (00:05:31 and 00:04:24, respectively) (two-tailed P value of 0.4532) (Table 1.2).

The copulation durations of all S.39 flies trials was consistent throughout the experiment regardless of their age and averaged together (00:03:14 ± 00:00:04). The single observed parthenogenetic mating at seven days old had a copulation duration of 00:03:15.

**Progeny of a single mating**

The mean numbers of progeny produced by a single mating in two sexual and two parthenogenetic strains are presented in Table 2.1. Each data set of progeny counts was normally distributed (P values: 0.6369, 0.2797, 0.7718, and 0.0846 of S.39, S.22, P.05, and P.03, respectively). While the parthenogenetic strains showed a clear tendency to produce fewer progeny when mated, there was considerable variability was observed within and among strains. A multivariate ANOVA (Table 2.2a) showed that all terms; reproductive type, strain, and replicate had a significant effect on progeny number.

The slight male bias in the progeny of sexual females contrasts with the female bias in the progeny of mated parthenogenetic females (Table 2.1). A chi-squared test with the null hypothesis that the female percentage should be 50% was
not significant for sexual strains S.39 and S.22 (P value: 0.2952 and 0.2366, respectively) but was significant due to an excess of females for parthenogenetic strains P.03 and P.05 (P value: 0.0002 and 0.0001, respectively).

**Progeny of double matings**

Sexual females that mated twice had more than double the progeny relative to females that mated only once (Table 3 & Figure 2). In the laboratory, *D. mercatorum* females will remate after 3-4 days after the initial mating.

**Progeny in a lifetime**

Of nine S.39 females that were kept with two mature males for a lifetime, 66.7% stopped producing eggs at around 20 days old, while 33.3% kept laying eggs past 64 days old when the experiment was stopped, although oviposition was at a much slower frequency compared to the rate at younger ages. P.05 females experienced early deaths, with only 22.3% living passed 20 days of age, compared to the isolated virgins in the egg laying experiment, in which 94.4% survived past 20 days. One P.05 female survived past 54 days when the experiment was terminated and was continuing to produce offspring. The progeny number between sexual and parthenogenetic was significantly different (P value: 0.0006)
Daily egg-laying of unmated females

An important aspect of parthenogenesis is the ability for females to lay unfertilized eggs. Egg-laying patterns between the sexual and parthenogenetic strains were variable. Unmated S.39 females were the most reluctant to lay eggs, with only 54% laying any eggs in the 20 days of observation. Of these females, 71% laid eggs on only one day and only three females laid more than 20 eggs. All other strains laid significantly more eggs than S.39 females (all P values < 0.0001) (Table 5.1).

S.22 females laid considerably more eggs than the S.39 females and were not significantly different in egg-laying from P.05 or P.03 females (Unpaired t-test P value: 0.2217 & 0.1165, respectively). However, the temporal pattern of egg-laying by P.05 females was very different from S.22 females. S.22 females frequently laid zero eggs but also had a higher maximum daily output (Figure 3b), while virgin P.05 egg-laying plateaued around 60 eggs/day/female at eight days old and stayed considerably stable (Figure 3c). Average number of eggs laid by parthenogenetic strains, P.05 and P.03, were not significantly different (P value: 0.9063), but the temporal pattern of egg-laying by P.03 females was much closer to S.22 females due to the large variance in egg oviposition from day to day (Figure 3d). ANOVA of the effect of reproductive type and strain on egg-laying showed both variables to be significant (Prob(<F): <0.0001) (Table 5.2)
**Mercatorum sperm length**

Average *D. mercatorum* sperm length from the longest four sperm bundles was 1.20±0.02mm
Discussion

The overall goal of my study was to determine what the effects of long-term parthenogenesis are on reproductive biology. In order to address this question, I compared several aspects of reproductive behavior in sexual and parthenogenetic strains of *D. mercatorum*.

*The age at sexual maturity, courtship latency and copulation duration*

The results of my comparative analysis of copulation between sexual and parthenogenetic *D. mercatorum* strains is comparable to Carson's (1977) experiments, in which he tested 99 seven-day-old P.03 females (then called K_{23·0-lm}) for mating propensity. Out of the 99 females, he found that 51.5% mated in 10 minutes, 29.3% mated in 10-30 minutes, and 19.2% did not mate. In comparison, P.05 females, where only 3.7% (ages 5, 7, and 11 days old) mated within an hour, were much less inclined to mate. Even when the exposure to males was extended to 24-hours, the mating percentage increased above 80% on only two occasions (Table 1.1).

However, at two days of age, the mating frequency was much higher in parthenogenetic females than in the sexual females. Presumably, if the sexual females had been exposed to mature males for 24 hours, the percentage mating would also increase. Given the sample size at the different ages, and the different
techniques I needed to employ for the sexual and parthenogenetic females, it is difficult to compare the age of sexual maturity.

A non-significant difference in the latency for males to start courtship suggests that long-term parthenogenesis has not made these females any less attractive to males. Yet the lower mating propensity of the parthenogenetic females shows that long-term asexual reproduction has made these females more reluctant to mate than females from sexual strains. Such behavioral changes show how a facultative parthenogenetic species can begin to drift toward obligatory parthenogenesis.

**Progeny of a single mating**

The parthenogenetic females produced fewer offspring on average than those of the sexual strain when mated with a male (Table 2.1 & Figure 1). A multivariate ANOVA shows that the reproductive type, sexual versus parthenogenetic, had the largest effect on the progeny number (Table 2.2). Without any sexual reproduction or recombination, long-term parthenogenesis is a rapid contributor to divergence within the species. The replicate difference between the sexual strains (Table 2.2c) can be most likely attributed to inexperienced handling of the flies; creating a lower survivorship to adulthood in the early replications.

Progeny from sexual strains showed a slight male bias while the parthenogenetic strains showed a female bias. This male bias was also observed in the subsequent experiment looking at the number of progeny produced in a lifetime.
suggesting that *D. mercatorum* has a natural male bias. This is contrary to other studies that have found a female bias of progeny in species such as *D. melanogaster* (Long, 2005). The mated parthenogenetic females were observed to produce only females for 5-15 days at the end of their progeny production, around 28 days after the mating. Thus, the female bias of the parthenogenetic strain is most likely attributed to parthenogenetic production after the females have depleted their stored sperm. Investigation of parthenogenetic progeny after sexual reproduction was not conducted in this study.

**Progeny of double matings**

Repeated matings have been shown to increase fecundity in *Drosophila* in many species (Ridley, 1988), and these results for *D. mercatorum* are consistent with those findings. Ridley’s review hypothesizes that species with a high productivity are less likely to receive a sufficient amount of sperm in one mating therefore those species benefit more from multiple matings. Others have found that males transfer nutrients along with sperm to females, which could potentially increase productivity of females when they mate more than once (Markow, 1990).

Because females mate multiply in the laboratory, they are expected to mate multiply in nature as well. While there are no data on the number of males siring the offspring produced by wild-caught females, the sperm load of wild caught females can be inferred by the number of progeny they produce. Unpublished data on progeny production (as a surrogate for sperm load) in wild-caught *D.
mercatorum females are presented in Figure 2 along with the reproductive output of mated sexual, mated parthenogenetic, and unmated parthenogenetic females.

The ability for females to produce a much greater quantity of progeny after a second matings suggests that D. mercatorum females are sperm limited in the wild. Because females do not receive a sufficient amount of sperm to produce its maximum number of progeny, it may have been advantageous for this species to evolve facultative parthenogenesis. Investigating the correlation of sperm limited species and the ability for parthenogenetic development may shed some new light to this matter.

Given that the average latency of re-mating was 3-4 days after the initial copulation, D. mercatorum females are not particularly promiscuous. The number of sperm carried by the wild caught females is lower than once-mated sexual females (in lab experiments), additionally supporting the hypothesis that D. mercatorum females are not promiscuous. These data suggest that D. mercatorum females will only remate when they have depleted most of their stored sperm from the initial mating. This low promiscuity may be due to (1) the sperm-storage organ, the seminal receptacle, being full and therefore the females do not have any incentive for further matings or (2) to persistence of a seminal protein, which inhibits female mating behavior (Avila, 2011).
Progeny in a lifetime

For the highest reproductive fitness, female flies would be expected to produce as many offspring as possible within their lifetime. I anticipated that females would keep mating and producing eggs until their death. However, most of the sexual females stopped producing eggs even in the presence of two healthy males and continued to live for days to weeks afterward. Why did these females stop producing offspring, whereas some of the other females of the same strain could produce over twice as many offspring? Did the females stop mating with the males? Did they run out of eggs? Or perhaps the males lost interest in these females.

As revealed by the “progeny from a single mating” experiment, mated parthenogenetic females also had fewer lifetime progeny than sexual females. The large difference can be accounted for in two ways. First, the mated parthenogenetic females oviposit eggs at a much slower rate than the mated sexual females. A vial containing a P.05 female and two males for two days produced 10-50 viable offspring, while a single S.39 female could rear over 130 offspring in the same time period. Second, both the sexual and parthenogenetic females’ lifespan was shortened relative to a virgin female in this experiment, but this was most severely pronounced in the parthenogenetic strain. The single P.05 female that was able survive in the presence of a male produced close to double that of the P.05 mean, suggesting that if P.05 could thrive with males, progeny production in a lifetime would increase substantially. However, this atypical P.05 female still produced less
than half of the mean of P.39 females. The slow rate of oviposition and the early mortality of the P.05 flies created a large difference between the sexual and parthenogenetic females in the total progeny production in a lifetime.

**Daily egg laying of unmated females**

One of the initial traits necessary for parthenogenesis is the egg-laying ability of virgin females. Although no parthenogenetic progeny were observed from the unmated S.22 females, the ability to lay large numbers of unfertilized eggs shows a greater potential for parthenogenesis in S.22 relative to the S.39 strain. Though the S.22 flies were established from the same location, and were the ancestral strain for both the parthenogenetic strains, the results of my experiment using S.22 flies differ from Templeton’s (1976) observations on sexual Hawaiian *D. mercatorum*. In Templeton’s experiment, 20 virgin females from sexually producing wild-caught Hawaiian *D. mercatorum* were put into a single vial and the number of oviposited eggs was counted 23 times out of 50 days. He found that females had a rapid increase in oviposition at 4 days old and steadily increased to peak around 50 eggs per day around 20-40 days of age (Figure 5). My results of the Hawaiian S.22 flies showed no increase in oviposition after 6 days of age, but the time scale of this experiment was much shorter than that of Templeton’s.

Templeton conducted his same egg laying experiment with eighteen parthenogenetic, K₂₈·₀·Im (P.03), flies of the fourth parthenogenetic generation of the strain. He observed that P.03 flies reached their egg-laying peak almost
immediately and their peak was higher than the sexual females (Figure 6).

Templeton’s observations mimic the behavior I observed for the P.03 and P.05 flies. One difference from the previous paper to my study is that Templeton got 27 viable adults from a total of 2,850 eggs (parthenogenetic rate of 0.94%), but for my experiment, 289 viable adults emerged from 10,726 eggs (parthenogenetic rate of 2.7%). These results suggest that the long-term parthenogenesis has not selected for increased oviposition, but for higher automictic success.

The parthenogenetic strains P.03 and P.05, as well as the geographically identical Hawaiian S.22 strain, do not need to be inseminated by males in order to oviposit large quantities of eggs. However, it is notable that both parthenogenetic strains laid more eggs than the S.22 strain, although this result was not statistically significant. An average P.05’s ability to lay 56.7 unfertilized eggs per day seems to contrast with the “progeny in a lifetime” data, which showed that mated P.05 only produce a maximum of 50 progeny in two days. One possible explanation for this is that the P.05 females may have become insensitive to the seminal proteins such as the sex peptide that has been shown to increase oviposition (Chen, 1988).

Alternatively, the absence of selection for more efficient fertilization and faster oviposition of fertilized eggs due to long-term parthenogenesis may have slowed down these rates in P.05 females.
Mercatorum sperm length

Sperm length in *Drosophila* greatly varies from 0.35mm in *D. subobscura* (Birkhead, 2002) to 56mm in *D. bifurca* (Pitnick, 1995). *D. mercatorum* sperm length at 1.2mm is roughly a little shorter than the median of the *Drosophila* genus. The shorter length of the sperm allows for faster sperm development and therefore, faster sexual maturity compared to the species with long sperm (Pitnick, 1995). The ability for *D. mercatorum* females to produce large quantities of progeny in a single mating suggests that the smaller mass of the sperm allows for larger quantities of sperm to be transferred in a single mating.

Conclusion

Investigation of the reproductive biology of sexually and parthenogenetically reproducing strains of *D. mercatorum* revealed many fascinating differences. Parthenogenetic females showed a large behavioral difference in mating propensity from the sexual strain. Furthermore, the difference between Carson's (1977) observations on mating propensity just a few months after the parthenogenetic strains were created, relative to the results of the current study, after 38 years of obligate parthenogenesis is interesting. Carson observed a significant (P value: <0.001) decrease in mating propensity compared to his control sexual flies, but not to the same extent my study did. A significant reluctance after a few generations for Carson, to an extreme refusal to mate after almost four decades gives insight to how an obligate parthenogenetic species could potentially evolve in nature. Instances
when natural populations decline and females must reproduce parthenogenetically for a several generations can cause reluctance to mate even in the presence of a male.

Although parthenogenesis is an advantageous trait in extreme conditions, such as not being able finding a mate, my experiments showed a large reduction in reproductive fitness for the parthenogenetic strains. Long-term parthenogenesis has reduced their reproductive output as well as longevity when in the presence of males. *Mature D. mercatorum* males aggressively court females and will chase females tirelessly, especially when two males can “tag-team” harassing a single female. I believe that the parthenogenetic females have become more sensitive to the stress of male harassment, and therefore, tire themselves to an early death.

Markow (2013) states that the first component to successful parthenogenesis is the ability for females to lay unfertilized eggs. A predisposition for the S.22 females to lay many unfertilized eggs presents an interesting inclination of Hawaiian females towards parthenogenesis. The temporal pattern of daily egg-laying (Figure 3) showed an intriguing progression from S.39 to S.22 to P.03 and P.05. Interestingly, the reverse progression is seen in the “progeny of a single mating”. P.05 and P.03 strains produced the fewest offspring, and increased from S.22 to S.39 strains. As the strains become “less inclined” for parthenogenesis, there appears to be a trade-off and they become better at sexual reproduction.

An analysis of pair-wise distances of unpublished mitochondrial *cytochrome oxidase* subunit I (COI) sequences of S.22 and S.39 showed high genetic divergence
(0.9%) (Table 6) (Richmond and Markow, unpublished), relative to the pair-wise comparisons between S.39 and all other strains. S.22 shows a relatively higher genetic divergence to all strains. This divergence could partially explain the differences in reproductive traits observed between S.22 and S.39 strains. Unfortunately, COI data for the parthenogenetic strains are unavailable.

Many types of parthenogenesis have arisen in a multitude of taxonomic lineages suggesting an evolutionary advantage of asexual reproduction. This study provides insight into the reproductive biology of a facultative parthenogenetic species. Further work on this system identifying the parthenogenetic progeny of mated parthenogenetic females or investigating the persistence of the asexual trait to the offspring of mated parthenogenetic females, could give a better understanding of a facultative parthenogenetic species. Because parthenogenetic females do not use the sperm storage organs, I expect changes to occur in the reproductive system of parthenogenetic females. A morphological study may reveal a physical difference between sexual and asexual females, which resulted in the divergence in sexual reproduction revealed in this study. Integrating morphological data with an analysis of the molecular aspects of long-term parthenogenesis, we could develop a more detailed perspective on the divergence of sexual and parthenogenetic *D. mercatorum.*
Table and Figures

Table 1.1 Age at which sexual and parthenogenetic *D. mercatorum* strains become sexually mature.

<table>
<thead>
<tr>
<th>SEXUAL FEMALES S.39</th>
<th>Age (days)</th>
<th>N mated /N tested</th>
<th>% Mated</th>
<th>PARTHENOGENETIC FEMALES P.05*</th>
<th>Age (days)</th>
<th>N mated /N tested</th>
<th>% Mated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0/15</td>
<td>0.0%</td>
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<td></td>
<td>2</td>
<td>6/9</td>
<td>66.7%</td>
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<tr>
<td>2</td>
<td>5/15</td>
<td>33.3%</td>
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<td></td>
<td>3</td>
<td>10/19</td>
<td>52.6%</td>
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<tr>
<td>3</td>
<td>10/19</td>
<td>52.6%</td>
<td></td>
<td></td>
<td>4</td>
<td>9/9</td>
<td>100.0%</td>
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<tr>
<td>4</td>
<td>10/13</td>
<td>76.9%</td>
<td></td>
<td></td>
<td>5</td>
<td>13/17</td>
<td>76.5%</td>
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<tr>
<td>5</td>
<td>9/9</td>
<td>100.0%</td>
<td></td>
<td></td>
<td>6</td>
<td>6/7</td>
<td>85.7%</td>
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<td>MALES S.39</td>
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<td></td>
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<tr>
<td>2</td>
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<td>3</td>
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<td></td>
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<td>4</td>
<td>11/12</td>
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<tr>
<td>5</td>
<td>12/14</td>
<td>85.7%</td>
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</tbody>
</table>

*No parthenogenetic females mated within the 60 minutes, subsequently parthenogenetic females were left with males for 24 hours and dissected for sperm in the seminal receptacle.

Table 1.2 The mean latency period before courtship was initiated.

<table>
<thead>
<tr>
<th>Female Age and Reproductive Type</th>
<th>Courtship Latency ± SE</th>
<th># of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 day old sexual S.39</td>
<td>5 min 31 sec ± 1 min 16 sec</td>
<td>7</td>
</tr>
<tr>
<td>5 day old parthenogenetic P.05</td>
<td>4 min 24 sec ± 47 sec</td>
<td>8</td>
</tr>
</tbody>
</table>
Table 2.1 Mean progeny produced by singly mated *D. mercatorum* females from sexual and parthenogenetic strains, and the percent of progeny that were female.

<table>
<thead>
<tr>
<th>Female type</th>
<th>Replication</th>
<th>Progeny Mean ± SE (n)</th>
<th>% Female ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexual strain S.39</td>
<td>a</td>
<td>239.7 ± 33.4 (7)</td>
<td>48.8 ± 1.5%</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>320.2 ± 22.6 (6)</td>
<td>51.0 ± 1.1%</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>359.5 ± 49.1 (6)</td>
<td>48.0 ± 1.1%</td>
</tr>
<tr>
<td>Sexual strain S.22</td>
<td>a</td>
<td>184.8 ± 29.1 (6)</td>
<td>50.2 ± 2.6%</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>259.3 ± 46.8 (7)</td>
<td>48.2 ± 0.8%</td>
</tr>
<tr>
<td>Parthenogenetic strain P.05</td>
<td>a</td>
<td>124.4 ± 10.0 (9)</td>
<td>55.9 ± 1.8%</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>157.2 ± 20.3 (6)</td>
<td>56.7 ± 2.6%</td>
</tr>
<tr>
<td>Parthenogenetic strain P.03</td>
<td>a</td>
<td>197.1 ± 24.9 (7)</td>
<td>51.3 ± 1.2%</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>215.89 ± 6.04 (9)</td>
<td>54.5 ± 1.2%</td>
</tr>
</tbody>
</table>

Figure 1. Box plot of the progeny separated by reproductive type and replicate. Parthenogenetic strain progeny numbers shown in red dots and sexual strain progeny shown in blue.
Table 2.2 Results from the ANOVA testing the effect of several variables on progeny number from a single mating.
(* Significant with Prob(>F) < 0.05  ** Prob(>F) < 0.005  *** Prob(>F) < 0.0001)
a. ANOVA of all reproductive types and strains

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum</th>
<th>Sq Mean</th>
<th>Sq F Value</th>
<th>Prob(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive type</td>
<td>1</td>
<td>134803</td>
<td>134803</td>
<td>23.078</td>
<td>1.24e-05 ***</td>
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<tr>
<td>Strain</td>
<td>2</td>
<td>73552</td>
<td>36776</td>
<td>6.296</td>
<td>0.00345 **</td>
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<tr>
<td>Replicates</td>
<td>5</td>
<td>96429</td>
<td>19286</td>
<td>3.302</td>
<td>0.01117 *</td>
</tr>
<tr>
<td>Residuals</td>
<td>55</td>
<td>321267</td>
<td>5841</td>
<td></td>
<td></td>
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</tbody>
</table>

b. ANOVA of parthenogenetic strains only

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum</th>
<th>Sq Mean</th>
<th>Sq F Value</th>
<th>Prob(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>1</td>
<td>38103</td>
<td>38103</td>
<td>21.330</td>
<td>8.49e-05 ***</td>
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<tr>
<td>Replicates</td>
<td>2</td>
<td>5238</td>
<td>2619</td>
<td>1.466</td>
<td>0.249</td>
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<tr>
<td>Residuals</td>
<td>27</td>
<td>48231</td>
<td>1786</td>
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</table>

c. ANOVA of sexual strains only

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum</th>
<th>Sq Mean</th>
<th>Sq F Value</th>
<th>Prob(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>1</td>
<td>35449</td>
<td>35449</td>
<td>3.635</td>
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<tr>
<td>Replicates</td>
<td>3</td>
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<td>30397</td>
<td>3.117</td>
<td>0.0419 *</td>
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<tr>
<td>Residuals</td>
<td>28</td>
<td>273036</td>
<td>9751</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.3 Results from the ANOVA testing the effect of the variables on female percentage of the total progeny from a single mating

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum</th>
<th>Sq Mean</th>
<th>S q F Value</th>
<th>Prob(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive type</td>
<td>1</td>
<td>0.04138</td>
<td>0.04138</td>
<td>21.450</td>
<td>2.33e-05 ***</td>
</tr>
<tr>
<td>Strain</td>
<td>2</td>
<td>0.00770</td>
<td>0.00385</td>
<td>1.996</td>
<td>0.146</td>
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<tr>
<td>Replicates</td>
<td>5</td>
<td>0.01133</td>
<td>0.00227</td>
<td>1.175</td>
<td>0.334</td>
</tr>
<tr>
<td>Residuals</td>
<td>54</td>
<td>0.10417</td>
<td>0.00193</td>
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</tr>
</tbody>
</table>

Table 3: Number of progeny produced by twice-mated sexual females (S.39)

<table>
<thead>
<tr>
<th>Female type</th>
<th>Replication</th>
<th>Progeny Mean ± SE (n)</th>
<th>Average Remating Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexual strain S.39</td>
<td>a</td>
<td>526.8 ± 37.3 (6)</td>
<td>3.8 days after initial mating</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>789.6 ± 74.3 (8)</td>
<td>3.9 days after initial mating</td>
</tr>
</tbody>
</table>
**Figure 2.** Summary of mean number of progeny (with standard error bars) produced by females in various conditions. *(Markow, unpublished)*

**Table 4.** Total progeny production by 3-day-old females (S.39 and P.05) kept with two 4-day-old males (S.39) for a lifetime.

<table>
<thead>
<tr>
<th>Female Strain</th>
<th>Mean ± SE</th>
<th>% FEMALE ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexual S.39 (9)</td>
<td>915.2 ± 135.2</td>
<td>48.6 ± 0.8%</td>
</tr>
<tr>
<td>Parthenogenetic P.05 (7)</td>
<td>213.5 ± 50.2</td>
<td>40.9 ± 6.4%</td>
</tr>
</tbody>
</table>

**Table 5.1** Average number of eggs laid in 20 days by virgin P.05 and S.39 females

<table>
<thead>
<tr>
<th></th>
<th>Ave. Eggs Laid ± SE</th>
<th># of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.39</td>
<td>52.92 ± 41.9</td>
<td>13</td>
</tr>
<tr>
<td>S.22</td>
<td>655.50 ± 47.8</td>
<td>10</td>
</tr>
<tr>
<td>P.05</td>
<td>749.88 ± 36.7</td>
<td>17</td>
</tr>
<tr>
<td>P.03</td>
<td>757.90 ± 47.8</td>
<td>10</td>
</tr>
</tbody>
</table>

**Table 5.2** ANOVA results of the effect of reproductive type and strain on egg laying

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum of Sq</th>
<th>Sq Mean</th>
<th>F Ratio</th>
<th>Prob(&gt;f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive type</td>
<td>1</td>
<td>2382036.8</td>
<td>2382037</td>
<td>36.83</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Strain</td>
<td>3</td>
<td>4434738.9</td>
<td>1478247</td>
<td>64.6753</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>
a. Virgin S.39

b. Virgin S.22

**Figure 3.** Number of eggs laid daily, expressed by individual females
c. Virgin P.05
Data are not available for 12 and 13 days old in some samples.

d. Virgin P.03
Data are not available for 18 and 19 days old.
**Figure 3.** continued
Figure 4. Average number of eggs laid per day by strain, with standard error bars.
Figure 5. Daily egg laying of sexual Hawaiian flies taken from Templeton (1976). Curves were estimated using a least-squares regression, explained in Templeton’s literature.
Figure 6. Daily egg laying of parthenogenetic (P.03) Hawaiian flies taken from Templeton (1976). Open circles indicate observed points, and a line is drawn to connect these points.
Table 6. Uncorrected COI distances of *D. mercatorum* generated in PAUP*. The text lists UCSD Drosophila Species Stock Center stock number with the location of which the stock was collected. Data from Richmond and Markow, unpublished.

<table>
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<th>15082-1521.40 Arizona</th>
<th>15082-1521.39 Arizona</th>
<th>15082-1521.38 Arizona</th>
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<th>5082-1521.33 Brazil</th>
<th>15082-1521.25 Brazil</th>
<th>15082-1521.22 Hawaii</th>
<th>15082-1521.02 Chile</th>
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<tbody>
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References


