Investigating Phenotypic Plasticity of Male Reproductive Investment using *D. Melanogaster*

Priya Bhatt
MS16121

A dissertation submitted for the partial fulfilment of BS-MS dual degree in Science

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Indian Institute of Science Education and Research Mohali
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Dedicated To My Parents

Certificate of Examination

This is to certify that the dissertation titled "investigating phenotypic plasticity of male reproductive investment using D. melanogaster " submitted by Ms. Priya Bhatt (Reg. No. MS16121) for the partial ful- filment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

Dr. Manjri jain

Dr. Rhitoban Ray Choudhury

Prof. N.G. Prasad
(supervisor)

Dated: May 30 2021

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Prof. N.G. Prasad at the Indian Institute of Science Education and Research Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Priya Bhatt

Dated: May 30, 2021

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Prof. N.G. Prasad

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Abstract

In promiscuous species, females mate multiple times which gives rise to post copulatory sexual selection (PCSS). The prediction of sperm competition theory is that males should tailor their ejaculate investment to suit the perceived level of sperm competition in their environment. Numerous studies have shown that changing the socio-sexual environment prior to mating results in plastic responses in male reproductive investment. Here, I investigated whether *Drosophila melanogaster* males can plastically modulate their reproductive investment in response to varying numbers of early life competitors and whether such responses are sensitive to the identity of competitors. I modulated the identity of competitors by either deriving males from populations evolving under male-biased or female-biased sex ratio, or by deriving competitor males with small or large body sizes. My results suggest that male reproductive investment, in the course of copulation duration and sperm defense ability, increases in response to the presence of competitor males in early life. However, I found no evidence to suggest that the magnitude of this increase is sensitive to the exact number or the identity of competitor males.

Chapter 1: Introduction

Evolution is the change that occurs in the heritable traits of biological populations over several generations. Charles Darwin and Alfred Russel Wallace (1858) were the first to give a mechanistic explanation of evolution by proposing a mechanism called natural selection. Expressed in modern terms, their argument was the following: populations could "adapt" to their environments through differential, heritable reproductive success. (Darwin 1871), in his famous treatise the Descent of Man, expanded the idea of natural selection by describing a novel phenomenon that was essential to explain several potentially "costly" male traits (eg, the peacock's tail). This mechanism was sexual selection. (Darwin 1871) argued that sexual selection 'depends on the advantage which certain individuals have over other individuals of the same sex and species, in exclusive relation to reproduction. There are two components selections. Intra-sexual selection is primarily mediated by male-male competition. On the other hand, inter-sexual selection is primarily mediated by female-choice (Darwin 1871),(Ferns 1995; Andersson 1994).

Traditionally, sexual selection research focused on differential mating success. However, it has become increasingly clearer that in many organisms sexual selection does not cease with mating. In some species such as Drosophila melanogaster, females mate multiply, and more importantly, have specialized sperm storage organs called spermatheca and seminal receptacle. Therefore, sperm deposited by more than one male can coexist and interact, creating the potential for post-copulatory sexual selection (Birkhead and Pizzari 2002). Post copulatory sexual selection is mediated by either female's choice for sperm (cryptic female choice) (Eberhard 1996; Gromko and Pyle 1978) and/or competition between sperm of multiple males to compete for the race to that particular egg (Sperm Competition) (Eberhard 1996). Sperm competition has evolutionary -morphological, behavioural and physiological traits- consequences (Snook 2005). The degree of sperm competition experienced by a male can be characterized by two parameters -- sperm competition risk (probability that females mate with more than one male) and sperm competition intensity (total number of ejaculates competing for access to a female's eggs). Sperm competition theory predicts that males should tailor their investment to suit the perceived risk and intensity of

sperm competition in their environment (Snook 2005; “Sperm Competition Games: Individual Assessment of Sperm Competition Intensity by Group Spawners” 1996), (Parker et al. 1997). A common way of experimentally manipulating the risk and intensity of

sperm competition in model organisms like D. melanogaster is to alter the socio-sexual environment by manipulating the number of competitor males experienced in early life [(Parker et al. 1997), (Parker 1970), (Wedell, Gage, and Parker, n.d.), (Wedell, Gage, and Parker, n.d.; Bretman, Gage, and Chapman 2011)]. Studies have shown that males can plastically alter their ejaculate investment based on the number of competitors experienced early in life. For example, (B. Nandy and Prasad 2011; Bretman, Fricke, and Chapman 2009) showed that males tend to increase their ejaculate investment when they are housed with rivals, relative to when they are held snugly. However, they found that this increase was not sensitive to the number of rival males, but was dependent solely on the presence or absence of rivals. On the other hand, (B. Nandy and Prasad 2011) reported a non-linear plastic pattern of male reproductive investment in order to vary the number of early adult life competitors. (Maggu et al. 2021) investigated whether the plastic patterns of male reproductive investment can evolve in response to varying levels of sexual selection. They held males from populations evolving under high or low levels of sexual selection with different numbers of rival males and reported that males evolving under higher levels of sexual selection continued to exhibit a non-linear pattern of reproductive investment, while males evolving under low levels of sexual selection exhibited a monotonically increasing pattern of reproductive investment. (Maggu et al. 2021) also showed that these patterns may be sensitive to the identity of competitor males.

During my thesis, I built upon these studies and investigated the nature of male plastic responses in reproductive investment. Specifically, I asked whether the pattern of male reproductive investment is sensitive to the identity of competitor males (ie high fitness competitors vs low fitness competitors). I manipulated the identity of competitors in two different ways. First I exposed baseline males to varying numbers of competitors derived from populations evolving under either high or low levels of sexual selection. Second, I exposed baseline males to either large competitors or small competitors. I measured male reproductive investment by measuring three fitness-related traits: mating latency, copulation duration and sperm defense ability (P1).

Chapter 2: Materials and Methods

2.1 Experimental Populations

2.1.1 Baseline Populations:

LH: LH is a large outbred population of Drosophila melanogaster that was founded by Larry Harshman in the 1990s. It is maintained on 1 14 day discrete generation cycle on cornmeal molasses yeast food medium at 20 degrees celsius and on 12 hour: 12 hour light:dark cycle. The total population size is around 2000 individuals. The detailed maintenance protocol can be found in (B. Nandy and Prasad 2011). Briefly, each generation starts with dispensing ~150 vials in a vial carrying 8-10 ml food. 60 such vials are set up for the LH populations. On the 12th day post egg collection, by which time all flies develop into adults, flies are transferred to conical flasks and then anesthetists using mild CO2 anesthesia. Flies are then sorted into groups of 16 males and 16 females. 60 food vials, each containing 16 males and females are set up. These flies are allowed to interact with each other for two days. On the 14th day post egg collection, flies are flipped into fresh food vials containing 8-10 ml food. Flies are allowed to oviposit in these vials for 18 hours, after which the adults are discarded. The eggs are trimmed to a density of about 150 eggs per vial, which starts the next generation.

LHst: LHst population is maintained in a manner identical to that of LH, except it has a total of 30 vials. LHst was set up by introgressing a benign, recessive autosomal eye colour marker into the LH populations. It is regularly backcrossed to the LH population to maintain genetic homogeneity between the two populations.

2.1.2 Selection Regimes:

In Experiment 1 (see below), we used populations belonging to two selection regimes (M and F). These selection regimes consist of a total of six populations of M(M1-3) and F(F1-3) which were derived from the LHst population. Two selection regimes are followed by these six populations - the male-biased (M) and female-biased (F). Three replicates - M1, M2, M3 and F1, F2, F3 - of these M and F populations are there. Both populations are maintained in the same manner except both of them are having different adult sex ratios. Male : female (m:f) is 3:1 for male-biased(M) and 1:3 for female-biased(F). For the maintenance of these population,14 days discrete cycle is followed at 250C and approximately 60% humidity and 12hours:12hours; Light: Dark cycle along with standard cornmeal–yeast-molasses food in standard vials (90mm× 30-mm; Length ✖ diameter). They are nurtured under a moderate density of 140-160 eggs in 8 dram vials containing 8-10ml of food. Flies are collected virgin at 9-10 days of their lifecycle in the density of 8 individual per single sex vial and on 12 days flies are alloted to their respective sex ratio regime in vials containing cornmeal - molasses - yeast food. After 2 days flies are transferred to new vials for oviposition. Within 18-22 hours eggs are laid, which are used for the next generation's egg collection. At the time of the experiments, M and F populations had undergone >250 generations of selection.

It has been previously shown that M males have evolved superior sperm competitive abilities (Bodhisatta Nandy et al. 2013), higher courtship frequencies and higher mate harming ability (Bodhisatta Nandy et al. 2012) relative to F males.

2.2 Standardisation:

In Experiment 2, before generating experimental flies we subjected M and F populations to one generation of common rearing or “standardisation” (Rose 1984). This equalized the effect of non-genetic parental effects across both regimes and allowed us to attribute differences observed (if any) to genetic differences between the two regimes.

2.3 Experimental Design:

Our experimental design was based on the design used by (B. Nandy and Prasad 2011). Our design was identical to theirs except that we replicated their design twice, once with each kind of competitor. Briefly, we held virgin LH males either singly or with 7, 15 or 31 competitors. The treatments with competitors were further divided into two treatments, where focal males were held with competitors of one kind or another (see below). In experiment 1 we manipulated competitor identity by deriving competitors from M or F populations. In experiment 2, we manipulated competitor identity by manipulating body size. After holding males with their competitors or singly, we assayed their reproductive investment by measuring three fitness-related traits: mating latency, copulation duration and sperm defense ability (P1). Below we describe these experiments in detail.

2.3.1 Experiment 1 (Male-biased M competitors or female-biased F competitors):

Here, we manipulated competitor identity by deriving competitors from populations evolving under stronger sexual selection (M) or populations evolving under weaker sexual selection (F).

Experimental flies (as competitors) were collected from these standardizing flies. Egg collection for the experimental males (LH), competitor males (M and F) females (LHst)was done on the same day. These eggs were reared in vials containing 6ml of cornmeal-molasses-yeast food at an exact density of 150. Considering egg collection on day 1, On 9-10th day virgin male flies from LH, F, M populations and virgin males and females from LHst were collected. M and F males were collected in groups of 7, 15 or 31 per vial. LHst males (it be used as second males in the sperm defense ability assay, see below) and LHst females were collected in groups of 8 per vial. Shortly after LH males enclosed (< 4 hours after eclosion) they were combined with M and F males or were held singly. Thus LH males were subjected to 7 different treatments::(a) 1LH♂ / vials (b) 1LH♂ ✖ 7M♂ / vial (c) 1LH♂ ✖ 15M♂ / vial (d) 1LH♂ ✖ 31M♂ / vial (e) 1LH♂ ✖ 7F♂ / vial (f) 1LH♂ ✖ 15F♂ / vials (g)1LH♂ ✖ 31♂F / vials . We aimed at having 35

replicate vials of each treatment; however in some cases, there were fewer vials. LH males were held in their respective treatments till the 12th day post egg collection. On the 12th day post egg collection, we measured the reproductive investment of LH males from each treatment by measuring three traits: mating latency, copulation duration and sperm defense ability. First, from each vial LH males were separated from competitor males under light CO_2 anesthesia. LH males were distinguished using their red eyes; the competitors had scarlet eyes. The competitors were discarded while LH males were transferred to fresh vials. After allowing these males to recover from anesthesia for an hour, each LH male was combined with a virgin LHst female. These vials were observed continuously till the pair finished mating. The time taken from combination to the beginning of mating was recorded as mating latency. The duration for which the pair remained in copula was recorded as copulation duration. The pairs that did not mate for 60 minutes were discarded. After the mating was over, LH males were aspirated out, LHst females were retained in the vials. Then, virgin LHst males were introduced in these vials. The LHst males and LHst females were allowed to interact for 20-22 hours, after which LHst females were transferred individually to test tubes (12mm ✖ 75mm) having 2 ml cornmeal - yeast - molasses food and allowed to oviposit for 18 hours. After this, the females were discarded and the test tubes were incubated under standard conditions for 12 days. When all the progeny had enclosed these test tubes, they were frozen at 20 degrees celsius. The progeny from each test tube were scored for eye color. Note that the scarlet eye color gene in the LHst population is autosomal recessive. Therefore, the progeny sired by the focal males (LH males subjected to each of the 7 treatments) were red-eyed, while the progeny sired by the competitor (LHst) males were scarlet. For each test tube, we calculated the proportion of progeny sired by the focal (LH) males and this was used as a measure of their sperm defense ability (P1). This entire experiment was repeated for two blocks (block 1 and block 2) of the M and F selection regimes.

2.3.2 Experiment 2 (Large low-density (LD) competitors or small, high-density (HD) competitors):

In this experiment, I manipulated the competitor identity by manipulating body size, which was done by altering the larval rearing density. Large competitor males (LD males) were obtained by collecting eggs from the LHst population at standard density (150 eggs per vial), while small

competitors (HD males) were obtained by collecting eggs from the LHst population at a higher density (300 eggs per vial). My pilot experiments suggested that LD males were about 20% larger than LD males.

Experimental flies were generated in the same way as Experiment 1. Ehhs were collected from the LH population (for focal males) at a density of 150 eggs per vial containing 8-10 ml food. For LHst males (to be used in the sperm defense assay as second males) and LHst females, eggs were collected from LHst population at a density of 150 eggs per vial. For LD competitors and HD competitors, as described above eggs were collected from LHst population at a density of 150 eggs per vial and 300 eggs per vial containing 8-10 ml food.

Considering egg collection on day 1, On 9-10th day virgin male flies from LD and HD and virgin male and female flies from LHst were collected. LD and HD competitors were collected in groups of 7, 15, 31 miles per vial. Shortly after LH males enclosed (< 4 hours post eclosion), they were either held singly or combined with LD or HD competitor males to set up the following seven treatments: a) 1LH♂ / vials (b) 1LH♂ ✖ 7LD♂ / vial (c) 1LH♂ ✖ 15LD♂ / vial (d) 1LH♂ ✖ 31LD♂ / vial (e) 1LH♂ ✖ 7HD♂ / vial (f) 1LH♂ ✖ 15HD♂ / vials (g)1LH♂ ✖ 31HD♂ / vials. For each treatment, we aimed to set up 35 replicate vials, although in some cases the sample size was lower. LHst males to be used in the sperm defense assay and LHst females were collected in groups of 8 per vial. LH males were kept in their respective treatments till the 12th day post egg collection. As described for Experiment 1, on the 12th day post egg collection, they were separated from their competitors (LH males were red-eyed, while LD and HD competitors were scarlet-eyed) and were assayed for three fitness-related traits (mating latency, copulation duration and sperm defense ability) in the same way as for Experiment 1. There were two replicates of this whole experiment.

Chapter 3: Statistical Analysis

All analysis was performed in R (version 4.0.2).

In both sets of experiments, using the R package “lmerTest", we analyzed each of the traits using a mixed model ANOVA, where treatment was a fixed factor while block (in Experiment 1) or replicate (Experiment 2) were fixed factors. Whenever we found a significant effect of treatment, we performed a post-factor Tokey’s HSD for multiple comparisons using the R package “emmeans' '. Note that the data for sperm defense ability were arc-sin-square-root transformed.

The exact models used for each trait were as follows:
Experiment 1: Measurable ~ Treatment + (1|Block)
Experiment 2 : Measurable ~ Treatment + (1|Replicate)

Chapter 4: Results

Experiment 1:

In this experiment, we held LH males with 0, 7, 15 or 31 competitors from either the M regime or the F regime. The outputs of our mixed models for Experiment 1 have been summarised in Table 1. For Mating latency, there was no significant effect of treatment (Figure 1A, $p = 0.3759$). For copulation duration, we found a significant effect of treatment (Figure 1B, $p = 0.0006$). Tukey's HSD (Table 2A) revealed that the 0 competitors' treatment had a significantly lower copulation duration than all other treatments, which were not significantly different from each other. The trend for sperm defense ability (P1) mirrored that of copulation duration, there was a significant effect of treatment (Figure 1C, $p = 0.0021$). Our post-factor Tukey's HSD, however, revealed that the 0 competitors' treatment was significantly different from the 7 F treatment and 31 M treatment. All other comparisons were not statistically significant (Table 2B).

Experiment 2:

In this experiment, we held LH males with 0, 7, 15, or 31 Lhst males reared under either low density (LD) conditions or high density (HD) conditions. The outputs of our mixed models for Experiment 2 have been summarised in Table 3. Just as in Experiment 1, here too, we could not detect an effect of treatment on mating latency (Figure 2A, $p = 0.8432$). There was a significant effect of treatment on copulation duration (Figure 2B, $p < 0.0001$). Our post-hoc Tukey's HSD (Table 4) revealed that just as in Experiment 1, the 0 competitors' treatment was significantly different from all other treatments, which were not significantly different from each other. There was no effect of treatment on sperm defense ability (P1) (Figure 2C, $p = 0.8432$).

Table 1. Output of mixed model ANOVA for Experiment 1 for mating latency (ML), copulation duration (CD) and sperm defense ability (P1)

	SS	MS	NumDF	DenDF	F value	Pr(>F)
ML	117.44	19.573	6	399.05	1.0764	0.3759
CD	353.8	58.967	6	400	4.05	0.0006***
P1	3.2002	0.5333	6	354.03	3.5332	0.0021**

Table 2A. Post-facto Tukey's HSD for copulation duration (Experiment 1)

contrast	estimate	SE	df	t.ratio	p.value
00 Males NA - 07 Males F	-1.9413	0.688	399	-2.82	0.074
00 Males NA - 07 Males M	-2.5194	0.691	399	-3.645	0.0056
00 Males NA - 15 Males F	-2.6192	0.723	399	-3.622	0.006
00 Males NA - 15 Males M	-2.8903	0.691	399	-4.183	0.0007
00 Males NA - 31 Males F	-2.3281	0.706	399	-3.297	0.0182
00 Males NA - 31 Males M	-2.7471	0.727	399	-3.78	0.0034
07 Males F - 07 Males M	-0.5781	0.683	399	-0.847	0.9797
07 Males F - 15 Males F	-0.678	0.715	399	-0.948	0.9643
07 Males F - 15 Males M	-0.9491	0.683	399	-1.39	0.8069
07 Males F - 31 Males F	-0.3868	0.698	399	-0.554	0.9979
07 Males F - 31 Males M	-0.8058	0.719	399	-1.121	0.9215
07 Males M - 15 Males F	-0.0999	0.718	399	-0.139	1
07 Males M - 15 Males M	-0.371	0.685	399	-0.541	0.9982
07 Males M - 31 Males F	0.1913	0.701	399	0.273	1
07 Males M - 31 Males M	-0.2277	0.722	399	-0.316	0.9999
15 Males F - 15 Males M	-0.2711	0.718	399	-0.378	0.9998
15 Males F - 31 Males F	0.2912	0.732	399	0.397	0.9997
15 Males F - 31 Males M	-0.1278	0.752	399	-0.17	1
15 Males M - 31 Males F	0.5623	0.701	399	0.803	0.9846
15 Males M - 31 Males M	0.1433	0.721	399	0.199	1

Table 2B. Post-factoTukey's HSD for sperm defense ability (P1)(Exp1)

contrast	estimate	SE	df	t.ratio	p.value

00 Males NA - 07 Males F	-0.27212	0.0749	354	-3.633	0.0059
00 Males NA - 07 Males M	-0.11284	0.0741	354	-1.524	0.7305
00 Males NA - 15 Males F	-0.10561	0.0786	354	-1.344	0.8308
00 Males NA - 15 Males M	-0.20779	0.0766	354	-2.713	0.0979
00 Males NA - 31 Males F	-0.10303	0.0782	354	-1.318	0.8432
00 Males NA - 31 Males M	-0.27685	0.0786	354	-3.524	0.0086
07 Males F - 07 Males M	0.15928	0.0719	354	2.216	0.2895
07 Males F - 15 Males F	0.16651	0.0766	354	2.175	0.3118
07 Males F - 15 Males M	0.06433	0.0746	354	0.863	0.9777
07 Males F - 31 Males F	0.16909	0.0761	354	2.222	0.2866
07 Males F - 31 Males M	-0.00473	0.0766	354	-0.062	1
07 Males M - 15 Males F	0.00723	0.0757	354	0.096	1
07 Males M - 15 Males M	-0.09495	0.0737	354	-1.288	0.8574
07 Males M - 31 Males F	0.00981	0.0752	354	0.13	1
07 Males M - 31 Males M	-0.16401	0.0758	354	-2.165	0.3174
15 Males F - 15 Males M	-0.10218	0.0783	354	-1.305	0.8493
15 Males F - 31 Males F	0.00258	0.0797	354	0.032	1
15 Males F - 31 Males M	-0.17124	0.0802	354	-2.135	0.3343
15 Males M - 31 Males F	0.10476	0.0778	354	1.346	0.8297
15 Males M - 31 Males M	-0.06906	0.0782	354	-0.883	0.9749
31 Males F - 31 Males M	-0.17382	0.0798	354	-2.179	0.3095

Table 3. Output of mixed model ANOVA for Experiment 2 for mating latency (ML), copulation duration (CD) and sperm defense ability (P1)

	SS	MS	NumDF	DenDF	F value	Pr(>F)
ML	55.199	9.1998	6	388.06	0.4524	0.8432
CD	893.95	148.99	6	389	9.6045	<0.0001
P1	1.3975	0.23291	6	166	1.5447	0.1666

Table 4. Post-facto Tukey's HSD for copulation duration (Experiment 2)

contrast	estimate	SE	df	t.ratio	p.value
00 MalesNA - 07 MalesHD	-2.7788	0.759	388	-3.664	0.0052
00 MalesNA - 07 MalesLD	-4.6711	0.753	388	-6.206	<.0001
00 MalesNA - 15 MalesHD	-4.0665	0.739	388	-5.506	<.0001
00 MalesNA - 15 MalesLD	-4.0665	0.739	388	-5.506	<.0001
00 MalesNA - 31 MalesHD	-4.6175	0.762	388	-6.059	<.0001
00 MalesNA - 31 MalesLD	-4.4089	0.785	388	-5.62	<.0001
07 MalesHD - 07 MalesLD	-1.8922	0.738	388	-2.563	0.1405
07 MalesHD - 15 MalesHD	-1.2877	0.724	388	-1.778	0.5634
07 MalesHD - 15 MalesLD	-1.2877	0.724	388	-1.778	0.5634
07 MalesHD - 31 MalesHD	-1.8386	0.748	388	-2.458	0.1778
07 MalesHD - 31 MalesLD	-1.6301	0.771	388	-2.115	0.346
07 MalesLD - 15 MalesHD	0.6045	0.717	388	0.843	0.9802
07 MalesLD - 15 MalesLD	0.6045	0.717	388	0.843	0.9802
07 MalesLD - 31 MalesHD	0.0536	0.741	388	0.072	1
07 MalesLD - 31 MalesLD	0.2621	0.764	388	0.343	0.9999
15 MalesHD - 15 MalesLD	0	0.702	388	0	1
15 MalesHD - 31 MalesHD	-0.5509	0.727	388	-0.758	0.9886
15 MalesHD - 31 MalesLD	-0.3424	0.75	388	-0.456	0.9993
15 MalesLD - 31 MalesHD	-0.5509	0.727	388	-0.758	0.9886
15 MalesLD - 31 MalesLD	-0.3424	0.75	388	-0.456	0.9993
31 MalesHD - 31 MalesLD	0.2085	0.774	388	0.27	1

Figure 1A) Effect of number and identity of competitors on the mating latency (Experiment 1). Red boxes indicate treatments with F competitors, while blue boxes indicate treatments with M competitors

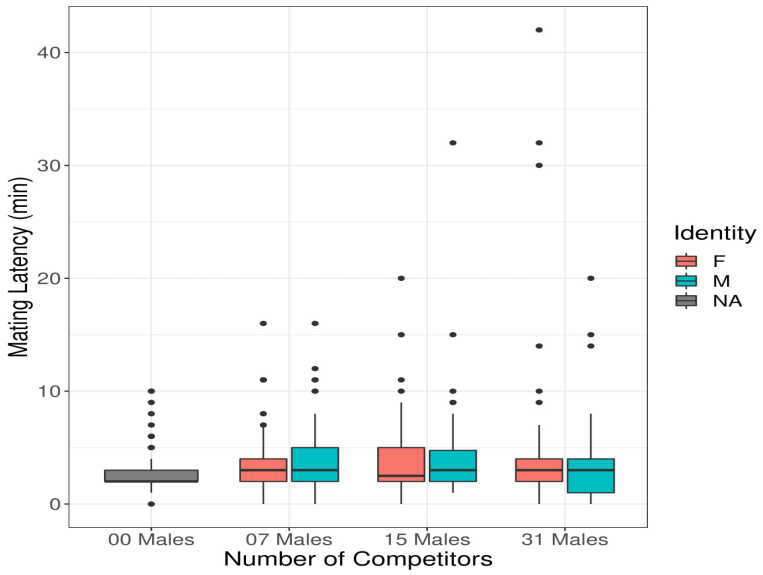


Figure 1B) Effect of number and identity of competitors on the copulation duration (Experiment 1). Red boxes indicate treatments with F competitors, while blue boxes indicate treatments with M competitors

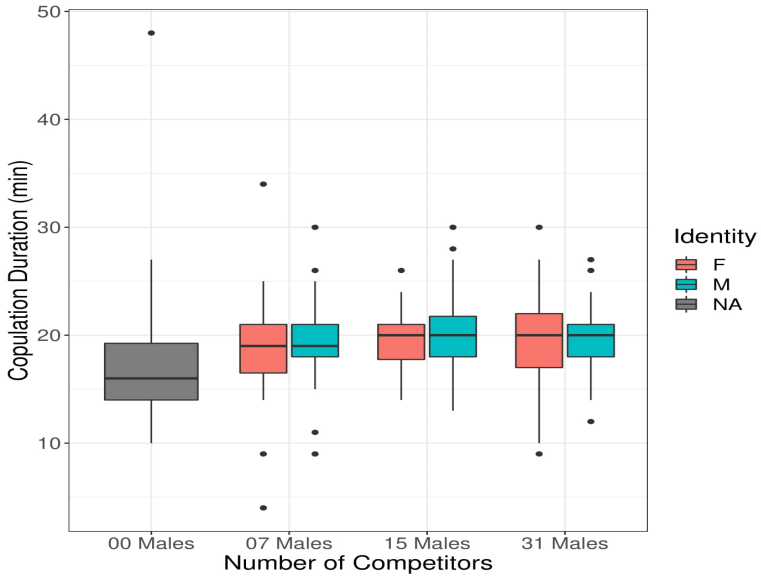


Figure 1C) Effect of number and identity of competitors on the copulation duration (Experiment 1). Red boxes indicate treatments with F competitors, while blue boxes indicate treatments with M competitors

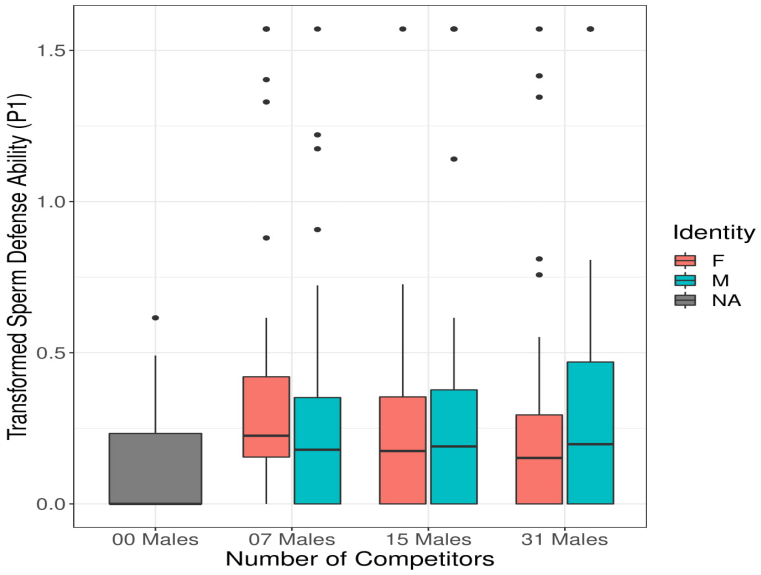


Figure 2A) Effect of number and identity of competitors on the mating latency (Experiment 2). Red boxes indicate treatments with HD competitors, while blue boxes indicate treatments with LD competitors.

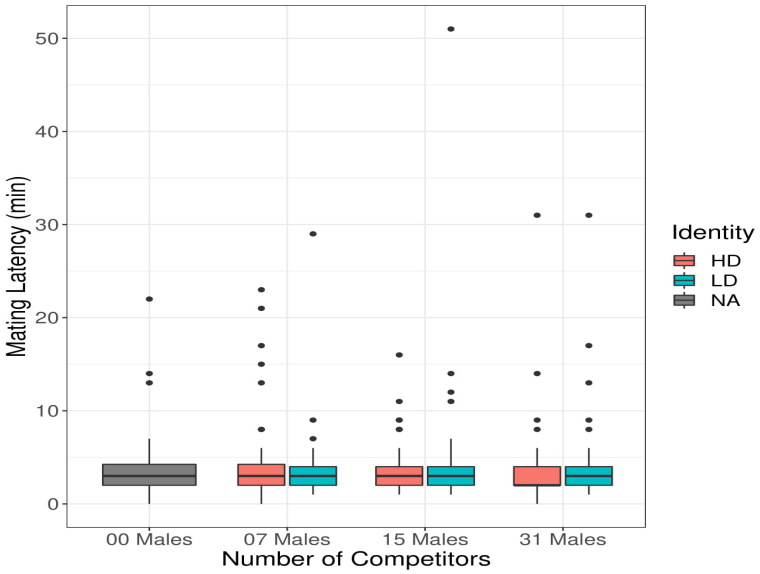


Figure 2B) Effect of number and identity of competitors on the copulation duration. (Experiment 2) Red boxes indicate treatments with HD competitors, while blue boxes indicate treatments with LD competitors.

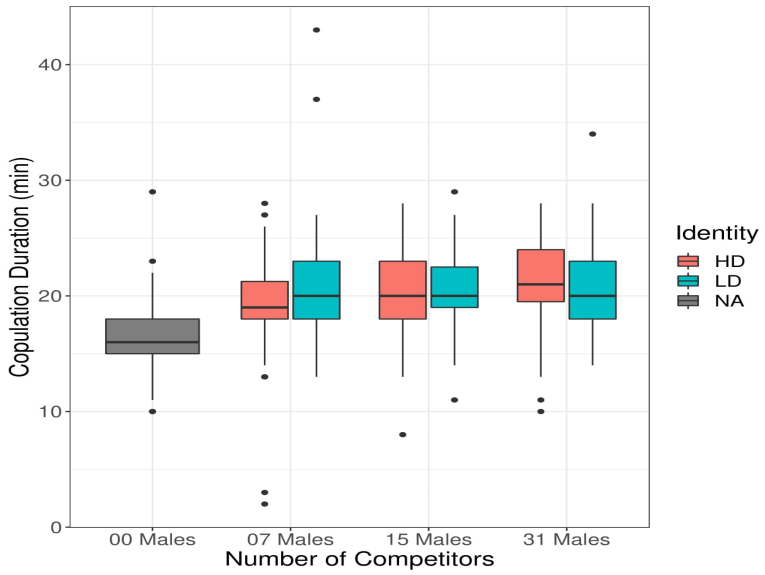
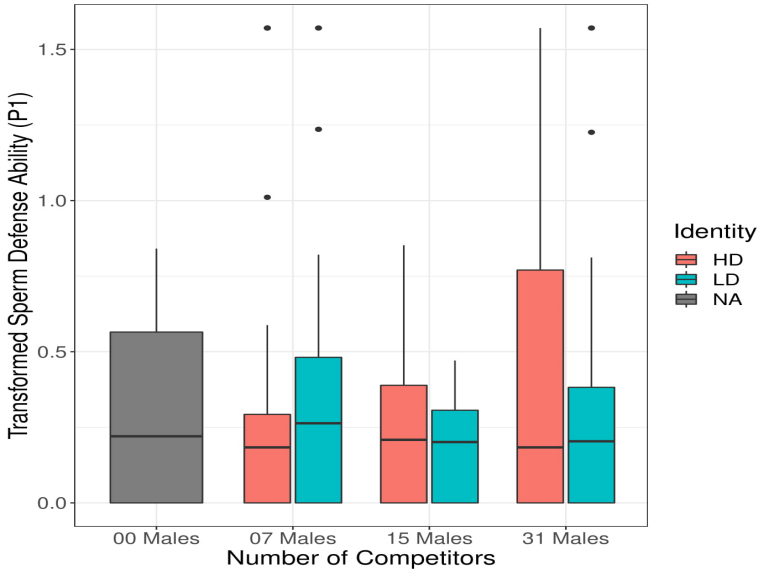


Figure 2C) Effect of number and identity of competitors on the sperm defense ability (P1) (Experiment 2). Red boxes indicate treatments with HD competitors, while blue boxes indicate treatments with LD competitors.



Chapter 5: Discussion

Previous studies showed that male reproductive investment varies with variation in identity (Bodhisatta Nandy et al. 2013; Maggu et al. 2021) and competitor number (Bodhisatta Nandy et al. 2013; Maggu et al. 2021), (B. Nandy and Prasad 2011). Manipulation of competitors results in the increased reproductive investment till an intermediate number of competitors but after that decline in reproductive investment takes place (B. Nandy and Prasad 2011) whereas manipulating the competitor's identity results in the continuous increase in reproductive investment for the population where male evolved under less competitive environment and nonlinear reproductive investment (increase till an intermediate number of competitors then decline in increase reproductive investment) could be seen for the population evolved under a highly competitive environment. Our findings show the increase in male reproductive investment when we introduce competitors to our focal male prior to the experiment but changing the number or identity does not make any difference to the male reproductive investment. Below, I discuss two important findings of my thesis.

1. No effect of the identity of competitor males:

We modulated the identity of competitor males in two separate ways, First, in Experiment 1, we used competitors from the M populations (evolving hider strong sexual selection) or from F populations (evolving under weak sexual selection). In both experiments, the plastic patterns of reproductive investment exhibited by our focal males were independent of the identity of the competitors they were housed with. This is a surprising result given that (Maggu et al. 2021) reported that such plastic responses may be sensitive to the identity of competitor males. Furthermore, the risk of level of sperm competition is also likely to be different based on the competitors. For example, (Bodhisatta Nandy et al. 2013) showed that M males have superior sperm competitive abilities compared to F males. Therefore, in my experimental treatments where the competitors were from M populations, the level of sperm competition experienced by the focal males is likely to be higher than the treatments where competitors were derived from the F populations. One of the reasons for my reasons could be that our focal males did not have

the ability to discriminate between M and F males, or were unable to gauge the elevated sperm competition posed by M males. Similarly, in experiment 2, we manipulated rearing density to alter the body size of competitors with LD males being on average ~12% larger. I speculate that a body size difference of 12% may not be sufficient to trigger changes in the plastic patterns of focal males. A follow up experiment where body size differences are even more extreme could help test this hypothesis.

These results also enable me to make an additional inference about the results of (Maggu et al. 2021). They have shown that the plastic patterns in the reproductive investment of M males are different from those of F males. They have argued that there could be two explanations for this. First, M and F males have evolved different plastic patterns per se. Second, M and F males are capable of exhibiting both kinds of plastic patterns, but the pattern they exhibit depends on the identity of their competitors. In (Maggu et al. 2021) experiments, M males were competing with M males, while F males were competing with F males. Therefore, they were not able to distinguish between these two explanations. My results indicate that baseline males (LH) respond identically to competitors from M and F populations; i.e. their plastic patterns do not change depending on whether the competitors were from M populations or F populations. These findings taken together with the results of (Maggu et al. 2021), provide strong evidence that M and F males have evolved divergent plastic responses to varying numbers of competitors in early life.

2. Reproductive investment increased in response to the presence of competitors, but was not sensitive to the number of competitors:

Both of my experiments suggest that male reproductive investment increased upon the introduction of competitors early in life. However, this increase was equal in treatments where there were 7, 15 or 31 competitors, suggesting that it is just the presence of competitors (within the range we studied) that stimulates an increase in reproductive investment and the actual number of competitors does not matter. These results are consistent with (Bretman, Fricke, and Chapman 2009) but are in contrast with (B. Nandy and Prasad 2011) and (Maggu et al. 2021), who had reported a non-linear matter of reproductive investment upon varying the number of early life competitors. This is surprising given that (B. Nandy and Prasad 2011) had used the

same experimental populations (LH) as this study. It is possible that this is a consequence of the long-drawn process of laboratory adaptation. The LH population was founded from 400 wild-caught females in the early 1990s. It has been maintained under the current maintenance regimen from the early 2000s. It can be argued that as generations go by the LH population is evolving from a state where it was adapted to its native, wild environment to a state where it is increasingly better adapted to the lab environment. (B. Nandy and Prasad 2011) carried out their experiments around 2010. I carried out my experiments nearly 10 years later, which is equivalent to nearly 260 fly generations. It is possible that as a consequence of lab adaptation the LH population used in this study is considerably different from the LH population used by (B. Nandy and Prasad 2011). More experiments are required to validate this hypothesis.

In conclusion, my results clearly show that D. melanogaster males can plastically modulate their reproductive investment in response to varying numbers of early life competitors. I showed that males increase their reproductive investment in response to experiencing competitors, early in life; however, this increase is not sensitive to the number of early life competitors but responds only to the presence or absence of competitors. Furthermore, I found no evidence to suggest that these plastic patterns can be influenced by varying the identity of early life competitor males.

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