Interlocus sexual conflict as an engine for post-zygotic isolation in *Drosophila melanogaster*

Martik Chatterjee

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Certificate of Examination

This is to certify that the dissertation titled "**Interlocus sexual conflict as an engine for post-zygotic isolation in *Drosophila melanogaster***" submitted by **Mr. Martik Chatterjee** (Reg. No. MS12079) for the partial fulfilment 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.Rhitoban Ray Chaudhury Dr.Manjari Jain Dr. N. G. Prasad

(Supervisor)

Dated: April 21, 2017

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr.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.

Martik Chatterjee

(Candidate)

Dated: April 21, 2017

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.

Dr.N. G. Prasad
(Supervisor)

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Abstract

Speciation, one of the most important biological processes, is a central theme of evolutionary biology. It happens –at least in sexually reproducing organisms - as individuals of a population acquire barriers that fully or partially impede them from reproducing with individuals from other similar populations. Of the many evolutionary processes that create such reproductive barriers (RB), inter-locus sexual conflict (IeSC) is put forwardas an important one.IeSC arises due to differential evolutionary interest of the two sexes in reproduction and results in rapid antagonistic coevolution of reproductive traits. This, as per verbal arguments and mathematical models, can lead to divergence in those traits and create RB in allopatric populations. This idea, however, remains controversial with little experimental support in its favour, especially from an experimental evolution perspective. Using replicate populations of laboratory adopted populations of *Drosophila melanogaster* selected for different levels of sexual conflict and known to show signs of pre-zygotic and post-mating pre-zygotic isolation, we investigated whether sexual conflict acts as a driving force for post-zygotic isolation or not. We do not find any compelling evidence of post-zygotic isolation in these populations. However, we noticed that in F2 generation, hybrids from the regime evolving under higher sexual conflict show signs of hybrid vigour for certain traits compared to their parental counterparts. This requires further investigation.

Introduction

In sexually reproducing species, males and females have different reproductive investment (Bateman 1948). In promiscuous species, inter locus sexual conflict (IeSC) is the phenomenon where this differential investment leads to different loci in the two sexes to evolve antagonistically (Parker 1979, Arnqvist and Rowe 2005). IeSC leads to antagonistic evolution of male and female physiology and behavior (e.g., mate harm caused by males due to their efforts to mate more and sire more progeny and female resistance towards mating) (Rowe et al. 1994, Chapman et al. 1995, Rice 1996, Nandy et al. 2013). According to verbal (Parker and Partridge 1998, Rice 1998, Rice 2005) and formal arguments (Gavrilets 2000, Gavrilets and Hayashi 2005), this antagonistic coevolution leads to divergence of genes in allopatric populations which on secondary contact will have reduced gene flow between them, demonstrating an instance of sexual conflict leading to speciation. These arguments can be tested by comparative phylogenetics or experimental evolution.

Arnqvist et al. showed that insect clades with sexual conflict had higher rates of speciation than those without (Arnqvist et al 2000). One can test these arguments by manipulating the degree of IeSC experimentally (either by enforcing monogamy or altering sex ratio) in allopatric populations for several generations and subsequently testing for reproductive isolation. Following this, allopatric populations with higher IeSC should show greater reproductive isolation compared to populations maintained under low IeSC. However, using this basic framework, there are only two studies that provide evidences of reproductive isolation (Martin and Hosken 2003, Syed et al. 2017(under preparation)). Several other studies using various model organisms, selection methods and methods of quantifying reproductive barriers have found no support for this theory (Plesnar-Bielak et al. 2013, Michalczyk Ł 2008, Bacigalupe et al. 2007, Gay et al.2009, Wigby and Chapman 2006, Hosken et al. 2009). After evolving replicate populations of *Sepsis cynipsea* under polygamy (presence of IeSC) and monogamy (removal of IeSC) for 35 generations, Martin and Hosken assayed proportion of successful mating as a measure of reproductive barrier. Allopatric pairs (males and females from different populations within the same regime) showed significantly less mating success compared to their sympatric counterparts in the polygamous, but not in monogamous regime (Martin and Hosken 2003).

Syed et al. on the other hand used laboratory adapted populations of *Drosophila melanogaster* maintained at varying levels of sexual conflict by manipulating sex ratio to test these arguments. They used three replicate populations each of a male biased (M) and a female biased regime (F). The M regime showed higher levels of IeSC and the F regime demonstrated low levels of IeSC (Nandy et al. 2013). According to theory, because of higher IeSC, when the replicate populations are brought into secondary contact, the M regime replicates should show higher reproductive isolation compared to F replicates. M males on given a choice, preferred to mate with females from the same replicate population over a different replicate population, whereas males from the F regime did not show any such preferences. Females from the M regime also tended to use sperm from males of same replicate population to fertilise eggs whereas no such bias was found in case of F females. Additionally, in the M regime, females showed reluctance to mate with heteropopulation males and males mated for a longer duration with conspecific females. All these evidences suggested that there were reproductive barriers emerging as a result of intense IeSC in the M populations.

However these barriers as seen by Syed et al. fall under the category of premating and post-mating pre-zygotic isolation. Another form of reproductive barrier is postzygotic isolation. It is a scenario where there is successful mating and fertilization between two different species or populations but the offspring are either unviable, sterile, or even if they develop properly, they fail to adapt ecologically and are not favored by natural selection (Coyne and Orr, 2004).

Using the same population of *Drosophila melanogaster* as in Syed et al., I investigate whether higher IeSC leads to formation of post-zygotic reproductive barriers. In allopatric populations, any mechanism which causes unique allelic combinations to become fixed has the potential to cause incompatibilities as novel allelic combinations come into contact and interact in hybrids (Dobzhansky Muller incompatibilities; reviewed in Johnson 2008). The incompatibilities tend to get magnified with subsequent generations.

I have created hybrids using two of the three replicate populations each of M and F regimes and look at the following traits in the offsprings in F1, F2 and F3 generations-

a. Dry Body weight at eclosion- to give an estimate of larval fitness

b. Desiccation and starvation resistance in both mated and virgin conditions- to give an estimate on how the parental and hybrid offspring do in stressful conditions
c. Total number of progeny produced- I believe that these populations have not diverged enough to show complete hybrid sterility and therefore we look into the number of progeny the hybrids sire to get an estimate of hybrid reproductive fitness. I looked at the fitness of all populations and saw whether there was a bias towards female progeny because the males might have developmental defect (Haldane's rule: Haldane 1922)
d. Mating latency and copulation duration- In some species, the hybrids are selected out because of lack of courting abilities (Stratton & Uetz, 1986). In order to look into that I investigated the time to start mating from time of introduction of females (mating latency) between parental and hybrids for the two regimes. I also calculated the copulation duration for each mating pair
e. Reproductive fitness of parental and hybrid offspring in competitive scenario- To give an idea of how the parental and hybrid strains perform in mate acquisition, sperm competition in presence of a competitor male.

Haldane's rule posits that males from hybridized populations are more likely to display developmental defects or loss of fitness. Thus, most of the above mentioned assays were performed on males. All these above assays, according to me will give an estimate of both extrinsic and intrinsic post-zygotic isolation if any.

Materials and methods

Fly maintenance:

Stock maintenance:

For details on the parental LH, LH_{st}, MCF population refer to chapter 2 of the thesis of Dr.Bodhisatta Nandy(2013).

Refer to Fig M1 for more details.

Generation of experimental flies and populations:

Eggs were collected at a density of 150eggs/vial from M1.M2, F1,F2 populations after a generation of standardization(refer to thesis of Dr.Bodhisatta Nandy for more details). Using these four populations we created 8 different populations-

a) Four parental populations-M1♂x M1♀, M2♂xM2♀, F1♂xF1♀, F2♂xF2♀

b) Four hybrid populations- M1♂xM2, M2♂xM1, F1♂xF2, F2♂xF1♀

All these above populations were created in between 176-182 generations of selection. Virgin flies were collected from M1.M2, F1, F2 populations on the 9-10th day of egg collection within 6hrs of eclosion on light CO_2 anaesthesia and kept at a density of 8 individual per vial in single sex vials with 2ml of cornmeal molasses food. Collected vials of virgin flies from the four populations were randomly assigned parental and hybrid treatments. On 12th day post egg collection, collected virgin flies were combined with the other sex (16 mating pairs) into fresh vials(vial A) supplied with 8ml of corn meal molasses food and limited live yeast. For parental crosses (eg. M1xM1 population), 2 vials of males from one population (eg.M1) were flipped along with 2 vials of females from the same population (eg.M1) into the same vial. For hybrid crosses (eg. F1♂ x F2♀), vials containing virgin males from one population(eg.F1) was combined with vials containing virgin females from a different population (eg.F2). After 48 hours, these flies were flipped into fresh vials(vial B) supplied with 8ml of cornmeal molasses food for egg laying. After 18 hours flies were flipped again into fresh vials (vial C) supplied with 8ml of cornmeal molasses food. Eggs in vials from which flies were flipped (vial B) were trimmed to a density of approximately 150 eggs/vial. These eggs were used to generate the next generation. The flies flipped into vial C

were allowed to lay eggs for 18 hours. Eggs were trimmed to a density of 150 eggs/vial and flies emerging from them were used to conduct experiments for that particular generation. For subsequent generations (F2 and F3 generations), no virgin collection was done, but on 12^{th} day of trimming eggs from vial B, vials from that particular population were pooled and adults were segregated into a density of 16 mating pair per vial supplied with 8ml of cornmeal molasses food and live yeast, thereby making them vial A for the next generation and subsequent protocol was followed for future generations.

Experimental assays:

Life history traits:

Dry body weight measurement:

Eggs for experimental flies were trimmed in vial C at a density of approximately 150 eggs/vial on 8-10ml food. Virgin males were collected very young (4-6hrs after eclosion) on 9^{th}day of egg collection under light CO_2 anaesthesia. Flies were flash frozen at -20^oC immediately after virgin collection. Flies were later dried at 60^oC for 48 hours and weighed in a high precision electronic balance (Sartorius CPA225D) to the nearest 0.01mg. A total of 50 males per population were measured for body weight distributed in groups of 5 each. Mean body weight of each group was calculated and taken as unit of analysis. Same was repeated for F2 and F3 generations.

Starvation resistance assay:

Eggs for experimental flies were trimmed in vial C to a density of approximately 150 eggs/vial on 8-10ml food. Virgin males were collected on 9^{th}day of egg collection under light CO_2 anaesthesia at an interval of 6hrs at a density of 8 individual per vial. On the 12^{th} day post egg trimming, virgin males randomly chosen from the pool of collected vials were assigned virgin and mated treatments. For virgin treatment flies were sorted into groups of 5 and aspirated into 8 dram vials with non-nutritive agar gel (1% Agar-agar solution + 1% p-hydroxy benzoate solution) and plugged with cotton. The agar gel provides moisture for the flies and the flies are only starved and not desiccated. There were ten vials containing 5 flies from each population. They were observed every 6 hours till all flies in a vial died. Time taken to death for each fly was considered as unit of analysis.

For mated treatment, eggs were collected from LH_{st} population at a density of 150 eggs/vial on the same day eggs were trimmed in vial C so that eclosion of LH_{st} and experimental flies were synced. On 9-10th day of egg collection, virgin females were collected as young as <6hrs from eclosion on light CO_2 anasthesia and housed at a density of 8 individuals/vial supplied with 2ml of cornmeal molasses food. On 12th day of egg collection, these females were flipped into fresh vials supplied with 2ml of cornmeal molasses food along with males from the experimental population which were randomly selected for the mated treatment. These mixed sex vials were kept for 48hours and on 14th day from egg collection/egg trimming, males were segregated from the mixed sex vials on light CO_2 anaesthesia and kept in starvation resistance vials. There were ten vials containing 5 flies from each population. They were observed every 6 hours till all flies in a vial died. Time taken to death for each fly was considered as unit of analysis.

Desiccation resistance assay:

Eggs for experimental flies were trimmed in vial C to a density of approximately 150 eggs/vial on 8-10ml food. Virgin males were collected on 9thday of egg collection under light CO_2 anaesthesia at an interval of 6hrs at a density of 8 individual per vial. On the 12th day post egg trimming, virgin males randomly chosen from the pool of collected vials were assigned virgin and mated treatments. For virgin treatment flies were sorted into groups of 5 and aspirated into empty 8 dram vials with a cotton plug and ~5gm silica gel and parafilm to seal the mouth of the vial. The silica gel acts as desiccators to absorb moisture from inside the vial. The parafilm acts as a barrier between the outside environment and the silica gel so that no moisture from outside comes into the desiccation vial. There were ten vials containing 5 flies from each population. They were observed every 1 hour till all flies in a vial died. Time taken to death for each fly was considered as unit of analysis.
For mated treatment, eggs were collected from LH_{st} population at a density of 150 eggs/vial on the same day eggs were trimmed in vial C so that eclosion of LH_{st} and experimental flies were synced. On 9-10th day of egg collection, virgin females were collected as young as <6hrs from eclosion on light CO_2 anasthesia and housed at a density of 8 individuals/vial supplied with 2ml of cornmeal molasses food. On 12th day of egg collection, these females were flipped into fresh vials supplied with 2ml of cornmeal molasses food along with males from the experimental population which were randomly selected for the mated treatment. These mixed sex vials were kept for 48hours and on 14th day from egg collection/egg trimming, males were segregated from the mixed sex vials on light CO_2 anaesthesia and kept

in desiccation resistance vials. There were ten vials containing 5 flies from each population. They were observed every hour till all flies in a vial died. Time taken to die for each fly was considered as unit of analysis.

Mating latency, copulation duration and total number of progeny:

Eggs for experimental flies were trimmed in vial C to a density of approximately 150 eggs/vial on 8-10ml food. Virgin males and females were collected on 9^{th}day of egg collection under light CO_2 anaesthesia at an interval of 6hrs at a density of 8 individuals per vial. On the day of egg trimming, LH_{st} eggs were collected in 8-10ml cornmeal molasses food vial, at a density of 150 eggs/vials. On 9^{th}- 10^{th} day of egg collection, virgin males and females were collected from the LH_{st} population at a density of 10 individuals/vial and kept in separate sex vials. On 12^{th} day, virgin males from parental and hybrid regimes were aspirated into fresh food vials containing 2ml of cornmeal molasses food along with a female from LH_{st} population. Similarly a female from parental and hybrid population was introduced with a male from LH_{st} population. In both cases the vials were coded to negate observer bias and volunteers measured mating latency (time taken to start mating from time of introduction of females/males) and copulation duration (total time spent in mating). After mating, males were aspirated out from the vials. Vials in which the flies did not mate within one hour of introduction were removed. After 48hrs from end of mating, females which mated were transferred into oviposition test tubes (dimensions:12mmx75mm) supplied with corn meal molasses food for 18hrs to lay eggs. On completion of 18hours, females were discarded and test tube kept in standard laboratory conditions for 12 days for progeny to eclose. On 12^{th} day, the test tubes were frozen at -20^{o}C and subsequently progeny were counted and sexed. Total number of progeny and proportion of female progeny were taken as unit of analysis.

Competitive mating success:

Eggs for experimental flies were trimmed in vial C to a density of approximately 150 eggs/vial on 8-10ml food. Virgin males were collected on 9^{th}day of egg collection under light CO_2 anaesthesia at an interval of 6hrs at a density of 8 individuals per vial. On the day of egg trimming, LH_{st} and LH eggs were collected in 8-10ml cornmeal molasses food vial, at a density of 150 eggs/vials. Virgin males were collected from LH population at a density of 10 individuals/vial and females from Lh_{st} at a density of 10 individuals/vial. On 12^{th} day of egg trimming, one male from the collected hybrid or parental population was aspirated into a fresh vial with 2ml cornmeal molasses food. Another rival male from the LH population was

aspirated into the same vial along with an LH_{st} female. The combination was kept like this for 48 hours. On 14th day from egg trimming, the LH_{st} females were separated on light CO_2 anaesthesia and transferred to oviposition test tubes (dimensions:12mmx75mm) supplied with corn meal molasses food for 18hrs to lay eggs. On completion of 18hours, females were discarded and test tube kept in standard laboratory conditions for 12 days for progeny to eclose. On 12th day, the test tubes were frozen at -20°C and subsequently progeny were scored for red or scarlet eye colour. Since scarlet eye colour is a homozygous recessive marker, all progeny sired by the focal males are scarlet eye coloured and those sired by LH are red eye coloured. Proportion of scarlet eyed progeny from each vial was used as unit of analysis. This was done only for F2 and F3 generations. The F3 data is not shown here.

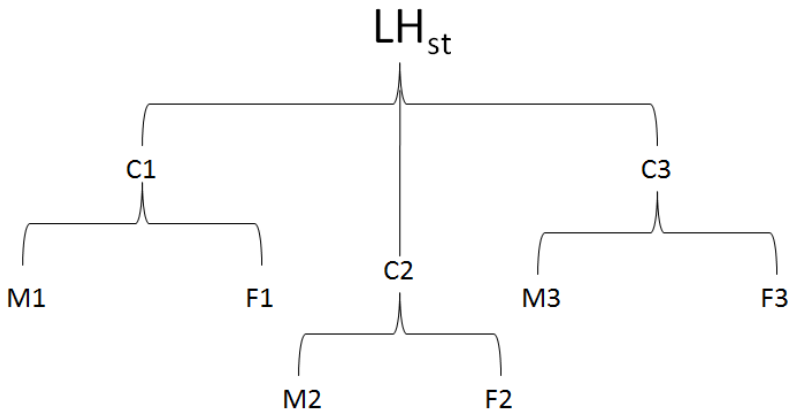

Fig M1: C(1-3) are populations with equal sex ratio derived from LH_{st} 1-3 denote separate blocks which are allopatric populations with no gene flow in between them. M denotes population with male-biased sex ratio(3♂:1♀) and higher IeSC. F denotes population with female-biased sex ratio(1♂:3♀) and low IeSC.

♀ \ ♂	M1	M2
M1	M1M1	M2M1
M2	M1M2	M2M2

FigM2: Crossing scheme within M regime. M1M1 are parental populations and M1M2, M2M1 are hybrids.

♀ \ ♂	F1	F2
F1	F1F1	F2F1
F2	F1F2	F2F2

FigM3: Crossing scheme within F regime. F1F1 are parental populations and F1F2, F2F1 are hybrids.

Data analysis and results

Data analysis

All data was analysed using JMP 7.

For dry body weight on eclosion, data was analysed using two way ANOVA using type (parental or hybrid) and selection regime(M or F) as fixed factors. It was done for F1, F2, F3 generations. I did a Tukey's HSD to look for significant interections. In F2 generation we did a one way ANOVA to compare between M1♂xM2♀ and M2♀xM1♂.

For desiccation resistance and starvation resistance assays (both virgin and mated treatment), data was analysed using two way ANOVA using type (parental or hybrid) and selection regime(M or F) as fixed factors. It was done for F1, F2, F3 generations. I did a Tukey's HSD to look for significant interactions. In case of difference between hybrid and parental type was found, I did a one way ANOVA keeping the cross as factor to see if there was a difference between reciprocal crosses.

For competitive mating success, since the data was non parametrically distributed, we analysed using a Kruskal Wallis test separately for M and F regimes keeping type (parental and hybrid) as a factor. We did this only for F2 generation. In case of significant difference within a particular type, I did a Kruskal Wallis test for that type and regime comparing the reciprocal crosses.

For mating latency(ML), data was analysed using two way ANOVA with type (parental or hybrid) and selection regime (M or F) as fixed factors. For copulation duration(CD), data was analysed using two way ANOVA using type (parental or hybrid) and selection regime (M or F) as fixed factors. For both ML and CD, it was done separately for males and females. In case of differences between parental and hybrid types for a regime, I did a one way ANOVA taking the type of cross as a fixed factor to see if there was difference between reciprocal crosses.

For total number of progeny produced by males and females from selected populations, data was analysed only for F2 generation, using two way ANOVA with type (parental or hybrid) and selection regime (M or F) as fixed factors. Assuming that there is no bias towards a

particular sex and that the proportion of female progeny produced is 0.5, I did a t-test to look for differences from the null hypothesis.

Results

Male Life History Traits:

Dry body weight on eclosion (Fig R1):

In F1 generation, there is no significant difference in between the hybrid and parental strains of both the regimes. In F2 generation, hybrids from the M regime have significantly higher body weight compared to M parental ($p=0.0014$) and F parental types but not F hybrids (dry body weight(mg) $\pm$ SE: M Hybrid: 0.161 $\pm$ 0.005; F Hybrid: 0.148 $\pm$ 0.005; M Parental: 0.130 $\pm$ 0.005; F Parental: 0.129 $\pm$ 0.005) (Fig R1). However this difference in M hybrids can be attributed to M2♂xM1♀ progeny crosses whose dry body weight on eclosion was significantly higher than M1♂xM2♀ progeny($p<0.0001$)(Data not shown). In F3 there is no significant difference between F hybrid and parental types and between M parental and hybrid types (Fig R1). Differences between M and F regime are not shown.

Desiccation resistance (virgin treatment) (Fig R2):

In F1 generation, there is no significant difference in mean time to death under desiccating conditions between M hybrid and M parental types. Also there is no significant difference between F hybrids and F parental types. In F2 generation, M hybrids survived for a longer duration significantly more than M parental types($p<0.0001$) and no such difference is found between F parental and hybrid types (mean time to death(hrs) $\pm$ SE: M Hybrid: 14.632 $\pm$ 0.236; F Parental: 12.91 $\pm$ 0.236; M Parental: 12.67 $\pm$ 0.236; F Hybrid: 12.55 $\pm$ 0.236). There is no significant difference between reciprocal crosses of M hybrids. In F3 generation, there is no significant difference between parental types within M regime as well as F regime (Fig R2). Differences between M and F regime are not shown.

Desiccation resistance (mated treatment) (Fig R3):

In F1 generation, there is no significant difference in mean time to death under desiccating conditions between M hybrid and M parental types. However in case of F hybrids and F parental types, there is a significant difference with F hybrids dying much faster than F parental types ($p=0.003$) (mean time to death(hrs) $\pm$ SE: M Hybrid: 9.176 $\pm$ 0.217; F Parental: 9.113 $\pm$ 0.217; M Parental: 9.105 $\pm$ 0.224; F Hybrid: 8.178 $\pm$ 0.205). However, there is a significant difference between the reciprocal crosses of the F hybrids ($p<0.0001$) (Data not shown). In F2 generation, there is a significant difference between M parental and M hybrids ($p<0.0001$) but no such difference is found in the F regime. The M hybrids live for a longer duration than parental types (mean time to death(hrs) $\pm$ SE: M Hybrid: 8.996 $\pm$ 0.309; F Parental: 7.907 $\pm$ 0.291; M Parental: 7.42 $\pm$ 0.299; F Hybrid: 8.087 $\pm$ 0.291). There is also no difference between the reciprocal crosses of the M hybrids. In F3 generation, F parental types survived for a longer duration than F hybrids ($p=0.0003$) and no such difference was found in the M regime (mean time to death(hrs) $\pm$ SE: M Hybrid: 8.307 $\pm$

0.158; F Parental: 9.001 ± 0.158; M Parental: 7.995 ± 0.158; F Hybrid: 8.060 ± 0.158). Differences between M and F regime are not shown.

Starvation resistance assay (virgin treatment) (Fig R4):

In all the three generations (F1, F2, F3), there is no significant difference between hybrid and parental types in both M and F regimes. Differences between M and F regime are not shown.

Starvation resistance assay (mated treatment) (Fig R5):

In F1 generation, M parental types live for a longer duration than M hybrids in starved conditions (p=0.01) (mean time to death (hrs) ± SE: M Hybrid: 54.64 ± 1.13; M Parental: 58.95 ± 1.13). No such difference was found between parental and hybrid strains in the F regime. In F2 and F3 generations, there was no difference between the parental and hybrid strains within a selection regime. Differences between M and F regime are not shown.

Competitive mating success (Fig R6):

In F2 generation, there is no significant difference between proportion of scarlet progeny sired in the parental and hybrid populations from the F regime. However within the M regime, the M hybrids sire significantly higher proportion of scarlet progeny than M parental types (p=0.0087) (KW Ranked Sum test, Score mean: M Hybrids: 76.154, M Parentals: 56.0211). When the reciprocal crosses in hybrid were compared (KW Ranked Sum test), no significant difference was found.

Male reproductive traits:

Mating Latency (Fig R7a):

In F2 generation, there is no difference in mating latency between hybrid and parental populations in both M and F regimes. In F3 generation, hybrids from the F regime take a significantly longer time to start mating compared to parental F males(p=0.0018) (mean mating latency(min) ± SE: F Hybrid: 9.17 ± 0.86; F Parental: 4.21 ± 0.86). When the reciprocal crosses were analysed, males from the F1♂xF2♀ crosses took a significantly shorter duration to mate with females compared to F2♂xF1♀ males (p=0.01). When the hybrid and parental types for M regime were compared, M hybrids took a longer duration to start mating compared to parental types (mean mating latency(min) ± SE: M Hybrid: 5.21 ± 0.87; F Parental: 3.77 ± 0.87). However it was not significant (p=0.0473).

Copulation Duration (Fig R7b):

In F2 generation, M hybrids mate for a longer duration than M parental type (p=0.0031) (mean copulation duration (min) ± SE: M Hybrid: 19.47 ± 0.66; M Parental: 16.57 ± 0.68). When analysis was done between reciprocal crosses of M hybrid population, it was seen that males from M1♂xM2♀ cross mated for a significantly longer duration than M2♂xM1♀ cross(p=0.002). However no such difference in duration of mating was found within F regime. In F3 generation, M parental types mate for a significantly longer duration than hybrid populations of the same regime (p=0.0063) (mean copulation duration (min) ± SE: M

Hybrid: 17.22 ± 0.35; M Parental: 18.62 ± 0.35), whereas no such difference was found within F regime.

Total number of progeny and sex-ratio bias:

In F2 generation, no difference between total number of progeny was found within M and F regimes (Fig R7c). Also, there was no evidence of sex ratio bias (data not shown).

Female reproductive traits:

Mating latency (Fig R8a):

In both F2 and F3 generations, no significant difference was found between hybrid and parental types in both F and M regimes.

Copulation duration (Fig R8b):

In both F2 and F3 generations, no significant difference was found between hybrid and parental types in both F and M regimes.

Total number of progeny and sex-ratio bias:

In F2 generation, no significant difference was found between total numbers within M and F regimes (Fig R8c). Also, there was no evidence of sex ratio bias (data not shown).

Figures:

Fig R1: Dry body weight on eclosion. Different alphabets denote significant difference within each generation. P=0.0014.Between regime differences are not shown.

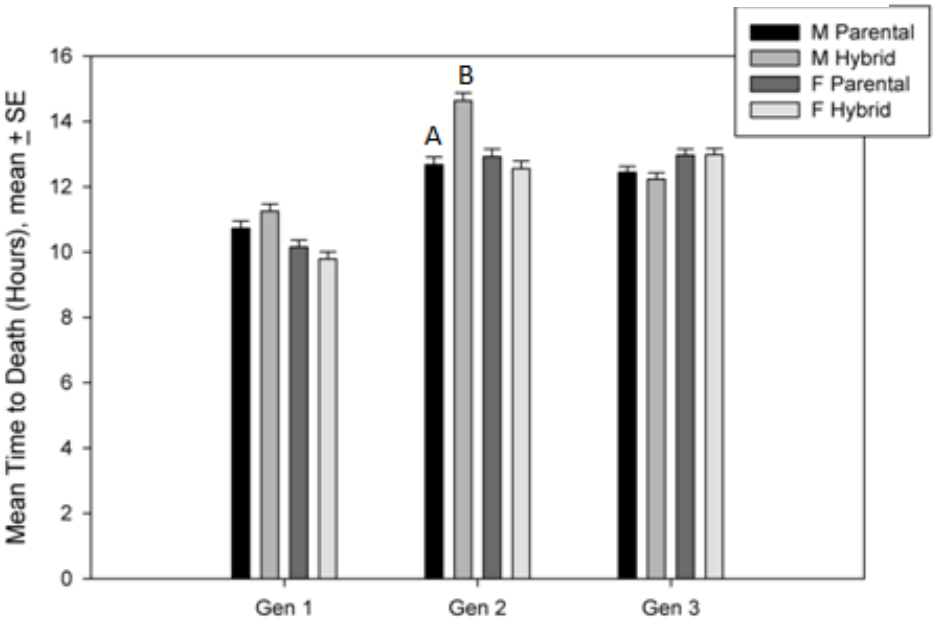

Fig R2: Desiccation resistance (Virgin treatment) Different alphabets denote significant difference within each generation. P<0.0001 . Between regime differences are not shown.

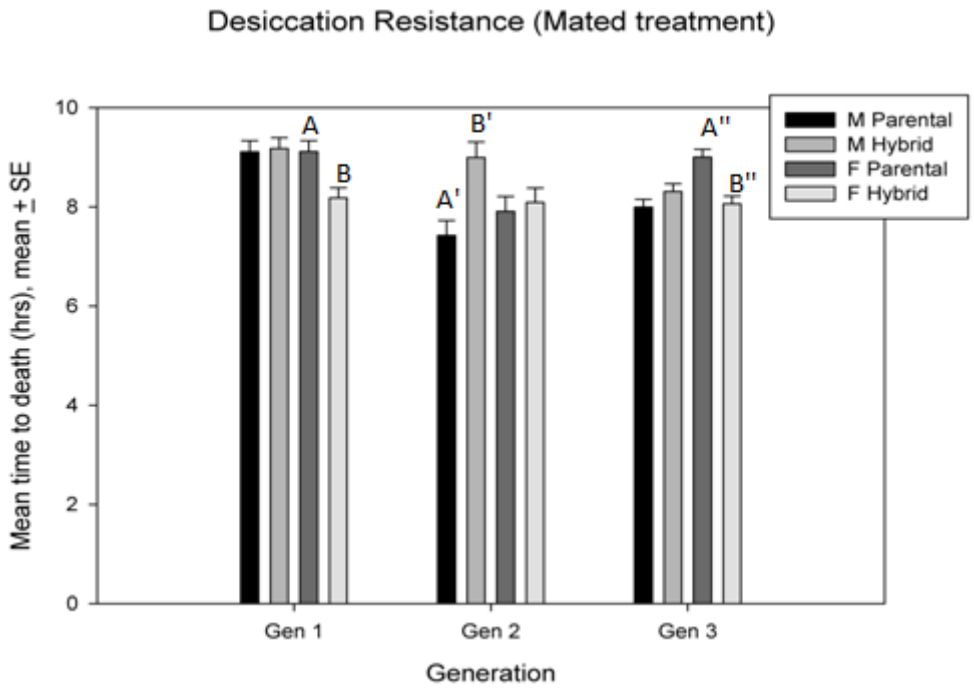

Fig R3: Desiccation resistance (Mated treatment) Different alphabets denote significant difference within each generation. For p-values refer to results. Between regime differences are not shown.

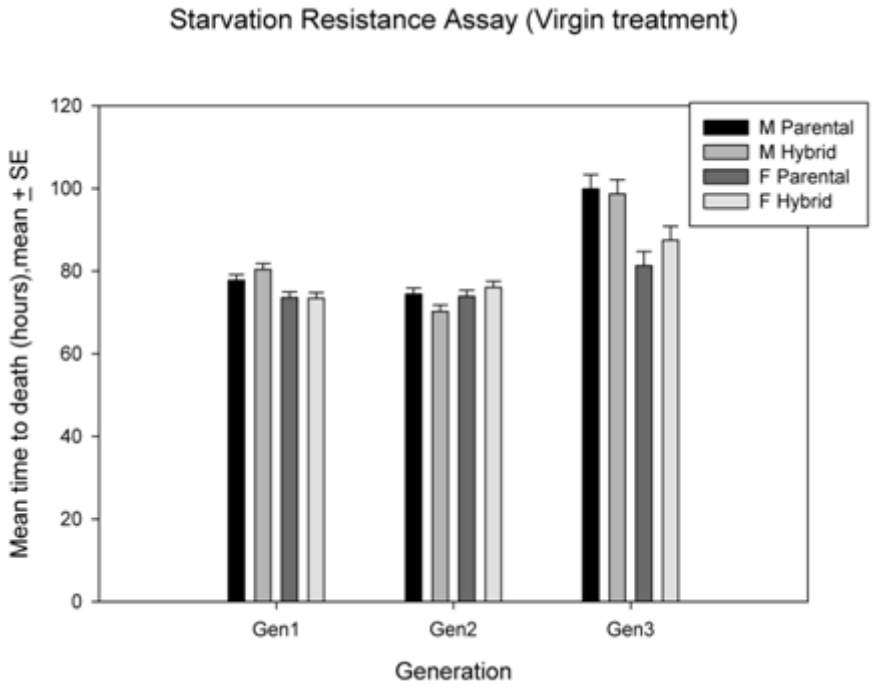

Fig R4: Starvation resistance (Virgin treatment). Between regime differences are not shown.

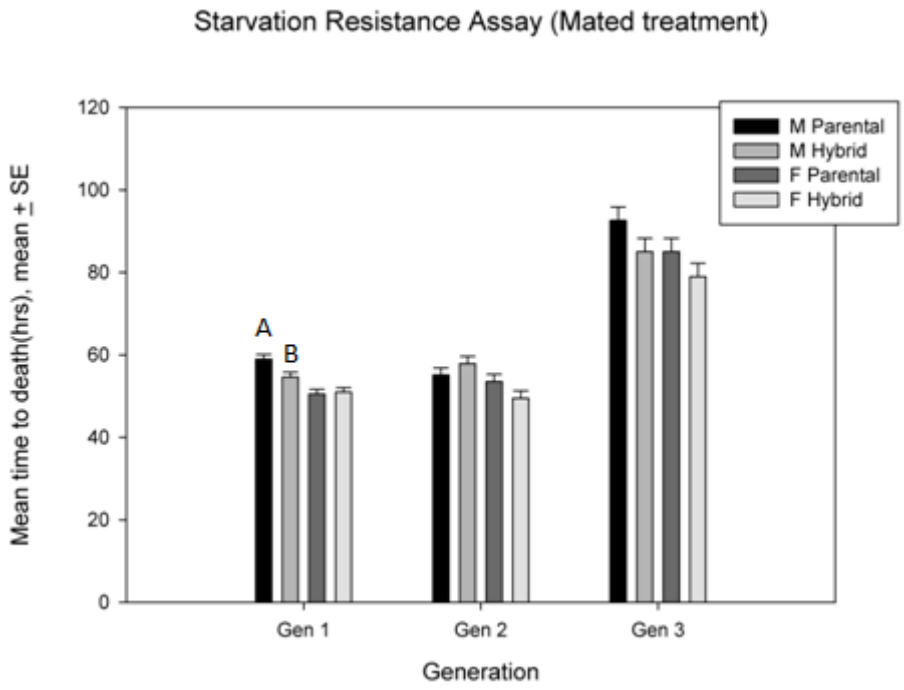

Fig R5: Starvation resistance (Mated treatment) Different alphabets denote significant difference within each generation. p=0.01. Between regime differences are not shown.

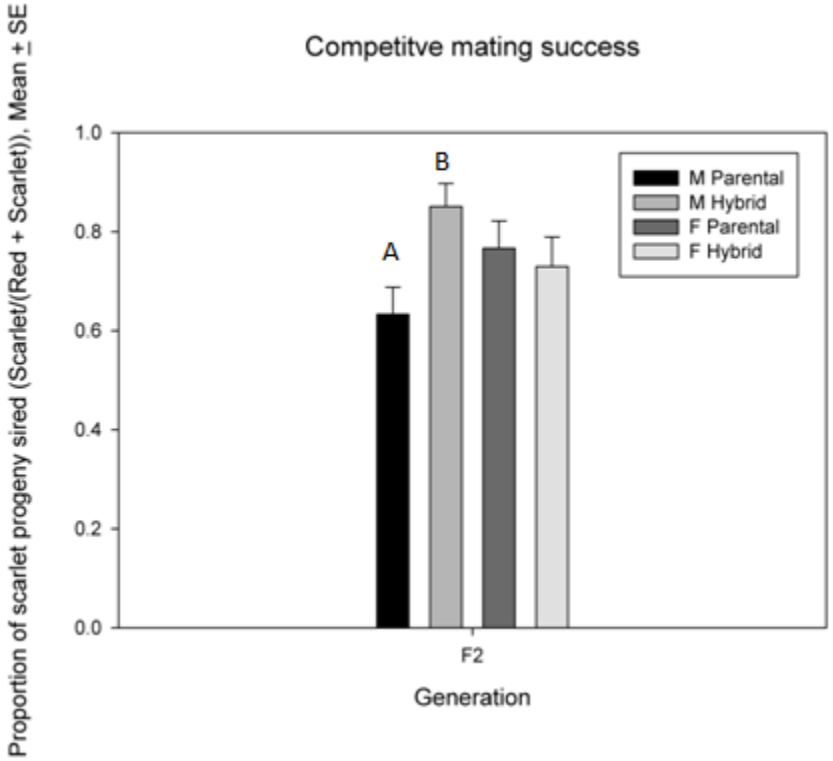

Fig R6: Competitive mating success: Different alphabets denote significant difference within each generation. p=0.0087

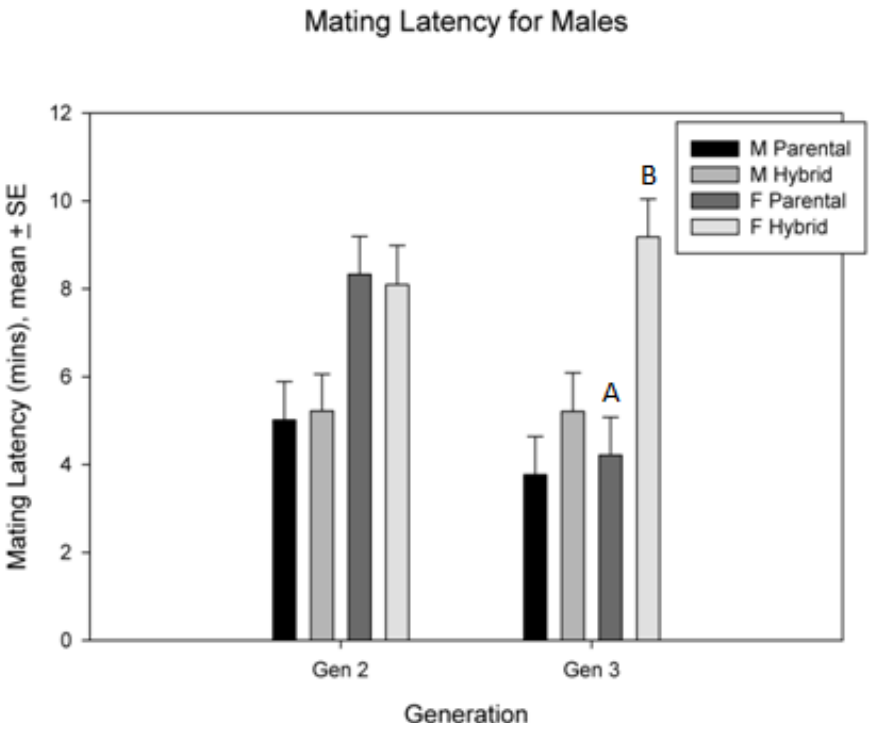

Fig R7a: Mating Latency for males from selected populations:
Different alphabets denote significant difference within each generation. P=0.0018. Between regime differences are not

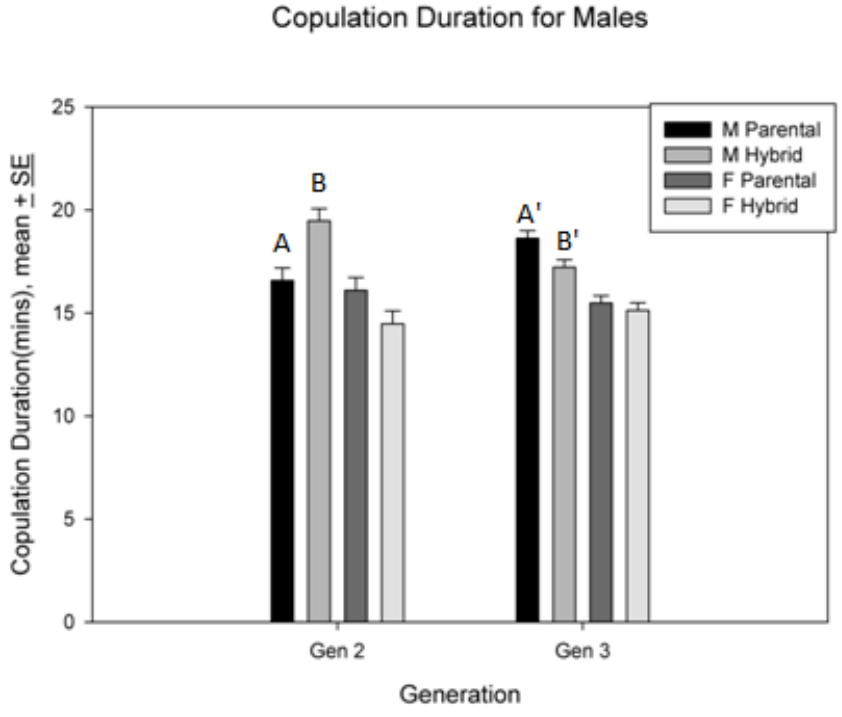

Fig R7b: Mating Latency for males from selected populations:
Different alphabets denote significant difference within each generation. Refer to results for p value. Between regime differences are not shown.

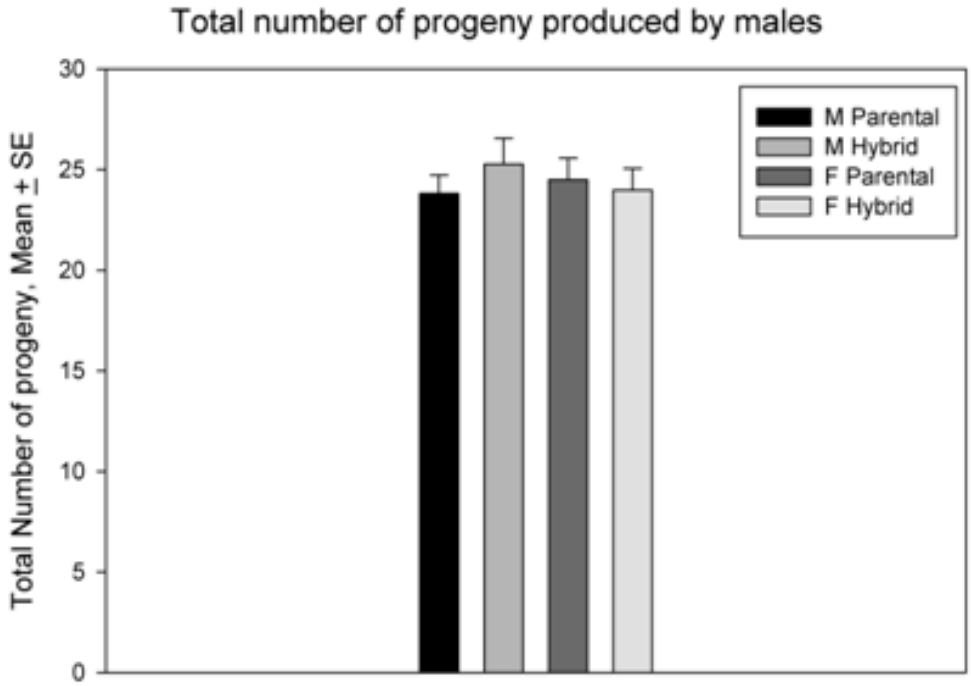

Fig R7c: Total Number of progeny produced by males from selected parental and hybrid crosses in F2 generation. Between regime differences are not shown.

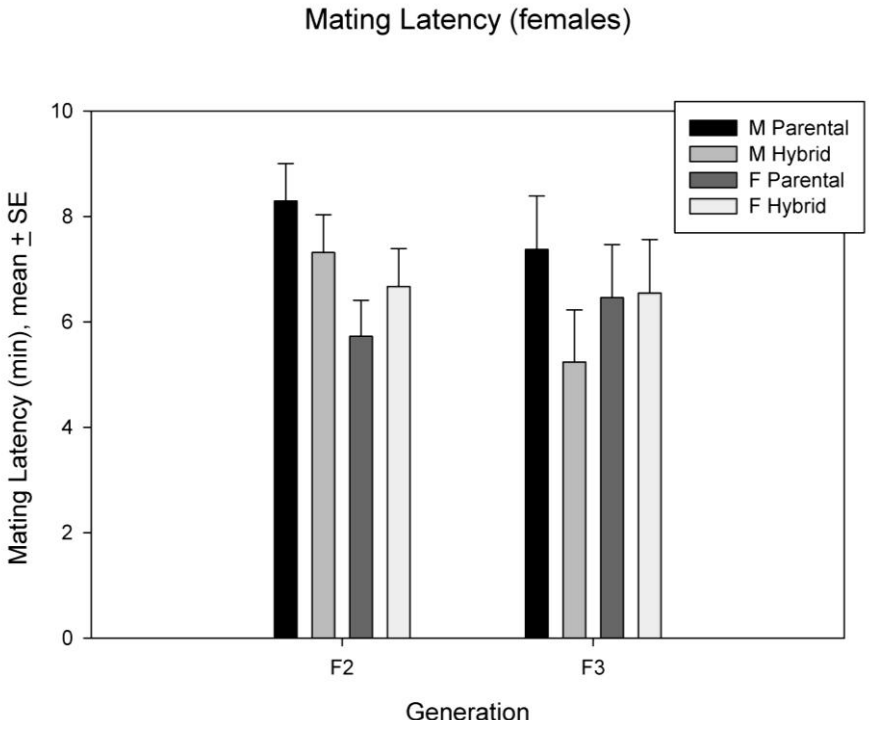

Fig R8a: Mating Latency for females from selected populations:
Between regime differences are not shown..

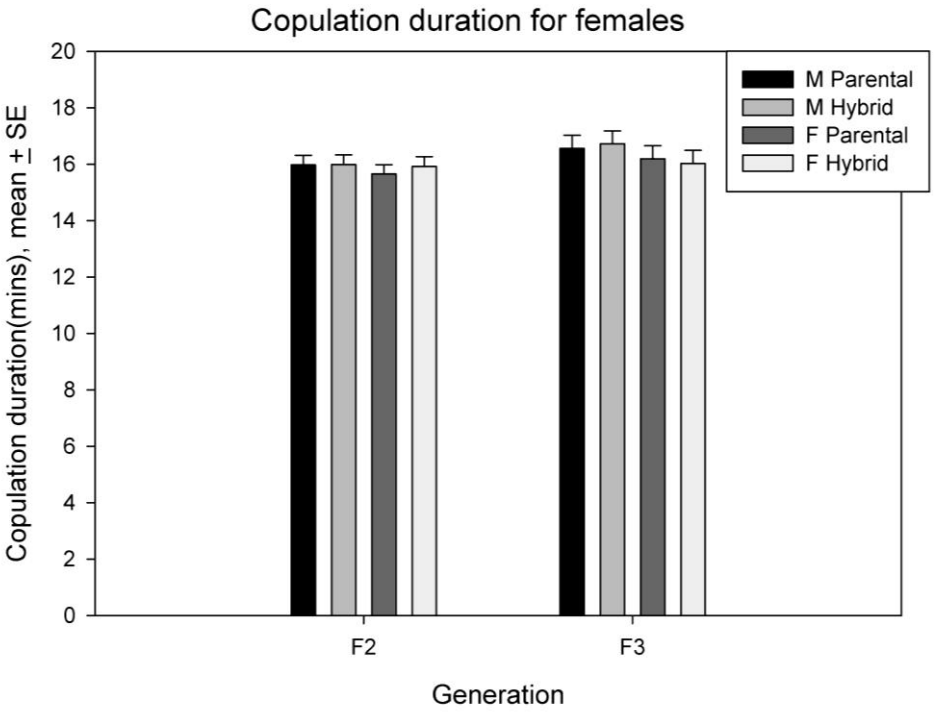

Fig R8b: Copulation duration for females from selected populations:
Between regime differences are not shown..

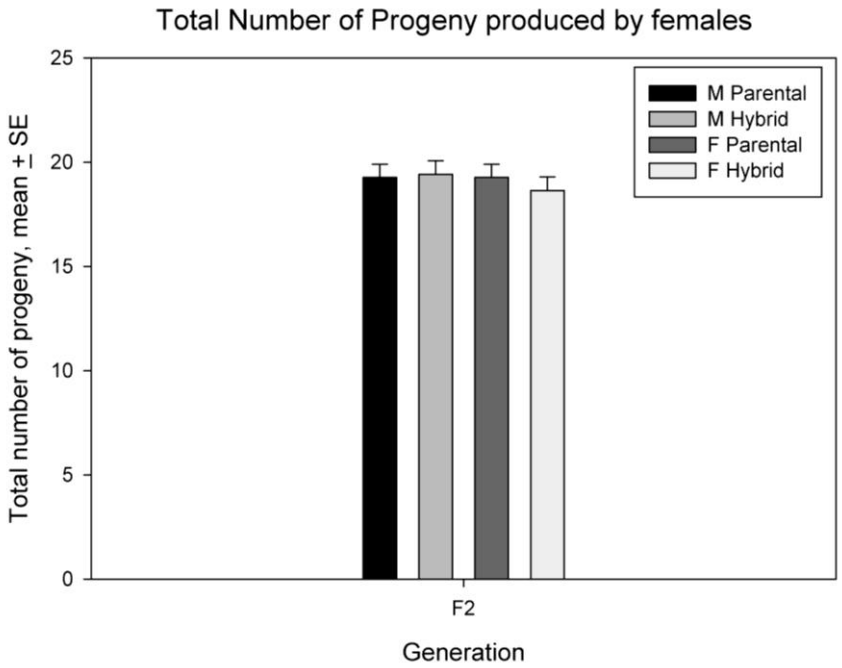

Fig R8c: Total Number of progeny produced by females from selected parental and hybrid crosses in F2 generation. Between regime differences are not shown.

Discussion

In order to investigate whether inter locus sexual conflict drives post-zygotic reproductive isolation, we used allopatric replicate populations of *Drosophila melanogaster* selected for varying levels of sexual conflict with the previous knowledge that the replicates of the population with higher IeSC show signs of incipient pre-mating and post-mating pre-zygotic isolations. Two studies till now has investigated post-zygotic isolation due to IeSC (Bacigalupe et al. 2007, Gay et al. 2009) with both of them finding no effect of sexual conflict on post-zygotic reproductive barriers.

Bacigalupe's study looked at inviability and sterility of F1 progeny from allopatric and sympatric crosses of *D.pseudoobscura* populations evolving under enforced monogamy and polygamy for 50 generations. Gay et al. used *C.maculatus* populations of two different effective population sizes where sexual conflict was enforced for 18 generations and saw no difference in fecundity and total number of offspring eclosed in both allopatric and sympatric crosses in each treatment. However, all the studies measured reproductive isolation after a relatively short time and thus it might have not been enough time for sexual conflict to accumulate changes in the populations so that isolation would be detectible. Also both these studies investigate traits (eg.- hybrid inviability, sterility) which are severe and very unlikely to emerge in populations diverging for a very short duration (<50 generations). Not only that, these studies looks at these hybrid traits in F1 generation only, whereas according to Dobzhansky-Muller incompatibilities hybrids are more likely to suffer with subsequent generations when novel allelic combinations are broken apart from compatible backgrounds. To address these issues, in our study, we investigated post-zygotic isolation in populations which had diverged for more than 175 generations in varying levels of sexual conflict. We also created hybrids within these populations and measured several fitness related traits to elucidate both extrinsic and intrinsic factors instead of just viability and sterility for F1, F2 and F3 generations. Because the heterogametic sex suffers more in case of hybridisation we do most of the assay using males.

We found no evidence of hybrid inviabilty or sterility for both sexes in F1, F2 and F3 generations. Within a selection regime, we did not find any difference between total progeny produced by hybrid and parental strains. There was also no evidence of Haldane's rule as we

did not observe sex-ratio biases. In F2 generation, parental populations of the M regime lived for a longer duration in starved conditions (after mating) compared to their hybrid counterparts. Also, males from the parental M populations mated for a longer duration compared to their hybrid counterparts in the F3 generations. On the other hand, F hybrids have a significantly lower trait value for desiccation resistance (when mated) than their parental types in F1 and F3 generations. These are the only instances where hybrids suffer a loss of fitness compared to their parental strains from same regime. There is also hybrid vigour for several traits (dry body weight at eclosion, dessication resistance when virgin and mated, competitive mating success, copulation duration) in M populations in F2 generation whereas nothing was observed in F populations. These results are interesting and open avenues to how certain alleles under sexual selection in different allopatric populations may interact on secondary contact. However, in line with previous studies, my study does not find compelling evidences of sexual conflict being a driving force for post-zygotic isolation. Given that these populations show pre-zygotic isolation, my study supports long standing arguments that pre-zygotic isolation appears faster than post-zygotic isolation (Kilias et al. 1980, Coyne and Orr 1989, 1997, Rice and Hostert 1993, Coyne and Orr 2004, Vines and Schluter 2006, Ghosh and Joshi 2012).

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