Does Sexual Conflict influence speciation through Postzygotic Reproductive Isolation?

Harshavardhan Thyagarajan

A dissertation submitted for the partial fulfillment of BS-MS Dual Degree in Science

Indian Institute Of Science Education and Research, Mohali

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Certificate of Examination

This is to certify that the dissertation titled “Does Sexual Conflict influence speciation through Postzygotic Reproductive Isolation?” submitted by Mr. Harshavardhan Thyagarajan (registration number MS13129) for the partial fulfillment 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. Manjari Jain **Dr. Rhitoban Ray Choudhury** **Dr. N. G. Prasad (Supervisor)**

Dated: April 20th, 2018

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 acknowledgements of collaborative research and discussion. This thesis is a bona fide record of original work done by me and sources listed within have been detailed in the bibliography.

Harshavardhan Thyagarajan
Dated: April 20th, 2018

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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Acknowledgements

The work detailed in this report is not an individual effort - mentally or physically, although only my name adorns the cover. I owe my gratitude to a large number of people, all of whom I hope to name in this section. I request that any oversight be pardoned.

Firstly and most crucially, I would like to thank Manas GA for personally overseeing my highly error-prone fly work, teaching me to analyse data and serving as an inspiration. A remarkably thorough, brilliant thinker and an incredibly kind human being; Manas has left a lasting image to strive towards.

To Martik Chatterjee I owe thanks, as my work has been largely derived from his master's thesis.

In the middle of competitive entrance exams and PhD applications, EBL has proved to be an absolutely stress-free environment to work in and a great place to simply be. For this I would like to thank all my labmates and appreciate aloud the ethos our guide has set this lab up upon.

Specifically, I would like to thank Karan Bhatt and TJ Chechi for being honest enough with me to eschew politeness and call me out on my mistakes.

Prakhar Gahlot, Bhatti, TJ and Manas have contributed majorly in the form of manpower when I've been overwhelmed by my workload.

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Vaibhvi, Reshma, Aatashi, Bhatti, and Prakhar have worked on their own master's projects alongside me. I'm lucky to have such an amazing cohort of batchmates to graduate with. I am also grateful to all the juniors and project students for helping with observations on experiment days and the like. Special mention to Amisha for being ever so resourceful, reading random nonsense I write and for being an awesome-sauce cook.

Last, and possibly as crucially as first, I'd like to thank Dr. NG Prasad. Before I thank him in his role as my guide and mentor, I must give thanks for the incredible job he has done from the bloodsucking office of the Dean (Students). Student life has improved by and large in his term; and although his hair have grown no whiter, the number of hours he has put in and the finesse with which he has handled his role have been exemplary. An encyclopedia, a brilliant teacher and an exceptional thinker, he is everything anyone can ask for in a guide both academically and otherwise.

Outside the lab, I'm lucky to have some amazing friends who have helped me stay on an even keel through the year. For helping with data entry and thesis submission panic, I am ever grateful to Riya Kothari. One those days when my stomach and wallet cried into the night together in despair, Sharfu bhai has been my lifeline. And when all else fails, my parents have always been there for me.

Thank you.

Contents

Abstract

Reproduction in sexually reproducing species was classically seen as a cooperative effort between individuals that benefited from it symmetrically. This canon has since been rejected on the back of theoretical and empirical evidence that suggest that it is instead a game of conflict between individuals with discordant interests, as a consequence of the different costs and benefits associated with the reproduction for each sex. This discord in interests is especially prominent in large, promiscuous populations. Verbal and formal models predict that this conflict can act as an engine for speciation between allopatric populations, but experimental evidence for the same remains inconclusive. A recently published study conducted on *Drosophila melanogaster* suggests that populations experimentally evolved at higher levels of sexual conflict do in fact show higher levels of prezygotic reproductive isolation between allopatric replicates than those in relaxed conditions (with respect to sexual conflict). Using the same model system, this study investigates the levels of postzygotic reproductive isolation that have evolved between allopatric replicates under both relaxed conditions and the stress of sexual conflict.

1. Introduction

In sexually reproducing species, the differential costs and benefits of mating and reproduction experienced by each sex results in them assuming different roles in the process of reproduction. In promiscuous species, this results in different fitness optima for males and females with respect to mating frequency and other traits such as level of parental care, offspring size etc. Consider mating frequency in a promiscuous population. In sexually reproducing species, males produce sperm, the cheaper gamete (in terms of cost in resources); while females produce eggs, which are both limited in number and costly to produce (Parker 1972). As a result, it is in the evolutionary interest of the male to mate as multiply as possible and spread his seed, while the female's fitness is best served by limiting the number of times she mates (which is a costly exercise). (see Bateman 1948 for an empirical demonstration)

Such a conflict of evolutionary interests is termed as sexual conflict, or specifically, interlocus sexual conflict. Sexual conflict also exists in another form, which occurs because both sexes share most of the genome, although selection pressures on males and females can be distinct. In this, the expression of a trait controlled by a single locus has different effects on fitness when expressed in each of the two sexes. This is referred to as Intralocus conflict (IaSC). In the case of interest to this study (Interlocus conflict (IeSC)); the expression of certain antagonistic alleles in a single sex results in opposite effects on the fitness of both sexes. Couched in terms of mating rate for instance, novel mutations that help males gain a greater number of matings will boost male fitness, all else being equal. Likewise, there are fitness benefits in novel mutations that enable females to minimize the costs arising from male interests. Both these cases will result in a depression of the fitness of the other sex. Clearly, to achieve such optima, IeSC must act as a selection pressure on the evolution of traits that influence pre- and postcopulatory reproductive success; although it may also play a role in other traits through pleiotropic routes. Inheritable behaviours that function as mating signals, preferences associated with such signals, (Debelle 2014), reproductive morphology and male ejaculate (including sperm and accessory gland secretions) are some of the traits that are directly influenced by this selection pressure. As a consequence of the male-male competition, mate-harming traits are frequently selected for (see Nandy 2013*a*), which indirectly forces females to evolve defensive mechanisms that are grouped under the umbrella term of mate-harm resistance.

Increased levels of IeSC can be resolved through a formation of an evolutionary stable strategy between the two sexes; or result in an evolutionary arms races, where the two sexes persistently co-evolve under the stress imposed by the other. In the latter case, this sexually antagonistic coevolution (SAC) is realised through perpetual change (a hallmark of coevolution) in

reproductive traits. For instance, male reproductive structures that come into physical contact with females, including sperm, show particularly rapid evolutionary change, because female reproductive tracts themselves undergo rapid evolution so as to allow females post-copulatory mate choice (Miller & Pitnick 2002).

In populations maintained in perfect allopatry (absence of exchange in genetic information), high levels of sexual conflict should result in the accumulation of a large number of changes in reproductive traits that need not be identical or even analogous (although there do exist cases of parallel evolution under sexual selection - Boughman 2005). Contrary to this, in conditions of low sexual conflict, accumulation of changes acts more as a measure of drift in the population. Consequently, it is predicted that the populations under high levels of IeSC display assortative mating on secondary contact, promoting speciation (Lande 1981; Parker & Partridge 1998; Gavrilets 2000) between allopatric replicates - at greater rates compared to populations experiencing low levels of IeSC. Evidence for this claim comes from two types of studies - comparative phylogenetics and experimental evolution studies.

Arnqvist et al (2000) tested this prediction by comparing the relative species richness of sister clades that differed in mating behaviour. He showed that polyandrous clades of insects are more speciose than monandrous clades, which suggests that the promiscuous species undergo speciation more frequently than monogamous species. However, Morrow & Arnqvist (2003) suggested that in bird species, clade size could not be explained by levels of sexual selection. Using data for spermatogenic investment (testes size) as a proxy for post-mating sexual selection, and sexual size dimorphism and sexual dichromatism for pre-mating sexual selection; they argue that none of the variables explained patterns of species richness.

Extant literature also suggests that the evidence from laboratory studies testing this prediction is equivocal. The standard method to test this prediction has been to experimentally evolve populations at different levels of sexual conflict (using promiscuity as a proxy for level of sexual conflict), and to measure the difference in reproductive isolation between allopatric replicates of high IeSc and low IeSC respectively. Operationally, level of IeSC is manipulated by adjusting sex ratio of the population or enforcing monogamy. Martin & Hosken (2003) used experimentally evolved populations of *Sepsis cynipsea* and showed that larger, more dense populations with more sexual conflict showed greater levels of reproductive isolation than small populations with relaxed conflict. Monogamous flies consistently showed the least levels of reproductive isolation, suggesting that the rate of speciation drops when that fitness optima for the two sexes are artificially rendered identical. Contrarily however; Wigby & Chapman (2006), Bacigalupe LD (2007) and Plesnar-Bielak (2013) demonstrated that experimentally evolved populations of *Drosophila melanogaster, Drosophila pseudoobscura* and *Rhizoglyphus robini* respectively did not show greater levels of reproductive isolation when subjected to higher levels

of sexual conflict. Most recently, under this general schema, Syed ZA et al (2017) showed that *Drosophila melanogaster* flies artificially evolved under the selection pressure of a male-biased sex ratio ('M' flies) diverge more from allopatric replicates than flies evolved under a female-biased sex ratio ('F' flies). M flies show: (a) An assortative mating preference for sympatric flies over allopatric individuals - a premating reproductive barrier (b) Higher levels of sperm defense with sympatric partners over allopatric partners that mirrors the investment in terms of copulation duration - a postmating prezygotic reproductive barrier.

Using the same system, this study aims to investigate if the replicates maintained at high levels of sexual conflict also evolve greater levels of postzygotic reproductive isolation. The canonical understanding of the existence of postzygotic reproductive isolation is the formation of incompatible allele complexes in hybrids as a consequence of novel mutations in the parental genotypes that are not well adapted to the other's genetic background (Johnson 2008). Given that IeSC is predicted to drive reproductive isolation through increased levels of assortative mating, it is ostensible to assume that the presence of elevated levels of sexual conflict makes no difference to the rate of development of postzygotic reproductive isolation. In general , a mechanism that generates prezygotic isolation need not by default result in the acceleration of postzygotic reproductive isolation while the two populations remain firmly in allopatry. However, as a selection force responsible for fixation of alleles, it may potentially act as a source of incompatibility. There may also be pleiotropic consequences resulting from the selection of antagonistic alleles.

To study postzygotic reproductive isolation, we generate hybrids between allopatric replicates of a treatment and measure their reproductive fitness. The hybrids are 'selfed' (hybrids from identical treatments are mated) and the reproductive fitness of their progeny is measured.

Female reproductive fitness is quantified as the number of progeny (at adult stage) ensuing from a single mating with a baseline male. In hindsight, a better experimental design would have used a continual exposure schema, where a female is merely housed with the male overnight - allowing for multiple matings. This schema would also account for the potential susceptibility of hybrid females to mate harm. Male reproductive fitness is studied using a Competitive Fertilisation schema, where focal males compete with baseline males to copulate with a limited number of baseline females. Proportion of offspring sired by focal males (determined through simple eye colour genetics) is treated as the measure of fitness. The reason to adopt this setup is to capture both mating ability and sperm competitive ability in a single comprehensive measure.

The presence of increased levels of postzygotic reproductive isolation between high sexual conflict lines would suggest that the sexual conflict is either directly (or pleiotropically) driving postzygotic divergence. The absence of increased levels of postzygotic reproductive isolation

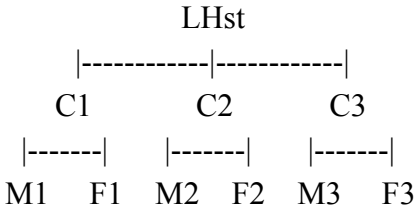
would suggest that sexual conflict accelerates speciation rates by singularly influencing prezygotic reproductive isolation. Prezygotic isolation has been implicated as the more critical factor in keeping populations separate (Kirkpatrick 2002), and such a result would align with this school of thought.

For the purpose of analysis, the null hypothesis of this experiment is the absence of any difference in the reproductive fitness of hybrids from the M regime (relative to M parentals) and the reproductive fitness of hybrids from the F regime (relative to F parentals).

2. Materials and Methods

2.1 Stock maintenance, Baseline populations

The male biased (M) and female biased (F) lines are derived from a baseline population called LHst as in the schematic below. The LHst population is itself derived from the LH population and is designed to differ in eye colour. The LH population expresses red eyes (dominant trait) whereas the LHst line and all derivatives express the recessive scarlet eye.



Stock population maintenance is detailed in Chapter 2 of Nandy 2013*b*. All populations used in this study are maintained on Cornmeal-Molasses food. In reference to amount of food, the following terms are used; Small Pour (SP) - 2mL, Large Pour (LP) - 6mL

2.2 Experimental Populations

All experiments were conducted between generations 190-204 of the MCF populations. Three iterations of the experiment were to be conducted as per the experimental design, in order to pair each block with both the other blocks (1-2, 2-3, 3-1) (Two iterations are complete, and the data from the same is analysed in this thesis). In each iteration of the experiment between block *i* and block *j*, crosses were set up as to generate hybrids (or parental controls) as follows:

♂ ♀	M_i	M_j	F_i	F_j
M_i	M_i	M_iM_j	-	-
M_j	M_jM_i	M_j	-	-
F_i	-	-	F_i	F_iF_j
F_j	-	-	F_jF_i	F_j

Table 1 - Experimental Populations

Eggs were collected from parental populations (M_i, M_j, F_i & F_j). Subsequent chronology is dated as the n^{th} day post egg collection. On the 9^{th} & 10^{th} days, virgin flies were sexed and collected within 6 hours of eclosion under light CO_2 anaesthesia. These sexually mature flies were then maintained (without flies of the opposite sex) at a density of 8 flies per SP vial.

On the 12^{th} day crosses were set up in order to generate hybrid flies. For each cross, 2 vials of male flies and 2 vials of female flies (32 individuals in all) were combined in a fresh LP vial supplied with active yeast paste and held for 48 hours. For each of the 8 crosses, 10 such yeasted LP vials were maintained.

These mated flies were then made to provide eggs in three separate sets of oviposition vials on days 14, 15 and 16. This was done to segregate the flies reared for male experiments, female experiments and producing generation F2, as below:

- Day 14 - Parental flies for F2
- Day 15 - Female experiments
- Day 16 - Male experiments

On the 14^{th} day, all the flies were flipped (transferred) into a fresh LP vial for egg laying for 18 hours. Subsequently, adult flies were flipped into a fresh LP vial (15^{th} day,) and the number of eggs in the original vial was trimmed approximately to the standard 150 eggs/6mL. 18 hours along, adult flies were flipped again into a third fresh LP vial (16^{th} day) and egg number was trimmed as before. At the 18 hour time-point, the adult flies were discarded and the egg number trimmed as before.

Eggs were collected from the baseline populations (LHst, LH) such that their eclosion peaks were synchronised with the respective focal flies.

Vials used for oviposition on the 14^{th} day are used to rear adults that produce F2 through "selfing" (flies are mated to the same treatment). On the 13^{th} day from oviposition (day 12 from egg trim), these mated flies are sorted under light CO_2 anaesthesia. They are subsequently housed as 16 males and 16 females per LP vial for oviposition. 10 such LP vials are maintained per treatment (cross). As in F1, subsequent flips are used to demarcate segregated populations for male and female experiments. The third flip is not conducted as there is no requirement for an F3 generation.

In the other two cases, flies emerging from eggs were sexed and collected as virgins within 6 hours of eclosion under light CO_2 anaesthesia on their respective 9^{th} & 10^{th} days. They are maintained at densities of 8 individuals per SP vial. On their respective 12^{th} days, the focal flies were subjected to experiments. The same procedure is repeated on F2 flies.

2.3 Experiments - Procedure and Measurements

Experiments conducted:

- Measurements of Mating Latency (ML), Copulation Duration (CD) (males and females)
- Female Fecundity
- Male Competitive Fertilisation Success (CFS)

2.3.1 Female Fecundity

After female focal flies are singly mated in the behavioural assay, the male is removed from the mating arena, and females are maintained in isolation for 48 hours. On the 14th day from egg trimming, the females are transferred to oviposition test tubes (12mmx75mm) for a period of 18 hours. Post oviposition, the adult female flies are discarded. The test tubes are maintained under standardized conditions until all the larvae in each test tube eclose, at which point (12th day) they are frozen at -20°C. The test tubes are subsequently scored for number of adult progeny.

2.3.2 Male Competitive Fertilisation Ability

For the competitive fertilisation success assay, 4 focal males, 8 baseline males (LH - red eyed) and 8 baseline females (LHst) are transferred to a single LP vial. They are allowed to compete and mate for a period of 48 hours, when the females are transferred singly into oviposition test tubes (14th day). The females are allowed to oviposit for 18 hours before being discarded. The test tubes are maintained under standardized conditions until all the larvae in each test tube eclose, at which point (12th day) they are frozen at -20°C. The test tubes are subsequently scored for proportion of adult progeny with scarlet eye colour. Each set of eight test tubes (females from the same competitive arena) were scored jointly.

2.3.3 Mating Behaviour Assays

Virgin focal flies are aspirated singly into SP vials along with a baseline (LHst) fly of the opposite sex. Time taken to commence mating (ML) and duration of mating (CD) are observed. ML, CD were noted for 100 flies (50♀, 50♂) for each treatment, in each generation - in a single iteration.

3. Results

3.1 Analysis

3.1.1 Fecundity; 3.1.2 Competitive Fertilisation Success

The number of adult progeny was subjected to an ANOVA test, using the following characters as factors in a full factorial model: Selection Regime (M or F), Cross Type (BR or WR). Generations F1 and F2 were analysed separately. Selection Regime and Cross Type are henceforth referred to as SR and CT respectively. The α value for all statistical tests in this study is 0.05.

3.1.3 Mating Latency; 3.1.4 Copulation Duration

Mating Latencies and Copulation Durations were analysed (separately for males and females) using the same fit model as described in the case of 3.1.1 and 3.1.2.

3.2 Observations

3.2.1 Fecundity

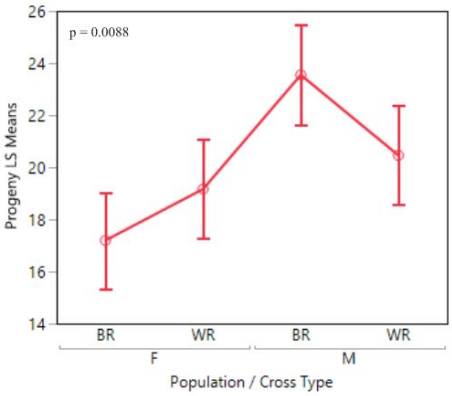
In both replicates of the experiment, and in both generation of each replicate; one observation holds consistently: There are no significant differences in the number of progeny produced by individuals derived from BR and WR crosses. This can be seen from the post hoc Tukey's HSD test.

B1 x B3:

- F1 gen: There is a significant effect of SR and the interaction of SR & CT. As a factor, CT alone does not have a significant effect.
- F2 gen: None of SR, CT or their interaction have a significant effect.
- In both generations, BR_M individuals display hybrid vigour in comparison to WR_M individuals, while BR_F individuals produce fewer offspring than WR_F individuals. Neither of these are significant results.

B2 x B3:

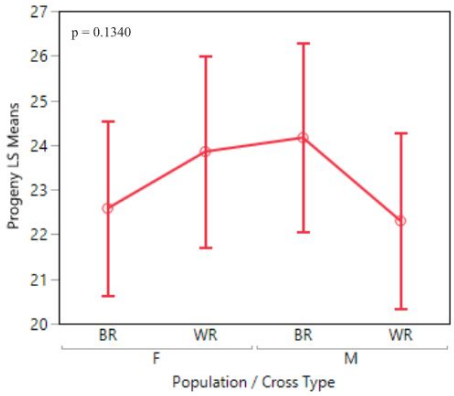
- F1 gen: There is a significant effect of the interaction of SR & CT. As a factor, neither SR nor CT alone have a significant effect.
- F2 gen: There is a significant effect of SR. CT, and the interaction of SR & CT do not have significant effects.
- In the F1 generation, BR_M individuals display hybrid vigour in comparison to WR_M individuals, while BR_F individuals produce fewer offspring than WR_F individuals. This resembles the result in B1 x B3, but the same cannot be said of the F2 generation, where this trend reverses diametrically. As in the previous case, none of these are statistically significant.
- Interestingly, in the F1 generation, females from the F parental treatments produce more offspring than the females from M parental treatments.



Level				Least Sq Mean
M3M1	A			24.162162
M1M3	A	B		23.023256
M3M3	A	B	C	21.190476
F1F1	A	B	C	20.902439
M1M1	A	B	C	19.700000
F1F3		B	C	17.909091
F3F3		B	C	17.476190
F3F1			C	16.452381

1x3 ♀s
F1 Generation

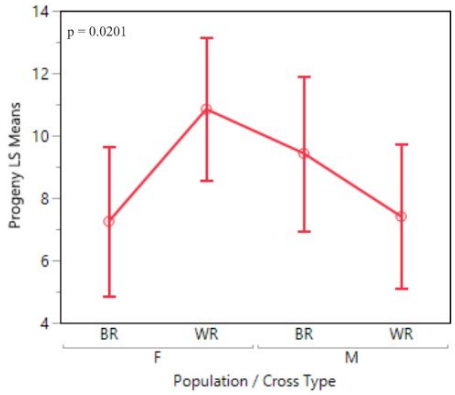
Tukey's HSD test



Level		Least Sq Mean
M1M3	A	0.44530052
M3M1	A	0.44145956
F1F1	A	0.40900761
M3M3	A	0.40073749
M1M1	A	0.38560246
F1F3	A	0.36842321
F3F1	A	0.28302560
F3F3	A	0.26514776

1x3 ♀s
F2 Generation

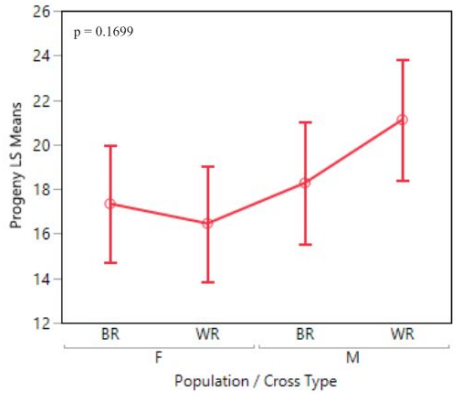
Tukey's HSD test



Level		Least Sq Mean
F2F2	A	11.763158
F3F3	A	10.000000
M2M3	A	9.848485
M3M2	A	9.029412
F2F3	A	8.378378
M2M2	A	8.333333
M3M3	A	6.064516
F3F2	A	6.057143

2x3 ♀s
F1 Generation

Tukey's HSD test



Level		Least Sq Mean
M2M2	A	23.177778
M2M3	A	20.058824
F3F2	A	18.317073
M3M3	A	18.303030
M3M2	A	16.833333
F2F2	A	16.813953
F2F3	A	16.418605
F3F3	A	16.095238

2x3 ♀s
F2 Generation

Tukey's HSD test

Fig. 1 - Female Fecundity Results

3.2.2 Competitive Fertilisation Success

From a quick glance at the data, it is evident that the experiment conducted on males from the F2 generation of the second replicate (B2xB3) has suffered from some error in handling. For all purposes of analysis, this particular dataset shall remain exempt.

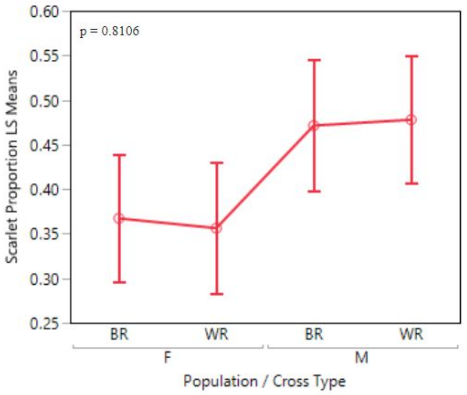
As with female fecundity, in both replicates of the experiment, and in both generations of the 1st replicate; one observation holds consistently: There are no significant differences in the number of progeny produced by individuals derived from BR and WR crosses. This can be seen from the post hoc Tukey's HSD test.

B1 x B3:

- F1 gen: There is a significant effect of SR. CT, and the interaction of SR & CT do not have significant effects.
- F2 gen: As in F1, there is a significant effect of SR. CT, and the interaction of SR & CT do not have significant effects.
- In the F2 generation, BR_M (between replicate individuals from M selection regime) individuals display hybrid vigour in comparison to WR_M individuals. In both generations, BR_F individuals sire an equal proportion of offspring as the WR_F individuals. Neither of these are significant results.

B2 x B3:

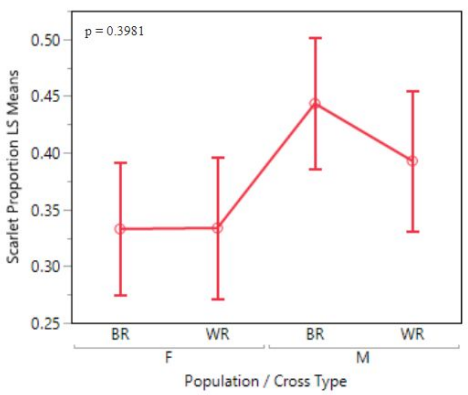
- F1 gen: There is a significant effect of CT. SR and the interaction of SR & CT do not have significant effects.
- Both selection regimes show hybrid vigour, rendering the question of RI moot.



Level		Least Sq Mean
M3M1	A	0.52250731
M1M1	A	0.52238418
F1F3	A B	0.45722092
M3M3	A B	0.42791891
M1M3	A B	0.42022658
F1F1	A B	0.39983460
F3F3	B	0.29620135
F3F1	B	0.28412472

1x3 ♂s
F1 Generation

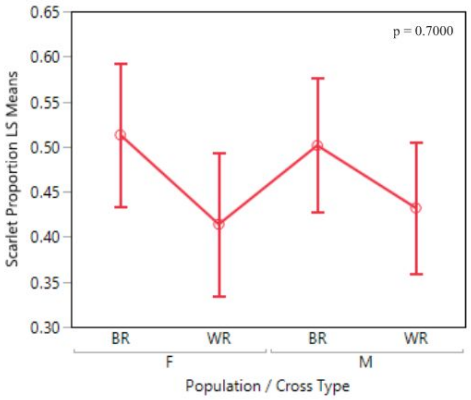
Tukey's HSD test



Level		Least Sq Mean
M1M3	A	0.44530052
M3M1	A	0.44145956
F1F1	A	0.40900761
M3M3	A	0.40073749
M1M1	A	0.38560246
F1F3	A	0.36842321
F3F1	A	0.28302560
F3F3	A	0.26514776

1x3 ♂s
F2 Generation

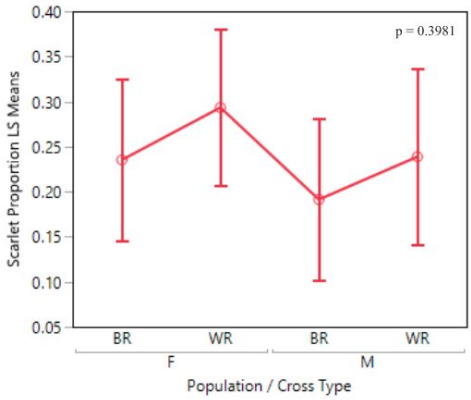
Tukey's HSD test



Level		Least Sq Mean
F2F3	A	0.57761425
M2M3	A	0.55408807
M3M3	A	0.45027640
F3F3	A	0.44829562
M3M2	A	0.44430280
F3F2	A	0.43572554
M2M2	A	0.41075317
F2F2	A	0.38529435

2x3 ♂s
F1 Generation

Tukey's HSD test



Level		Least Sq Mean
F3F2	A	0.45987822
M3M3	A	0.43985550
M3M2	A	0.39882137
F2F2	A	0.29706976
F3F3	A	0.29004854
M2M2	B	0.08818630
F2F3	B	0.05889890
M2M3	B	0.00000000

2x3 ♂s
F2 Generation

Tukey's HSD test

Fig. 2 - Male Competitive Fertilisation Success Results

3.2.3 Mating Latency; 3.2.4 Copulation Duration

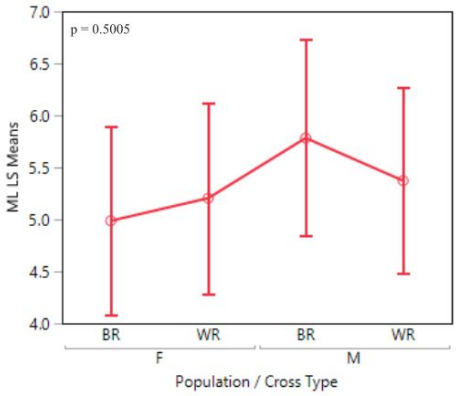
The following are observations from the replicate B2xB3. Data from B1xB3 is still under analysis.

Mating Latency:

- There are no consistent trends over two generations, in both the male and female datasets.
- Amongst males, SR has a significant effect on mating latencies, with F males having significantly longer latency periods. CT and interaction have no significant effects on the mating latency, in both generations.
- Amongst females, none of the factors have a significant in the first generation, but as a consequence of outlier latencies in hybrid F populations, all three factors appear significant in the second generation. M females have lower mating latencies between the two selection regimes, and BR individuals have longer latency periods.

Copulation Duration:

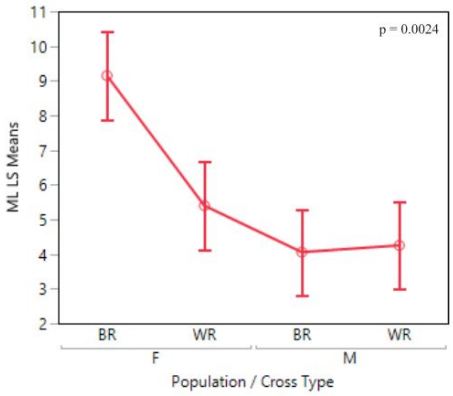
- Like in the case of mating latencies, there are no statistically significant trends over the two generations. However, there is a striking visual resemblance between the SR-CT interaction plots of Copulation Duration and Fecundity/Competitive fertilisation success.
- Amongst females, SR has a significant effect on the copulation duration in the second generation, with M females mating considerably longer than F females. No other factor has significant effects on the copulation duration.
- Amongst males, there are no significant factors affecting copulation duration. The general trend remains the same as in females, with M males generally mating for longer periods of time (not statistically significant).



Level		Least Sq Mean
F3F3	A	6.7948718
M3M3	A	6.6486486
M2M3	A B	6.1764706
M3M2	A B	5.4500000
F3F2	A B	5.0487805
F2F3	A B	4.9230769
M2M2	A B	4.3478261
F2F2	B	3.6153846

2x3 ♀s
F1 Generation

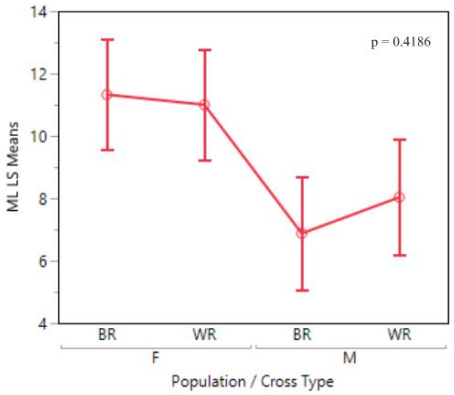
Tukey's HSD test



Level		Least Sq Mean
F3F2	A	10.536585
F2F3	A B	7.692308
F3F3	A B C	7.333333
M2M2	B C	4.897959
M2M3	B C	4.513514
M3M2	C	3.714286
F2F2	C	3.560976
M3M3	C	3.323529

2x3 ♀s
F2 Generation

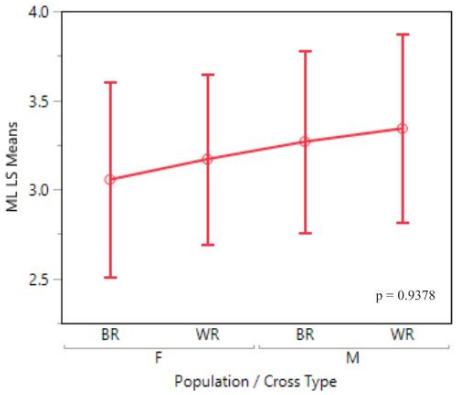
Tukey's HSD test



Level		Least Sq Mean
F2F2	A	15.978261
M3M2	A	15.613636
F2F3	A	15.574468
M2M3	A	15.555556
M2M2	A	15.325581
F3F2	A	15.266667
M3M3	A	14.525000
F3F3	A	14.212766

2x3 ♂s
F1 Generation

Tukey's HSD test

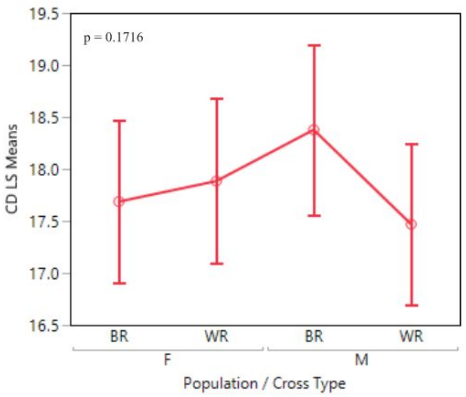


Level		Least Sq Mean
M2M3	A	25.181818
M2M2	A	19.315789
F3F3	A	17.960000
M3M2	A	17.421053
F2F2	A	17.363636
F3F2	A	16.789474
F2F3	A	16.352941
M3M3	A	15.578947

2x3 ♂s
F2 Generation

Tukey's HSD test

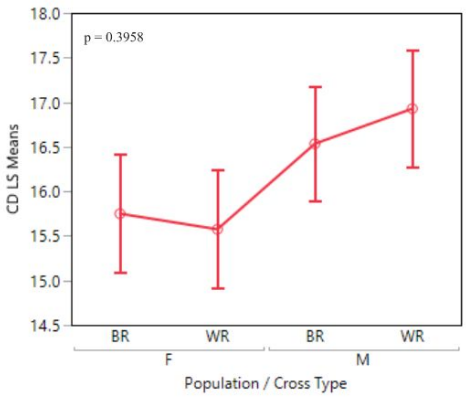
Fig 3. Mating Latency Results



Level		Least Sq Mean
M3M2	A	18.800000
M3M3	A	18.540541
F2F3	A	18.256410
F3F3	A	17.948718
M2M3	A	17.882353
F2F2	A	17.820513
F3F2	A	17.146341
M2M2	A	16.608696

2x3 ♀s
F1 Generation

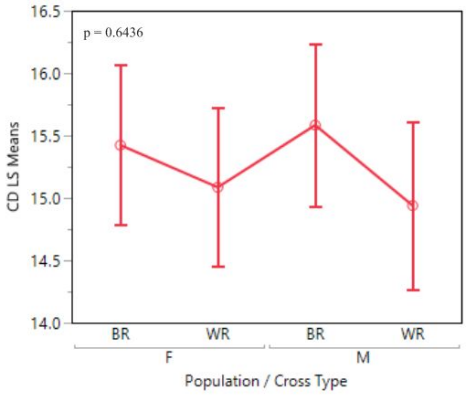
Tukey's HSD test



Level				Least Sq Mean
M3M3	A			18.000000
M2M3	A	B		17.459459
M2M2	A	B	C	16.183673
F3F3	A	B	C	16.153846
F2F3	A	B	C	16.102564
M3M2		B	C	15.836735
F3F2		B	C	15.414634
F2F2			C	15.024390

2x3 ♀s
F2 Generation

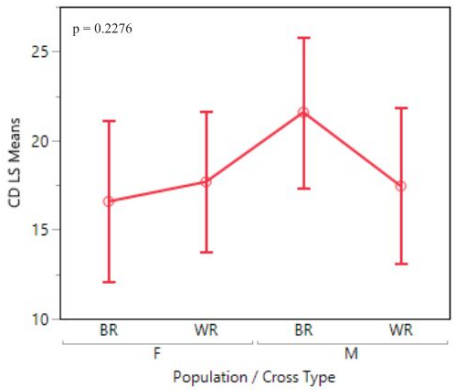
Tukey's HSD test



Level		Least Sq Mean
F2F2	A	15.978261
M3M2	A	15.613636
F2F3	A	15.574468
M2M3	A	15.555556
M2M2	A	15.325581
F3F2	A	15.266667
M3M3	A	14.525000
F3F3	A	14.212766

2x3 ♂s
F1 Generation

Tukey's HSD test



Level		Least Sq Mean
M2M3	A	25.181818
M2M2	A	19.315789
F3F3	A	17.960000
M3M2	A	17.421053
F2F2	A	17.363636
F3F2	A	16.789474
F2F3	A	16.352941
M3M3	A	15.578947

2x3 ♂s
F2 Generation

Tukey's HSD test

Fig 4. Copulation Duration Results

4. Discussion

This study is designed to test whether exposure to increased levels of sexual conflict results in postzygotic reproductive isolation between allopatric replicates. We report no significant differences in female fecundity or male competitive fertilisation success between hybrids and parentals in either the M selection regime or the F selection regime, and the trends inferred even indicate that there is some level of hybrid vigour in the hybrids of the M selection regime.

This analysis shows that the exposure to increased levels of sexual conflict does not affect speciation rates through post-zygotic RI, while Syed ZA et al (2017) conclusively demonstrate that there is a significant increase in levels of prezygotic RI in the presence of SAC, using the same system. In allopatric populations of *Drosophila*, the standard cannon suggests that both prezygotic and postzygotic RI are expected to evolve at the same rate (empirically demonstrated by Coyne & Orr, 1989).

Besides this central result, there are some interesting asides to take away from this experiment; and a major error to discuss. The first is the close parallels between the shapes of the interaction plots for copulation duration and fecundity/competitive fertilisation. This result, neatly keeps with our understanding that copulation duration serves as an efficient proxy for male postmating investment (Nandy, B. et al 2013*c*).

Secondly, although not statistically significant, BR individuals of the F regime consistently show lower fitness than the parental controls. In contrast, BR individuals of the M regime frequently show hybrid vigour (again not statistically significant). The mechanistic basis of such a trend is worth investigating further, especially if the third and ongoing replicate of this experiment produces results on similar lines.

Lastly, in the male competitive fertilisation success experiment of the replicate B2 x B3 (F2 generation), two populations (M2M2 and M2M3) displayed zero or near zero fitness. It is unlikely that any of these populations display such low levels of reproductive fitness, especially M2M2, which as a parental population is unlikely to show a random drop in fitness. Moreover, as they were housed simultaneously with flies of all the remaining populations, it is unlikely that they selectively experienced a random event that resulted in such a drastic result. One common sense argument to explain this absurdity could be a contamination in collection and maintenance of experimental fly populations, where an interchange between scarlet eyed baseline females and red eyed baseline females (through label exchange). Regardless of this, it is worth redoing this block, to have a clearer picture of the results.

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