Evolution of reproductive behaviour in response to selection for increased pathogen resistance in *Drosophila melanogaster*

Thesis submitted in partial fulfilment of the requirements of
five year BS-MS Dual Degree Programme

By

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Certificate

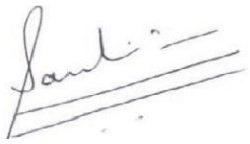
This is to certify that this dissertation entitled "**Evolution of reproductive behaviour in response to selection for increased pathogen resistance in *Drosophila melanogaster***" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents original research carried out by **V. Saudamini** at **IISER, Mohali** under the supervision of **Dr. N. G. Prasad, Associate Professor, Department of Biological Sciences** during the academic year **2014-2015**.

Signature of Supervisor Date: 25/03/2015

(N. G. Prasad)

Declaration

I hereby declare that the matter embodied in the report entitled "**Evolution of reproductive behaviour in response to selection for increased pathogen resistance in *Drosophila melanogaster***" are the results of the investigations carried out by me at the **Department of Biological Sciences, IISER, Mohali**, under the supervision of **Dr. N. G. Prasad** and the same has not been submitted elsewhere for any other degree.



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ABSTRACT

Both immune response and reproductive behaviour are important life-history related traits that govern the fitness of the organism. Given that both of these are costly traits, trade-offs between the two are to be expected when resources are limiting. In this study I explored evolution of sexual behaviour in a population of *Drosophila melanogaster* that has been selected for higher survivorship against a pathogen *Pseudomonas entemophila.* I found that immune response is positively affected by higher sexual activity in selected populations, whereas mortality in control populations remained unaffected by the degree of sexual activity. I have also shown that under infected condition males from selected population mate and court less frequently than the control populations under the same conditions. Further study has been conducted exploring if this is a trade-off between reproductive effort and immune response or an evolved reproductive strategy deployed by selected males. The study shows that there is no fitness benefit of higher survivorship post-infection in the selected males compared to control males. There are evidences suggesting a possible trade-off between reproductive behaviour and mounting an immune response.

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INTRODUCTION

The life history of an organism maps the events of birth, reproduction and death over the organism's lifetime (Stearns, 1992). Traits such as fecundity, life-span, age at reproductive maturity, growth pattern etc., that shape this schedule of survival and reproduction and consequently govern the fitness of the organism, are termed as life-history traits. One such life-history related trait is the immune response. The ability to defend oneself against a pathogenic attack is an important component of an organism's fitness.

Reznick (2010) has aptly stated, "Life-history theory predicts how natural selection should shape the way organisms parcel their resources into making babies". Most life-history traits are expensive and require resources for their maintenance and deployment. Evidence indicating that the immune response is a costly trait has been found in vertebrates as well as invertebrates (Hamilton and Zuk, 1982; Sheldon and Verhulst, 1996). When resources are limiting, such costly life-history traits, can potentially trade-off with other life-history traits such as fecundity, life-span, sexual behavior, etc.

De Jong and Noordwijk (1992) have explained trade-offs with the help of Y- model of resource allocation. The model suggests that increased investment in one trait will result in decreased investment in some other fitness related trait under conditions of limited resources. Immunity has been shown to trade-off with fecundity (Gwynn et al., 2005), longevity (Moret and Schmid-Hempel, 2000), egg viability (Ye et al., 2009) and development time (Rantala and Roff, 2005), etc.

Evidence from recent investigations has suggested that immunity and reproductive behaviour may be functionally linked (Zuk and Stoehr, 2002). Hamilton and Zuk (1982) in their seminal paper suggested that more immunocompetent males can have more elaborate secondary sexual characters and hence, secondary sexual characters will be an honest signal of a healthy male. This will result in sexual selection favouring more immunocompetent males. Several studies with insects also suggest similar links between sexual display and immunity (Rantala et al., 2000; Siva-Jothy, 1999).

Survival and fecundity are arguably two of the most important determinants of Darwinian fitness. While a strong immune response is essential for survival against pathogens, reproductive activity almost directly determines fecundity and consequently the fitness of the organism. Given that both these traits are resource expensive, trade-offs between the two are to be expected. Indeed, trade-off between immunocompetence and sexual behaviour has been observed in vertebrates (Norris and Evans, 2000) and invertebrates. In insects, increased sexual activity has been shown to trade-off with components of immune function such as phenoloxidase activity, hemolytic activity, etc. (Fedorka et al., 2004; McKean and Nunney, 2001; Siva-Jothy et al., 1998). It has also been shown that infected males have reduced ejaculate quality and secondary sexual characters (Demuth et al., 2012; Simmons and Roberts, 2005). In *Drosophila melanogaster* increased sexual activity in males results in a lower ability to clear bacteria (McKean and Nunney, 2001).

However, trade-offs between sexual activity and immune response are not ubiquitous. In a study on male crickets, lytic activity remained unchanged even when sexual activity was increased (Dowling and Simmons, 2012) . Another study of *Drosophila melanogaster* reported increased resistance to bacteria, in males with increased sexual activity (Gupta et al., 2013). It is clear that sexual activity and immune response may even be positively correlated with one another and that the trends are highly specific to the host-pathogen system.

For my study, I have explored how sexual behaviour in *Drosophila melanogaster* has evolved in response to selection for increased resistance against a pathogen. Previous studies (Ye et al., 2009) have attempted to address trade-offs between immunity and other life-history traits using *Drosophila melanogaster* populations that have been selected for better immune response against a bacterial pathogen. However, no relation has been uncovered between reproductive behaviour and immunity in these studies.

In order to understand how immune response and sexual behaviour are related, I have focused on a long term selection line of *Drosophila melanogaster*. These populations are selected for survivorship against a gram-negative pathogen *Pseudomonas entemophila* and have consequently evolved higher survivorship against this pathogen compared to the control populations. Other studies of this

nature have reported trade-offs between the evolved immune response and other life-history traits in their selected lines (Ye et al., 2009; Ma et al., 2012). No such trade-offs have yet been observed in my focal selection line, which is what prompted me to focus on reproductive traits in these populations.

Further, I have restricted the scope of my questions to male *D. melanogaster*. Drosophila melanogaster is a promiscuous species, where substantial resources are invested by males into pre-copulatory traits like courtship and mating and in post-copulatory traits like sperm offense and defense (Gupta et al., 2013). Maintaining a costly immune system along with these sexual traits will likely result in trade-offs between the two (Sheldon and Verhulst, 1996; Zuk and Stoehr, 2002).

In this study, I have asked two major questions in order to address the question of potential trade-offs between immune response and reproductive behaviour in the evolved populations-

1. What is the effect of sexual activity on the immune response of the evolved and control populations?
2. How does mounting an immune response consequently affect the reproductive behaviour in the evolved and control population?

METHODOLOGY

Model System

I have used the fruit fly, *Drosophila melanogaster*, as the model system for my thesis work. Due to their short life-cycle and ease of handling and manipulation of their ecology in the laboratory, *D. melanogaster* have become popular for studies of life-history evolution (Prasad and Joshi, 2003).

The fruit flies are holometabolous insects that undergo complete metamorphosis. They have an adult life span of typically 35-40 days (Prasad and Joshi, 2003). The Drosophila life cycle consists of 4 stages: egg, larva, pupa and adult. Eggs hatch into 1^{st} instar larvae around 18-24 days after they are laid. The larvae molt into 2^{nd} instar larvae after a day, and into 3^{rd} instar larvae after another day. They remain at this stage for 2-3 days, until they pupate. Pupal stage lasts for about 4-5 days after which flies eclose as adults. The flies attain sexual maturity after about 6-8 hours post-eclosion (Prasad and Joshi, 2003).

All population of flies used in this thesis have been reared in standard laboratory conditions of 25º C, 55% relative humidity and 12h:12h light-dark cycle.

Bacterial Stock

Pseudomonas entemophila strain L48 is the gram-negative bacteria used to infect flies for the experiments and the selection regime. *P. entemophila* is reported to have been isolated from *Drosophila melanogaster* and is considered as it natural pathogen (Vodovar et al., 2005). For experiments and selection, bacteria are cultured in Luria Bertani Broth medium overnight at 27 ºC, 150 rpm. The overnight culture is then sub-cultured by diluting it 1000 fold. The culture is monitored till it obtains OD 2.0 (±0.1) at 600nm, after which it is pelleted and re-suspended in an equal volume of 10mM $MgSO_4$ solution. This bacterial suspension is eventually used for infections.

Ancestral populations

For my thesis, I have used populations of *D. melanogaster* flies derived from the following ancestral populations

BRB: Blue Ridge Baseline (BRB) is a large outbred population of *Drosophila Melanogaster* that was established from 19 iso-female lines. The iso-female lines were founded by 19 females caught in the wild from Blue Ridge, USA. The populations are maintained in cages on a 14-day discrete generation cycle. There are five replicate populations (BRB1-5) comprising 2500 adults per generation for every replicate (Gupta et al., 2013).

LH: Named, after Larry Harshman, this large outbred population was founded from 400 wild females caught at Central California, USA, and has been reared in laboratory conditions for about twenty years. The populations are maintained in vials (150 eggs per vial) on a 14-day discrete generation cycle (Chippindale and Rice, 2001). LH_{st} population has been derived from LH by introgressing an autosomal recessive *st* (scarlet-eye) allele through repeated back-crossing (Chippindale and Rice, 2001).

Experimental populations

From BRBs, three separate selection regimes (**I**, **S** and **U**) were set-up (Gupta, V, unpublished data). Males from **I** and **S** selection regimes have been the focus of my experiments in this thesis. Following are the particulars of each regime:

I (Infected with pathogen): 12^{th} day post egg-collection, 150 males and 150 females are lightly anaesthetised under CO2 and then infected with the pathogen *Pseudomonas entomophila*. Infection is achieved by inserting a fine needle dipped in a bacterial suspension of the pathogen into the thorax of the flies.

S (Sham-infected): 12^{th} day post egg collection, 100 males and 100 females are anaesthetised and pricked in the thorax with a needle dipped in sterile MgSO4 (10mM) solution.

U (Unhandled controls): 12 days post egg-collection, 100 males and 100 females are sorted under light CO2 anaesthesia and transferred to cages.

These populations are bred on a medium of banana-jaggery food (composition given at the end of the section) and maintained on a 16-day discrete generation cycle. 12 days post egg collection, flies are transferred from vials to cages after being treated according to their selection regime. Food plates are changed after two days. Four

days post treatment, fresh food plates are provided for egg laying. After 18 hours, eggs are collected in 6mL food vials at a density of 70 eggs per vial. Four replicate sets of the selection regimes (I,S,U 1-4) have been derived from BRB(1-4) respectively. Therefore, I1 and S1 are more closely related to one another as compared to I1 and I2 and so on. Each replicate set is treated or assayed on the same day and the four sets of selection regimes are handled on four different days. The replicates are independent of each other and are treated as statistical blocks.

Standardization

Fitness related-traits may be heavily affected by non-genetic parental effects. Therefore, for conducting experiments, flies are generated from parental flies that have been maintained under similar conditions for one generation. This process is called standardisation (Rose,1984). Eggs are collected from stock populations at a density of 70 eggs per vial. On 12^{th} day, around 250 male and 250 female flies are transferred to cages. For generating experimental flies, eggs are collected from these standardised flies.

Experiment 1: Effect of sexual activity on immunity

In order to understand how sexual activity affects the ability of the host to mount an immune response against the pathogen, virgin and mated flies from I and S regimes were infected with *P. entemophila*. Post-infection their survivorship was monitored for 4 days.

Eggs (70 eggs/vial) were collected from I and S selection regimes after one generation of standardisation. 75 males per regime were collected as virgins on 9^{th} day and 10^{th} day post egg-collection from half of the vials. For mated males, 75 males and 75 females per regime were collected and housed in vials (8 mating pairs/vial) on 10^{th} day. On 12^{th} day, both virgin and mated male flies were infected with a needle dipped in bacterial suspension (OD 1.5 ± 0.1) and transferred to cages. Mortality was monitored - every 4 hours for the first 24-36 hours and every 6 hours thereafter - for 96 hours post-infection. This experiment was performed for Blocks 2, 3, and 4 of I and S regimes.

Bacterial concentration of OD 1.5 could be toxic to the S populations as they typically do not encounter any pathogenic challenge over the course of their selection regime.

This may lead to accelerated mortality in S, effectively masking any effects of sexual activity on male mortality, even if they were present. Therefore, the experiment was conducted again as described above but the concentration of the bacterial suspension was reduced to OD (1.0 ± 0.1).

Experiment 2: Sexual behaviour post-infection

To understand how sexual behaviour of the host may be affected when an immune response is mounted in the host, males from I and S regimes were challenged with pathogen and housed with a common baseline (BRB) female.

Eggs (70 eggs per vial) were collected from BRB and standardised I and S populations. BRB females were collected as virgins on the 9^{th} day post egg-collection. On 12th day, I and S males were given one of two treatments – infected (OD 1.5 ± 0.1) or sham infected. 6 hours post-treatment one un-infected BRB female was combined with one I and one S male in an observation vial, resulting in the vials containing the following combination of flies:

1. BRB Female + I infected male + S infected male
2. BRB Female + I sham-infected male + S sham-infected male

The males were dusted with micronized dust of two different colours to differentiate between each other.

Mating latency, copulation duration and the colour of the mating male was recorded. This was done for all four block of I and S regimes.

For measuring courtship frequency, the colour of the male(s) courting the female was recorded every 30 seconds till a pair started mating. After copulation the males were discarded and the females were held in the vials for egg-laying. After 18 hours, the female was discarded and the vials were maintained for development of eggs. After 12 days, the number of progeny per vial was counted. This was done for Block 1, 3 and 4 of the selection regime.

Experiment 3: Post-infection competitive fitness

As per the selection regime of I and S, egg collection for the next generation is done 4 days post treatment. In the following experiment I and S males were housed with a

common competitor male (LH-st) and a common female (LH-st) for four days post infection. Their sexual activity over the four days was measured in terms of number of progeny produced by the focal male per day.

Eggs were collected from standardised I and S regimes at a density of 70 eggs per vial and from LH-st population at a density of 150 eggs per vial. Female LH-st flies were collected as virgins on the 9^{th} and 10^{th} day post-egg collection. On the 12^{th} day, I and S males were given one of two treatments-infected (OD 1.5 ± 0.1) or sham infected. After treatment, one LH-st male and one LH-st female was combined with either one I (infected or sham-infected) or one S (infected or sham-infected) male in a vial. Each female in the vial was allowed to mate and oviposit in the vial for 24 hours after which flies from each vial were flipped into fresh food vials. The older vial was retained for development of progeny. This was repeated for 4 days. On the 5^{th} day flies were discarded from the vials.

Eye colour marker was used to differentiate between the progeny of focal (I or S) male and that of the competitor male. I and S are red-eyed which is the dominant allele, whereas, LH-st flies are scarlet-eyed which is the recessive allele. Progeny of I or S male with LH-st female will therefore always be red-eyed whereas the progeny of LH-st male with LH-st female will always be scarlet-eyed.

Number of red and scarlet progeny from the vials used for oviposition, were counted, in order to measure the lifetime fitness of the focal male.

Statistical Analyses

Experiment 1: Percentage mortality was analysed using Mixed Model ANOVA treating sexual activity and selection as fixed factor and block as random factor. Survivorship curves were generated using Kaplan-Meier estimator and compared using Gehan-Breslow chi-square test.

Experiment 2: Single sample t-test was carried out to check if proportion matings by I males were significantly different from 0.5. Courtship frequency was analysed for infected and sham-infected treatments using Mixed Model ANOVA with selection as fixed factor and block as random factor.

Experiment 3: Mixed Model ANOVA was used for analysis, with treatment and selection as fixed factor and block as random factor.

All statistical analyses were done using JMP for Windows, version 10 (SAS Inc. 2012).

***Table**: Composition of 1 litre standard Banana-Jaggery food*

Ingredient	Amount
Banana (g)	205
Barley flour (g)	25
Jaggery (g)	35
Yeast (g)	36
Agar (g)	12.5
Ethanol (mL)	45
Water (mL)	180
p-Hydroxymethyl benzoate (g)	2.4

RESULTS

Effect of sexual activity on immunity

When infected with bacterial concentration of OD 1.5, selection had a significant effect (p=0.0061) on mortality of males post infection with *P. entemophila* i.e. mortality in I males was significantly lower than the mortality in S control males (Fig.1). There was an effect (p=0.092) of interaction between selection and sexual activity (Table 1, Fig.2).

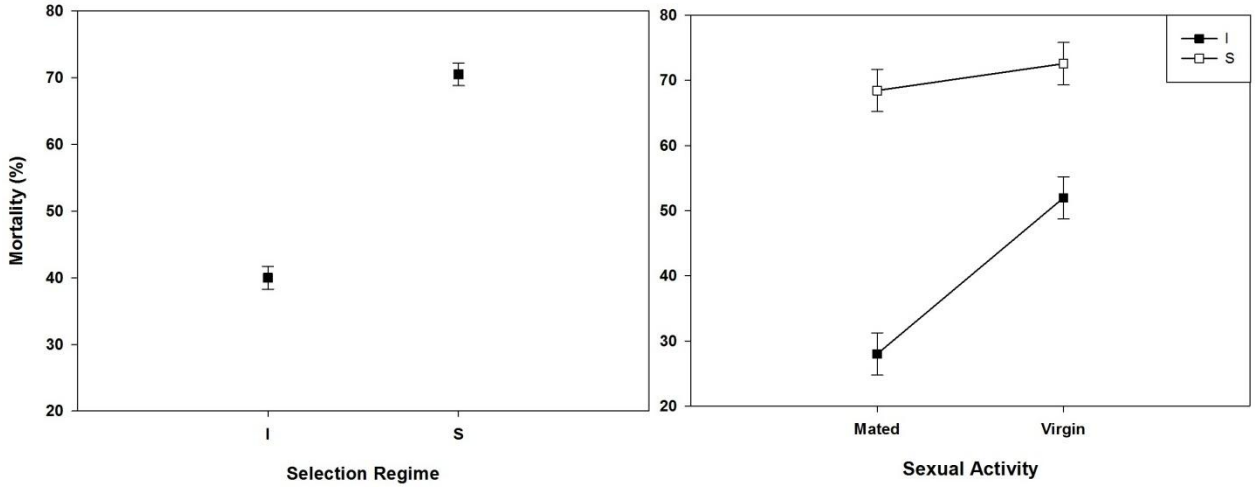

Fig. 1 & 2*: Percentage mortality at 96 hours post infection. Fig. 1 (left) gives mean mortality post-infection in I and S selection regimes. Fig. 2 (right) shows mean mortality in Mated and Virgin males. Filled squares (■) depict I selection regime whereas blank squares (□) depict S selection regime*

Table 1*: Effect of sexual activity on percentage mortality in males over 96 hours post infection. Summary of results from three-way mixed model ANOVA treating sexual activity (Virgin vs. Mated) and Selection (I vs. S) as fixed factors and block as random factor.*

Source	DF	Den DF	MS	F Ratio	p>F
Selection	1	2	2794.75	161.78	0.006**
Sexual Activity	1	2	590.79	5.52	0.14
Block&Random	2	1.3483	164.17	1.77	0.42
Selection × Sexual Activity	1	2	295.37	9.35	0.09*
Block × Selection	2	2	17.27	0.55	0.65
Block × Sexual Activity	2	2	107.09	3.39	0.23

***p<0.05, *p<0.1*

Further survivorship analysis was done separately for each block. Significant differences between virgin and mated males was observed in all three blocks when analysed block-wise using Kaplan-Meier Estimator. (Table 2)

Similar analyses were done when males were infected at a lower bacterial concentration (OD 1.0 ± 0.1). No effect of sexual activity on mortality was seen in I as well as S selection regimes. There was a significant effect (p=0.015) of selection on percentage mortality.

***Table 2**: Summary of p-values of Wilcoxon (Gehan-Breslow) Test of effect of sexual activity (Virgin vs. Mated) on survivorship in I and S regimes. Analysis was done using Kaplan-Meier Estimator.*

Block	Selection	Sexual Activity	Median time to death(hrs)	Mean time to death(hrs)	p value
2	I	Virgin	…	57.99	0.0525*
		Mated	…	77.53	
	S	Virgin	32.5	52.19	0.52
		Mated	38	45.62	
3	I	Virgin	33	89.11	<0.001**
		Mated	…	54.95	
	S	Virgin	28	46.99	0.63
		Mated	28	49.33	
4	I	Virgin	…	65.74	0.002**
		Mated	…	79.50	
	S	Virgin	47	57.53	0.07*
		Mated	78.25	65.81	

*"…" Mortality was less than 50%, **p<0.05, *p<0.1*

Sexual behaviour post-infection

Several reproductive traits like mating latency, copulation duration, and courtship frequency were recorded under infected and sham-infected conditions in both I and S males.

There was no significant difference in mating latency and copulation duration observed. However, there was a significant difference in mating frequency of I and S males under infected conditions (Single sample t-test, μ_0= 0.50, p=0.018). I males mated significantly less frequently compared to S males (Table 3, Fig. 3). There was no difference between mating frequency of I and S males under sham-infected conditions (Single sample t-test, μ_0= 0.50, p=0.97). Also I males mated significantly less frequently under infected conditions as compared to uninfected conditions; and vice versa for S males (Paired two-tailed t-test, p=0.003) (Table 3).

Table 3*: Proportion of successful mating by I male under infected and sham-infected conditions*

Block	Infected	Sham-Infected
1	0.35	0.49
2	0.39	0.48
3	0.32	0.43
4	0.44	0.60

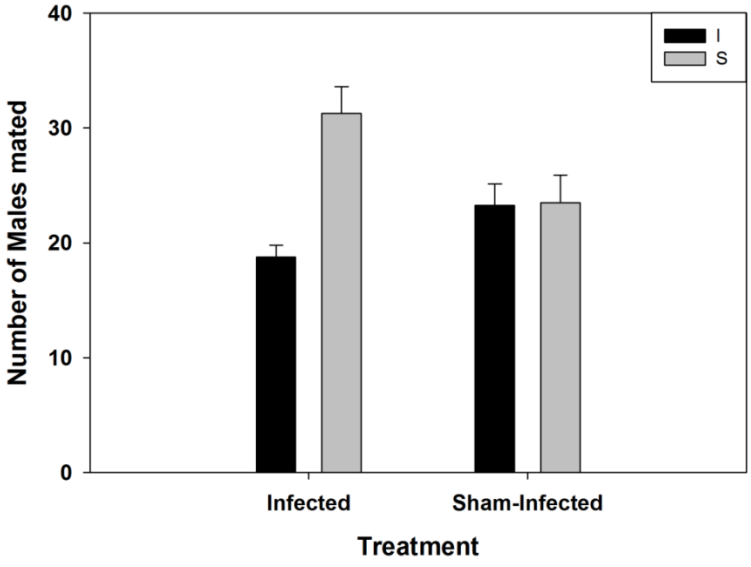

Fig. 3*: Number of successful mating by I and S males under infected and sham-infected conditions*

Analysis was done separately for infected and sham infected treatments. There was a significant effect (p=0.06) of selection (I vs. S) on the courtship frequency of infected males (Table 4). Under infected conditions I males courted less frequently compared to S males. There was no difference in the courtship frequency of the two under sham-infected conditions (Fig. 4).

No difference was found between number of progeny of I and S males under infected as well as uninfected conditions.

***Table 4**: Effect of selection on courtship frequency of males 6 hours post infection. Summary of results from two-way mixed models ANOVA treating selection (I vs. S) as fixed factor with block as random factor.*

Source	DF	Den DF	MS	F Ratio	p>F
Selection	1	2.28	182.44	11.66	0.06*
Block&Random	2	2	75.69	4.99	0.17
Selection × Block	2	263	15.18	0.52	0.60

**p<0.01*

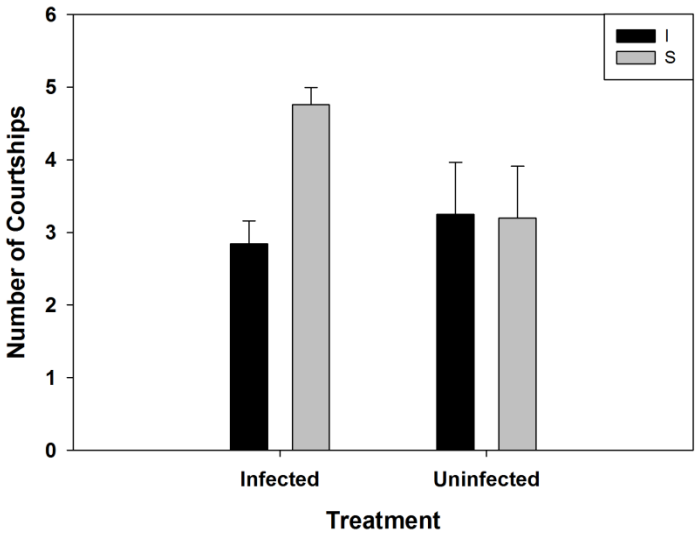

***Fig. 4**: Courtship score of I and S males under infected and uninfected conditions.*

Post-infection competitive fitness

Proportion of progeny sired by I and S males under infected and sham-infected condition, when housed with a common competitor over a period of four days was compared. Analysis is being presented for Block 2 and Block 4 only as most vials of Block 3 yielded no progeny.

There was a significant selection by treatment interaction effect (p=0.027) on progeny sired by I and S males as a proportion of total progeny produced over four days (Table 5). S males sired higher proportion of progeny under sham-infected conditions compared to I males, whereas there was no difference in proportion progeny sired under infected conditions (Fig. 5). Day-wise comparisons showed a similar significant selection by treatment interaction (p=0.029) only on Day 1 (Fig. 6). On all other days there was no significant difference in proportion of progeny sired by I and S males.

Table 5: *Effect of selection and treatment on proportion progeny sired over four days post infection. Summary of results from three-way mixed models ANOVA treating selection (I vs. S) and Treatment (infected vs. sham infected) as fixed factors crossed with random blocks.*

Source	DF	Den DF	MS	F Ratio	p>F
Selection	1	1	0.17	1.69	0.42
Treatment	1	1	0.006	0.14	0.77
Block&Random	1	1.70	1.10	7.70	0.13
Selection × Treatment	1	1	0.38	540.31	0.03**
Block × Selection	1	1	0.10	142.79	0.053
Block × Treatment	1	1	0.04	60.67	0.08
Selection × Treatment × Block	1	1	0.001	0.01	0.94

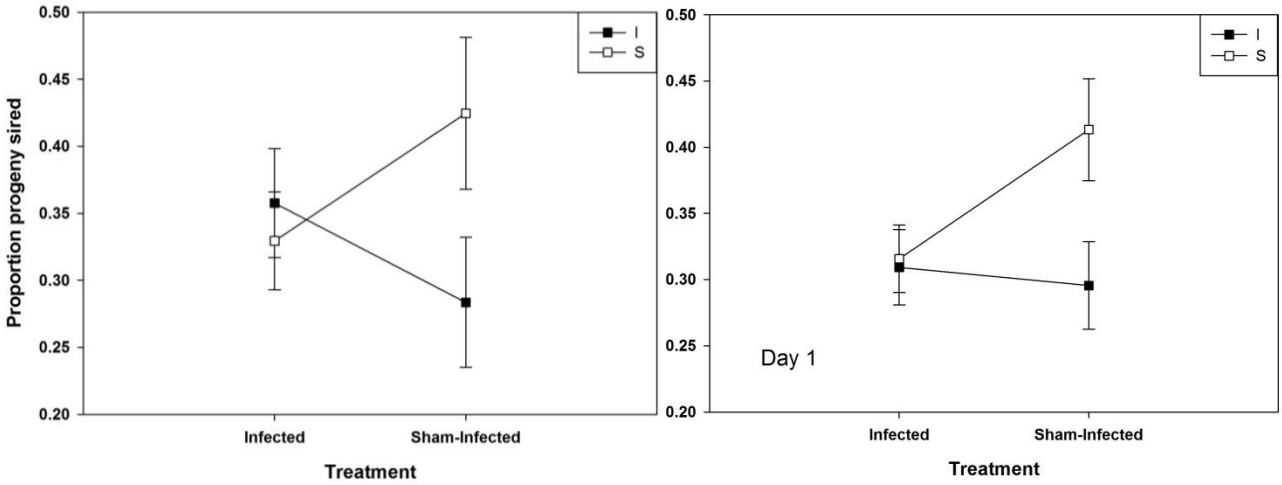

Fig. 5 & 6*: Proportion of progeny sired by I (■) and S (□) males under Infected and Sham-Infected condition over four days (left) and on Day 1 (right).*

DISCUSSION

Effect of sexual activity on immunity

The study indicates that mating confers a benefit in terms of a higher survivorship against pathogen infection (at OD 1.5) in I males whereas there is no effect of mating status in S males. In BRBs, the ancestral populations of I and S, mated males had significantly greater survivorship against *P. entemophila* compared to virgin males (Gupta et al., 2013). It was shown that sexually active males were able to clear bacteria or suppress the growth of pathogens better than virgin males (Gupta et al., 2013).

Although BRB and I males have greater resistance when sexually active, this trend seems to be absent in S males. To account for the possibility that the bacterial concentration (OD 1.5) used to infect the S males may have been toxic for them, the experiment was repeated using a lower concentration of bacteria (OD 1.0). The same concentration was used to infect BRB males (Gupta et al., 2013). No effect of sexual activity was seen in either S males even in this case.

Laboratory adaptation could explain why no difference was seen between post infection survivorship of virgin and mated S males. Change in life-history traits are observed as populations established from wild flies get adapted to laboratory conditions (Sgrò and Partridge, 2001). It has been shown, for example, that *D. melanogaster* populations lost the ability to resist environmental stresses under adaptation to laboratory culture (Hoffmann et al., 2001). I, S and U populations have been reared in the laboratory for more than 60 generations and BRB, the ancestral populations, for more than 80. Previous study on post-infection survivorship of virgin and mated BRB males was conducted after 20 generations of laboratory adaptation. It is possible that BRB, S and U males have now lost the ability to mount an efficient immune response against pathogen as a result of long term adaptation to sterile laboratory conditions. I males on the other hand, are exposed to infection every generation and hence may have retained the trend from their ancestral populations.

This loss of difference in survivorship between virgin and mated S males could also be a result of their selection regime itself. Flies from the S regime are pricked with a needle every generation. It is known that some common pathways and their

consequent responses are activated during wound healing as well as while mounting offense against a pathogen (Lemaitre and Hoffmann, 2007). It is possible that components of the immune response trade-off against one another. Males from S regimes may have traded-off the ability to defend against a pathogen to evolve more efficient wound healing.

Similar survivorship experiments have to be carried out with U and BRB males in order to confirm either of the two hypotheses.

Mate preference 6 hours post infection

In this study, I have also found that there is lower mate preference towards I males compared to S males under infected condition. Infected I males also court less frequently compared to infected S males. However, there was no difference in pre-copulatory traits such as copulation duration and mating latency in I and S males. Although, I males mated and courted less frequently compared to S males under infected conditions, there was no difference in their individual fitness measured as progeny produced per mating.

It is known, from gene expression studies, that flies from I and S regimes mount an immune response within six hours post infection (Gupta, V unpublished data). It is possible that six hours post infection, I males have evolved to invest energy in clearing bacteria from their system, and therefore decrease their investment in courting and mating, i.e. immune response and courtship behaviour may be trading-off with one another. Another possibility, although unlikely, could be that S males court and mate excessively post infection, as a strategy of terminal reproduction.

As per the I and S selection regimes, in order to contribute to siring the next generation, flies from both populations need to survive for four days after their respective treatments. The previous experiment gave insight on sexual behaviour of I and S males 6 hours post infection. At this time point there may not be ultimate fitness consequences of the observed differences in mating and courtship behaviour between I and S males.

Post-infection competitive fitness

This experiment was carried out to understand if differences in mating behaviour result in a difference in number of progeny sired over the lifetime of the individual. We observed that there was no difference in proportion of progeny sired over the four days under infected conditions. However, under sham-infected conditions S males sired higher proportion of progeny over four days, compared to I males. Results also indicate that it is perhaps the first 24 hours post infection that contributes to this difference in proportion of progeny sired. There was a significant difference in proportion of progeny sired under sham-infected conditions only on Day 1.

The results indicate that although I males survive better over the four days than S males under infected conditions, it seems they gain no resultant fitness due to their survivorship. Although most S males are dead by the end of Day 3, there is no consequent effect of their mortality on their fitness. They perform as well as I males under infected conditions. There is a hint of a possible trade-off between reproductive behaviour and immune response given that S males sire greater proportion of progeny compared to I males under sham-infected conditions.

It is important to note however, that in the above experiment infected I and S males were competing with healthy individuals for a healthy female. In their natural conditions, infected males are competing with other infected males to procure an infected female. Differences in sexual behaviour or I and S males under infected conditions may have been masked due to the presence of a healthy competitor over the four days. It would be prudent, therefore, to repeat this experiment with an infected competitor. Also, the results of the experiment need to be accepted with caution as they have been compiled from only two blocks of I and S regime. Data from the remaining two blocks will help to strengthen the results.

CONCLUSION

In this study I have shown that sexual activity increases survivorship post-infection in I males but not in S males. Further assays with BRB and U males will help understand why this is so. I have also shown that 6 hours post-infection I males mate and court less frequently compared to S males. In presence of a healthy competitor, S males sire higher progeny compared to I males. These are evidences that suggest that there is a trade-off between immune response and reproductive effort. However, further experiments need to be performed to confirm this.

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