SYNOPSIS

In sickness and in health: Exploring the evolution of immune response using *Drosophila melanogaster*

Most organisms are likely to face pathogenic challenge in their life time. The ability to mount an immune response has direct consequences on the host's fitness. Therefore, organisms have evolved an elaborate and complex immune response system. While the molecular mechanisms underlying immune response have been worked out in relatively great detail, the evolution of immune response has received relatively less empirical attention. The aim of my study was to understand the evolution of immune response using laboratory populations of *Drosophila melanogaster*. Towards this end, I first quantified the genetic variation in immune response using isofemale approach. Then, I successfully selected replicate populations of *Drosophila melanogaster* for increased ability to survive pathogenic infection using experimental approaches. I used the selected and control populations to explore the correlation of immune response with life-history traits, reproductive activity and resistance to multiple pathogens. I also addressed the mechanistic and genetic basis of evolved immune response.

Various studies have found genetic variation for immunity in populations of *Drosophila melanogaster*. This variation can be maintained due to a number of reasons like varying environment that the hosts inhabit, genetic correlation across host traits or due to differences in the pathogens. Most of these studies have used chromosome extraction lines or inbred lines to assess genetic variation. Using a set of isofemale lines of *D.melanogaster*, I addressed the following questions – a) Is there any genetic variation for immune response, fecundity and longevity, b) how are these life-history traits correlated with each other, c) does sexual activity have an effect on these traits and d) how do fecundity and immune response change with age. For the experiment, I used 18 isofemale lines that were established from flies caught in wild from Blue Ridge Mountains, USA. The immune response and fecundity of these lines was measured at three different ages. I measured immune response against bacteria *Providencia stuartii* by estimating the bacterial CFUs (Colony forming Units) present in the flies 24 hours post-infection. Longevity of un-infected flies was assayed by monitoring mortality in cohorts till the death of the last fly. My results indicate that there was a significant genetic variation across isofemale lines for immune response, longevity and fecundity. I found a negative correlation of

fecundity with both longevity and immunity. I observed that sexual activity had an effect on life-span but not on the immune response. I found that age specific changes in immune response varied across lines- an evidence for variation in immunosenescence across lines. Therefore, I concluded that substantial genetic variation exists in all the three traits and that senescence rates are not uniform across lines.

After this, I established a new laboratory population (Blue Ridge Baseline, BRB) of *Drosophila melanogaster* by mixing the flies from 19 isofemale lines and maintained them in the laboratory for ten generations to allow adaptation to laboratory conditions. I then used this newly created laboratory population (BRB) along with a long-term laboratory adapted population (LH) and inbred population (Canton S) to assess the effect of sexual activity on immune response. I subjected these populations to infection from two pathogens (*Pseudomonas entomophila* and *Staphylococcus succinus*). Previous studies with *Drosophila melanogaster* show that male's ability to clear bacteria decreases with increase in sexual activity. However, I found that sexual activity enhanced male survivorship in a pathogen specific manner. Sexually active males showed higher resistance than virgins upon infection with *Pseudomonas entomophila*. Interestingly, the beneficial effects of sexual activity increased with time of co-habitation with females and declined when access to females was restricted. I did not observe change in male survivorship upon experimentally varying the number of sexual interactions. My results showed that the sexual activity-immunity trade-off in males cannot be generalised. The trade-off is potentially mediated through complex interactions between the host, pathogen and the environment experienced by the host.

In the next part of my work, I studied the evolution of immunity by using long term Experimental Evolution protocol. For the study, I created three selection regimes using the BRB populations: – **I** (Infected with pathogen *Pseudomonas entomophila*), **U** (Unhandled Controls) and **S** (Sham Infected). Each selection regime consists of four independent replicate populations. I populations are challenged with bacterial infection through septic injury every generation. Flies are held for 4 days post infection during which time about 50% of the flies die. The next generation is started from the survivors. Flies from S populations are subjected to injury without infection while U populations are the unhandled controls. Within ten generations of selection, I observed that selected I males and females had higher survivorship post-infection compared to

controls. I have carried on the selection for more than 60 generations. After 45 generations of forward selection, I also established a set of reverse selected populations. The forward selected and reverse selected populations were maintained separately and were used to address several questions:

1. What is the mechanistic basis of evolved immune response?

2. Is cost of evolved immune response manifested in other life-history traits?

3. Is the evolved immune response generic or specific?

Protection against pathogenic attacks can be achieved by either eliminating the pathogen (resistance) or by protecting own system from the pathogenic damage (tolerance). Experimental and theoretical evidence suggest that tolerance and resistance have different fitness consequences along with variable effect on disease prevalence in the population. Therefore, these mechanisms can shape the life-history of populations differentially. In this study, I looked at evolution of resistance and tolerance mechanisms in *Drosophila melanogaster* populations evolved for increased ability to survive infection by a gram-negative pathogen *Pseudomonas entomophila.* After 29 generations of selection, I quantified mortality and bacterial load post infection in the selected and control populations. I found that males have evolved increased tolerance to infections whereas females have evolved increased resistance. To understand the genetic basis of the evolved response I also looked at gene expression of humoral genes. I did not find any effect of selection on the expression of the genes studied. I conclude that immune mechanisms in the same population can evolve in a sex-specific manner which might not be mediated by humoral immune response.

Maintenance and deployment of immune system are costly and are hence predicted to trade-off with other resource demanding traits, such as reproduction. However, very few empirical studies have investigated this trade-off. I subjected this long standing idea to test using laboratory experimental evolution approach. In the present study, three selection regimes: I, S and U were used. After 30 generations of selection, a number of life-history traits such as longevity, fecundity, egg hatchability or development time were assayed. However, contrary to expectations and previous reports, I did not find any evidence of trade-offs between immunity and any of the studied life-history related traits. In order to understand the fitness consequences

of evolved immunity, I established two reverse selection regimes where I and S populations were returned to U-type of regime. I observed that reverse selected I populations continued to exhibit higher post-infection survivorship even after ten generations of relaxed selection. I speculate that either evolved immune response has a feeble negative effect on fitness of the selected populations or that trade-offs occur with respect to traits like reproductive behaviour or other immune mechanisms that I have not investigated in this study.

All organisms are susceptible to pathogenic attack and most organisms face attack from more than one kind of pathogen. It is often observed that immune response against a pathogen affects the immune response against another pathogen. Therefore, directional selection of a population for better immune response against one pathogen may affect the ability to mount immune response against other pathogens. However, direct empirical evidence that validate whether evolved immune response against one pathogen would confer protection against other unrelated pathogens or not is scanty and inconsistent. For this study, I subjected the populations from the three selection regimes – I, S and U to two other pathogens – *Providencia retgerii* (gram-negative pathogen) and *Staphylococcus succinus* (gram-positive pathogen) to address whether evolved resistance is general or specific. Interestingly, I found that both males and females of selected I populations survived better than controls when challenged with these pathogens. Also, I observed that I females clear more bacteria compared to controls. However, even though I males showed increased survivorship against both pathogens, I observed a pathogen specific response in terms of their ability to clear bacteria. I also quantified mRNA expression of two genes from the humoral pathway and did not find any effect of selection. I conclude that evolved immune response against pathogen selected for, can result in better immune response against multiple pathogens.

In my thesis I have tried to address some of the long standing questions related to evolutionary ecology of immunity. I have used different approaches to understand evolution of immune response. From my work, I establish that substantial genetic variation for immune response exists in the population which can further be subjected to selection under laboratory conditions. This thesis provides the first empirical evidence that immunity can evolve in a sex-specific manner. My results indicate that trade-offs between immunity and other life-history traits should

be generalized with caution. Overall, my thesis has important implications in evolution of immunity, its trade-offs with other life-history traits and effect of sexual activity on immunity.