RESEARCH COMMENTARY

Immunocompetence in *Drosophila*: linking genetic to phenotypic variation

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Immunity can be classified into two types, namely innate and adaptive immunity. Innate immunity provides a generalized response against the invading pathogens, whereas adaptive immunity is mediated through the production of antigen-specific antibodies against the antigen, and includes a 'memory' that makes future response against any particular antigen more efficient. Invertebrates typically exhibit only innate immune responses, whereas vertebrates show both innate and adaptive immune responses. Despite lacking an adaptive immune system, *Drosophila* mounts a quick and efficient immune reaction against invading pathogens, involving (a) cell-mediated immunity, in which specific cells like macrophages and lamellocytes kill the pathogens by engulfing or encapsulating them, and (b) humoral immunity, through the production of antimicrobial peptides in the fat body that are released into the circulating hemolymph. It has been found that two distinct signal transduction pathways mediate specific immune responses in *Drosophila*: the Toll pathway, which acts against fungal and gram-positive bacterial infections, and the Imd pathway, which defends against gram-negative bacterial infections (De Gregorio *et al*. 2002). Activation of these two pathways ultimately leads to the expression of antimicrobial peptides which lyse the invading microbial cells, and nearly 50 different such peptides have been identified in *Drosophila* (Hoffmann 1995). In the past few years, the molecular details of the *Drosophila* immune system have been studied extensively (Hoffmann and Reichhart 2002), and there have also been a few attempts to link immune function to life-history related traits or to abundance in the wild (McKean and Nunney 2001; Sharmila Bharathi *et al*. 2003). There have also been a few molecular population genetics studies of polymorphism and divergence in immune function related genes of *Drosophila* (Lazzaro *et al*. 2003; Schlenke and Begun 2003), but studies connecting natural phenotypic variation in immune function and underlying genetic variation at loci involved in immune function have thus far been lacking.

In a recently published study, Lazzaro *et al*. (2004) have for the first time linked variation in genes involved in the immune response to naturally occurring variation in immunocompetence in a wild population of *D. melanogaster*. They focused on 21 candidate genes located on the second chromosome that can be grouped into three broad classes according to their putative function in the immune response of *D. melanogaster*:

1. Genes thought to be involved in microbial recognition (four class C scavenger receptors, and a three gene cluster coding for proteins involved in peptidoglycan recognition).
2. Genes believed to be involved in signal transduction (three Toll like receptors, a *rel* transcription factor, a *rel* inhibitor and two different intracellular signaling genes).
3. Seven genes actually coding for different antibacterial peptides.

In this study, Lazzaro *et al*. (2003) examined 101 lines of *D. melanogaster*, each rendered homozygous for an independent second chromosome. All lines shared a common genetic background, except for the second chromosome, ensuring that all phenotypic variation among them, ascribable to genes, would be a reflection of the naturally occurring variation at loci on the second chromosome in the wild population. Genetic variation across the 101 lines was assessed by examining a number of alleles at the 21 loci studied, through genotyping 127 sites situated within more than 100 kb of unique sequence, including introns and coding regions, as well as upstream regulatory regions.

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To assess the phenotypic variation in immunocompetence across the 101 test lines, over 16000 adult flies were manually infected with the gram-negative bacterium *Serratia marcescens* at different times of the day (morning or evening). The pathogen load was subsequently determined by crushing the flies followed by quantitative plating (colony count) at 7, 15, 26 and 39 h after infection. The results showed that line (second chromosome) was a significant determinant of bacterial load post-infection, with extreme lines differing by up to 10 phenotypic standard errors, indicating considerable variation in immunocompetence among individuals in the wild source population. Bacterial load, however, did not vary much at the different assay times, indicating a post-infection plateau in bacterial density in the infected flies over the time range of 7–39 h. A more detailed examination of the results indicated that the polymorphic sites most significantly associated with variation in suppression of *S. marcescens* were those situated within the genes involved in pathogen recognition and intracellular signaling. Polymorphism found in genes coding for actual antibacterial peptides, on the other hand, did not appear to exert significant effects on variation in immunocompetence. The authors suggest that this pattern might be due to the fact that the proteins involved in pathogen recognition and signaling exercise regulatory control over a variety of cellular processes related to the immune response, and even small changes in the expression or activity of these proteins are likely to have large effects on the phenotype. The antimicrobial peptides, on the contrary, are components of immune regulation at the end of the pathways, and are also partially redundant. Consequently, genes coding for these peptides may not show large effects on phenotypic variation in immune function. Overall, it appears that much of the variation in immunocompetence in the wild population studied derives from mutations of small phenotypic effect, although some mutations of larger effect are seen in genes coding for intracellular signaling molecules. Of greater interest, perhaps. is the authors' finding of significant epistatic interactions between different intracellular signaling loci, and between intracellular signaling loci and loci coding for antimicrobial peptides. Thus, loci coding for antimicrobial peptides that did not contribute much to variation in immunocompetence when considered singly, did interact with other immune system loci to affect immunocompetence levels. Additionally, a strong effect of the time of infection (morning or evening) was observed, with a number of sites upstream of the *imd* gene showing strong associations with bacterial loads sustained by flies infected in the morning but not those infected in the evening. This finding is perhaps not surprising, given previous studies indicating that the expression of immune genes in *Drosophila* shows circadian cycles even in the absence of infection (McDonald and Rosbash 2001). However, time of infection did not show any significant interaction with line. Similarly, no effects of gender or gender × line interaction on bacterial load were observed.

In recent years, there has been an increasing recognition that investments in immune function are likely to be involved in shaping tradeoffs among life-history related traits (Zuk and Stoehr 2002; McClean and Nunney 2001). At the same time, much information has now become available about the genetic mechanism underlying immune response in *Drosophila* (Hoffmann and Reichhart 2002; De Gregorio *et al*. 2002). Given the central role of *D. melanogaster* as a model system in studies of life-history evolution, this recent paper by Lazzaro *et al*. (2004) is a timely and welcome study, being the first to link patterns of genetic variation at loci involved in *Drosophila* immune response to phenotypic variation in immunocompetence in a wild population. We hope that further studies in this area will help integrate considerations of immune response to pathogens into our understanding of the factors and forces shaping life-history evolution in *Drosophila*.

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