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Research paper

A newly evolved *Drosophila Cytorace-9* shows trade-off between longevity and immune response

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ABSTRACT

Species with an efficient immune system would be at an advantage to evade pathogenic challenges and adapt to an ever changing ecological niche. The upkeep of immunity is a costly affair, thus trade-offs between immunity and other life history traits are expected. However, studies on the relation between immunity and life span have yielded paradoxical results. *Drosophila Cytoraces*, being at different stages of evolutionary divergence, provide an excellent experimental model system to study how evolving populations gain novel traits in the absence of selection. We found that in the absence of pathogenic infections, the *Cytorace-9* flies lived longer than those of *Cytorace-3*. However, when these *Cytoraces* were challenged with different pathogenic microbes, the trend was opposite. After infection with pathogens, the long-lived *Cytorace-9* survived worse than the short lived *Cytorace-3*, which can be attributed to a reduction in its immune response. This study provides evidence to support the existence of a trade-off between life span and immunity.

1. Introduction

Immunity has been described to affect the fitness of an organism both positively and negatively. During a microbial invasion, while an immune response bestows the responder with the benefit of survival to infection, in the long run the enormous expenditure of energy in mounting immune responses is expected to entail a reduction in other fitness related traits (such as longevity) of the individual (Alonso-Fernandez and De la Fuente, 2011; Anderson et al., 1985; Hahn and Smith, 2011; Lazzaro and Little, 2009; Zera and Harshman, 2001). However, experimental studies on longevity-immunity trade-off have yielded paradoxical results.

Though an activated immune system attempts to avoid premature death due to infection, reports suggest that chronic activation of immune responses leads to reduced fitness and life-span (Fernando et al., 2014; Hahn and Smith, 2011; Libert et al., 2006; Ye et al., 2009). The immune system itself deteriorates with age (reviewed in Ponnappan and Ponnappan, 2011). While these studies suggest an immunity-longevity trade-off, contrasting reports also exist. Several long lived animals – even humans – have better immune system and reduced effects of ageing on immune system (Balistreri et al., 2012; Candore et al., 2010; Libert et al., 2008; Weindruch et al., 1986). Also, animals unable to mount an immune response die if infected and have shorter life-spans (Le Bourg et al., 2012). Thus, despite several decades of work, a clear understanding of the impact of immunity on ageing and fitness and *vice versa* has not yet been established.

Studies done with animals collected from the wild are important and yet the absence of knowledge regarding their genetic make-up reduces the amount of information one can gain from these studies; hence the requirement of a good model system. *Drosophila* is an elegant choice for studies on life-history related trade-offs involving the immune system (Bharathi et al., 2007; Fellowes et al., 1998; Libert et al., 2008). In *Drosophila*, several alleles have been shown to increase stress tolerance, longevity and immunity, while entailing lower reproductive ability (Flatt and Kawecki, 2007). Moreover, longevity was shown to have trade off with immunity in natural populations of *Drosophila* (Gupta et al., 2013; Lazzaro et al., 2004; Parker et al., 2014; Ye et al., 2009), which stem from genetic variations that exist as a result of ecological differences.

In order to look at the evolution of the life history traits, we have used *Drosophila Cytorace* – true-breeding introgressed hybrids of two cross-fertile races *Drosophila nasuta nasuta* (2n = 8) and *Drosophila nasuta albomicans* (2n = 6) (Ranganath, 2002; Wilson et al., 1969). Without any deliberately-imposed selection pressure some of these *Cytoraces* have evolved as long-lived races in laboratory within 650 generations. While the *Cytorace-3* (*C-3*) has maintained the lifespan of the ancestor/parent approximately, *Cytorace-9* (*C-9*) diverged to have a

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longer lifespan (Ranjini and Ramachandra, 2009). Since these *Cytoraces* are cytogenetically closely related members, still diverging for different sets of life history traits, they offer a unique opportunity to witness the evolutionary changes in real time and to throw light on the processes and patterns of events in nature. *C-9* lives longer than *C-3* but has similar body size and fecundity, suggesting that there is no trade-off between longevity and fecundity (Harini and Ramachandra, 2003; Ranjini and Ramachandra, 2009). In this study, we show that there is a trade-off between longevity and immunity. The short-lived *C-3* showed better resistance and tolerance when challenged with multiple pathogens compared to long-lived *C-9*.

2. Materials and methods

2.1. Fly strains and maintenance

Fly lines used in this study are *C-3* and *C-9*. *C-3* (2n = 8; mean lifespan = 50 days) was generated through hybridization between *D. nasuta nasuta* (2n = 8; mean lifespan = 51 days) and *D. nasuta albomicans* (2n = 6; mean lifespan = 55 days) (Ramachandra and Ranganath, 1990). *C-9* (2n = 6; mean lifespan = 109 days) is a by-product of hybridization between *D. n. nasuta* and *C-2* (2n = 6; progeny of *D. n. nasuta* and *D. n. albomicans*) (Ramachandra and Ranganath, 1996; Tanuja et al., 2003). Thereafter, they were maintained under similar conditions without being subjected to any selection pressure. The lifespan of *C-3* is comparable to the ancestral races, suggesting that *C-9* has diverged from the parental populations. Stocks were maintained on standard cornmeal-sugar-agar medium supplemented with yeast at 25 °C on 12/12 hours light/dark cycle.

2.2. Lifespan assessment

Life-span was assessed following a modified protocol of Luckinbill and Clare (1985). Newly eclosed, synchronized, virgin flies were kept in fresh vials (n = 30) at 25 °C. The number of dead flies was recorded every day, while flies were transferred to fresh media vial every 2 days. Survival data were assessed for statistical significance using both Log-rank test and Wilcoxon test.

2.3. Stress survival assays

Flies were subjected to a heat stress of 37 °C and the number of dead flies was recorded every 10 min till all flies were dead (n = 30). The data were statistically assessed using both Log-rank test and Wilcoxon test. For oxidative stress, flies were fed 30 mM Paraquat in 5% sucrose and their survival assessed similarly at regular intervals (n = 30). Control flies were fed 5% sucrose. The difference in mean survival was tested for statistical significance using two-tailed unpaired *t*-tests.

2.4. Microbial strains and culture

Bacterial strains used were *Serratia marcescens* (MTCC #8702), *Enterobacter cloacae* (MTCC #7097), *Bacillus subtilis* (MTCC #441) and *Staphylococcus aureus* (MTCC #3160). Entomopathogenic fungal strains used were *Beauveria bassiana* (MTCC #6291) and *Metarhizium anisopliae* (MTCC #3210). All strains were procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh, India. Strains were cultured according to MTCC directions.

2.5. Infection survival

Bacterial infections were done by pricking fly thoraces with a tungsten needle dipped in concentrated cultures (~10^{12} colony forming units (CFU)/ml) (Apidianakis and Rahme, 2009). Control flies were pricked with sterile needle. For natural fungal infections, flies were anesthetized and rolled over a sporulating fungal lawn (Neyen et al., 2014). Control flies were treated similarly on empty plates. Survival rates of flies after infection were measured under identical conditions for each test genotype. Dead flies were counted at regular intervals (n > 50). Survival data were statistically assessed using both Log-rank test and Wilcoxon test.

2.6. Basal microbial load estimation

To calculate basal microbial load, hemolymph and gut components were collected from uninfected flies (n = 50, 25 males and 25 females) by centrifuging at 5000 rpm for 10 min in a perforated 0.5 mL tube within a 1.5 mL tube. The collected sample was serially diluted, plated on Luria Bertani (Himedia, India) agar plates and incubated at 37 °C for bacterial growth. CFUs were counted after visible colonies could be detected (~12 h). The data were assessed statistically using two-tailed unpaired *t*-tests.

2.7. Bacterial survival in fly hemolymph

Flies were infected as mentioned above. Six hours post infection the hemolymph was collected from groups of 30 decapitated flies (15 males and 15 females) as explained in Section 2.6. 100 μL of hemolymph-like solution (HL3 buffer) was added to the collected hemolymph. Diluted cultures of *S. marcescens* and *S. aureus* (~100 CFU) were incubated with the diluted hemolymph for 2 h at 25 °C (ambient temperature for fly) after which 100 μL of the culture was plated and incubated at ambient temperatures for bacterial growth. CFU counting was done after visible colonies could be detected (~12 h). Statistical analyses were done using two-tailed unpaired *t*-tests.

2.8. Quantitative RT–PCR analysis

Total RNAs were extracted from 10 flies (1–2 day old, 5 males and 5 females) using TRIzol (Sigma, India). cDNA was synthesized by using

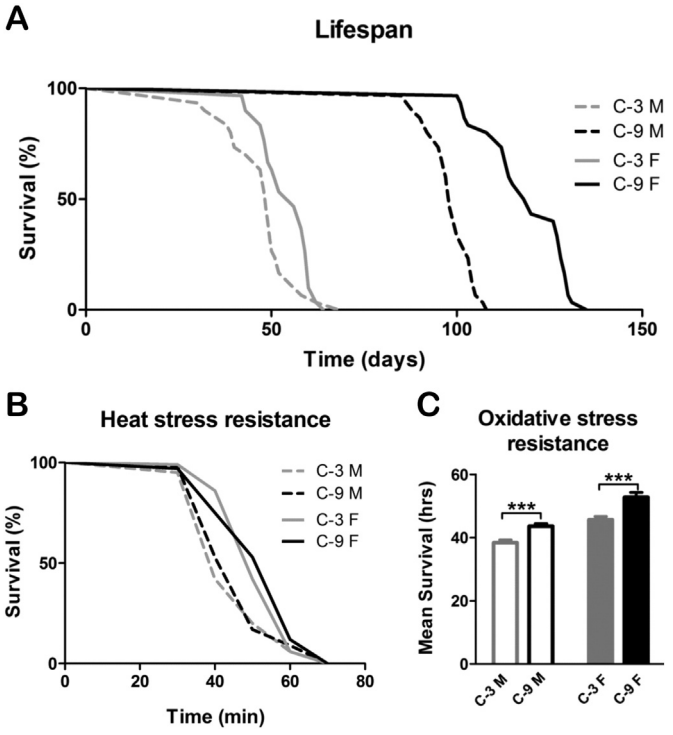

Fig. 1. Longevity and stress tolerance of *Cytorace-9* and *Cytorace-3*. (A) Lifespan (B) Heat stress resistance and (C) Oxidative stress resistance of *Cytorace-3* and *Cytorace-9*. (A, B) Dashed lines represent males and solid lines, females. (C) Open bars represent males and solid bars, females. *C-3*: *Cytorace-3*, *C-9*: *Cytorace-9*, M: males, F: females. Grey: *Cytorace-3* and black: *Cytorace-9*.