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Longevity for free? Increased reproduction with limited trade-offs in *Drosophila melanogaster* selected for increased life span

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ABSTRACT

Selection for increased life span in *Drosophila melanogaster* has been shown to correlate with decreased early fecundity and increased fecundity later in life. This phenomenon has been ascribed to the existence of trade-offs in which limited resources can be invested in either somatic maintenance or reproduction. In our longevity selection lines, we did not find such a trade-off. Rather, we find that females have similar or higher fecundity throughout life compared to non-selected controls. To determine whether increased longevity affects responses in other traits, we looked at several stress resistance traits (chill coma recovery, heat knockdown, desiccation and starvation), geotactic behaviour, egg-to-adult viability, body size, developmental time as well as metabolic rate. Longevity selected flies were more starvation resistant. However, in females longevity and fecundity were not negatively correlated with the other traits assayed. Males from longevity selected lines were slower at recovering from a chill induced coma and resting metabolic rate increased with age, but did not correlate with life span.

1. Introduction

Longevity is frequently used as a proxy for ageing, where ageing is defined as the total effect of those intrinsic changes in an organism that adversely affect its vitality and that renders it more susceptible to the many factors that can cause death (Zwaan, 1999). The mutation accumulation theory of ageing as coined by Medawar (1952), suggests that ageing is the result of late acting deleterious mutations that have accumulated in the genome during times where life span was limited due to high mortality resulting from non-optimal environments. Other theories, the antagonistic pleiotropy and the disposable soma theory of ageing (Kirkwood, 1977; Williams, 1957), predict a negative correlation between early and late gene effects, especially between longevity and early reproduction; and several definitions of ageing relate it directly to reproduction (Partridge et al., 1999; Prowse and Partridge, 1997; Roper et al., 1993). The disposable soma theory, and more generally the optimality theory of ageing (Partridge and Barton, 1993) suggest that energy and resources invested in the maintenance of the soma come at the expense of energy invested in reproduction.

Trade-offs in life history traits are a well-described phenomenon (reviewed in Stearns, 1989). They are the product of evolution of increased fitness using limited resources where beneficial changes in one trait are associated with detrimental changes in another. A frequently studied trade-off is the association between increased life span and reproduction (reviewed in Flatt, 2011). A wide variety of other traits, such as body size, metabolic rate and stress resistance have also been shown to correlate with life span in organisms ranging from mammals (Migliaccio et al., 1999) to worms (reviewed in Olsen et al., 2006) and yeast (Klass, 1977; reviewed in Fabrizio and Longo, 2003). The model organism *Drosophila melanogaster* has been studied extensively for correlations between longevity and reproduction, metabolic rate and resistance to a wide range of stressors (e.g. Arking et al., 1991; Rose et al., 1992; Service et al., 1985; reviewed in Vermeulen and Loeschcke, 2007). For most of these traits however, the direction of an association with life span is not consistently positive or negative (e.g. Arking et al., 1988; Force et al., 1995; Khazaeli and Curtsinger, 2010; Leroi et al., 1994a).

For female *D. melanogaster*, studies have shown that mating is detrimental for life span (Fowler and Partridge, 1989), that seminal fluids harbour proteins harmful for the female (Chapman et al., 1995; reviewed in Chapman, 2001; Wigby and Chapman, 2005) and that egg production is costly, too (Partridge et al., 1987). For *D. melanogaster* males on the

Abbreviations: LS, longevity selected; C, control; MDMR, mean daily metabolic rate; MMMR, mean minimum metabolic rate.

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other hand, courtship behaviour has been shown to limit life span (Cordts and Partridge, 1996), and reproduction early in life limits both life span and subsequent fertility (Prowse and Partridge, 1997). In *D. melanogaster* several lines selected for increased life span in different laboratories have shown an altered reproductive pattern compared to their relative controls (Luckinbill et al., 1984; Partridge et al., 1999; Rose, 1984; Zwaan et al., 1995). Generally, reduced reproduction early in life is found to be a correlated response of increased life span, though other studies have shown that this is not consistently so (Leroi et al., 1994b). Whether recovery of early life fecundity during later generations of the selection procedure is an artefact of the experimental setup rather than an actual correlated response of longevity remains unresolved (Arking et al., 2002a,b; Leroi et al., 1994a,b; Vermeulen and Bijlsma, 2006). Recent studies on recombinant inbred lines (RIL) based on the Luckinbill lines suggested that early life reproduction and life span can be uncoupled (Khazaeli and Curtsinger, 2010, 2012), a finding also described in *Caenorhabditis elegans* (Anderson et al., 2011).

A trait studied extensively in relation to both longevity and reproduction is metabolic rate. The oxidative stress theory of ageing (Harman, 1956; Sohal and Weindruch, 1996) suggests that a higher oxygen metabolism leads to a higher production of reactive oxygen species (ROS) in the mitochondria. If left uncompensated, this will result in more somatic damage, resulting in an increased failure to maintain somatic homeostasis. The subsequent breakdown of the soma leads to ageing and eventually death of the organism. Several studies have shown circumstantial evidence for this suspected relation between metabolic rate and life span. Dietary restriction (DR), suggested to decrease metabolic rate (Sohal and Weindruch, 1996), has been shown to increase life span and decrease lifetime as well as age specific fecundity in *D. melanogaster* (Chapman and Partridge, 1996; Magwere et al., 2004). Studies dissecting the role of calories per se versus specific nutrients to elucidate mechanisms underlying life span extension indicate that it is the latter that mediates longevity, and that a simple reallocation of resources from reproduction to somatic maintenance does not explain the effect of DR on longevity (reviewed in Tatar, 2011). Service (1987) has shown a decreased respiration rate in young *D. melanogaster* flies selected for increased life span. Conversely, studies on RIL derived from the lines described in Luckinbill et al. (1984), as well as lines of *Drosophila simulans*, have shown that while age-specific metabolic rate is at least moderately heritable and has a tendency to increase with age, it does not correlate with life span (Khazaeli et al., 2005; Melvin et al., 2007; van Voorhies et al., 2003). Other studies have shown no evidence of a correlation between life span and metabolic rate either (Arking et al., 1988; Hulbert et al., 2004; Lee et al., 2008). Additionally, increased life span and lower fecundity are correlated with increased storage of lipids and carbohydrates in *D. melanogaster* (Djawdan et al., 1996; Service, 1987), though a discrepancy has been shown in the energy saved by delaying reproduction and that actually stored for later use (Djawdan et al., 1996). Female *D. melanogaster* are more susceptible to oxidative stress and are less starvation resistant when they are more fecund (Salmon et al., 2001).

Several other traits correlate with longevity and reproduction. Well-studied examples include starvation and desiccation resistance. Both types of resistance correlate with increased life span in some studies on *D. melanogaster* (Hoffmann and Parsons, 1993; Rose and Archer, 1996; Rose et al., 1992; Service et al., 1985) but not in others (Force et al., 1995). Furthermore, Harshman and Schmid (1998) found no evidence for an association between starvation or desiccation resistance and metabolic rate. Resistance to extreme temperatures, in particular cold resistance, has also been shown to correlate with life span, likely due to an increased level of glycerol in long lived flies (Luckinbill, 1998). Other stresses, such as ethanol vapour (Service et al., 1985), oxidative stress (Arking et al., 1991; Mockett et al., 2001), as well as geotactic and phototactic behaviour have also been studied in relation to longevity (Arking and Wells, 1990).

Overall, these findings highlight the versatile nature of the ageing process. Correlated responses in reproduction, metabolic rate and stress resistance appear to depend on the selection procedure, experimental environment and/or the genetic background of the flies investigated, an observation which is supported by transcriptomic studies, where detected candidate genes are strongly dependent on the genetic background (Sarup et al., 2011a). This could indicate that multiple pathways are available to attain an increased life span.

The present study investigates correlated responses to selection for increased life span to gain insight into the potential mechanisms that underlie the longevity phenotype. This was initiated after we found a fecundity pattern that was positively affected by selection for increased life span and timing of reproduction. Besides fecundity, we examine male reproduction and other life history traits as developmental time and body size as well as metabolic rate and four types of stress resistance for both males and females. No trade-off for living long became apparent in the traits under investigation here.

2. Materials and methods

2.1. Origin and maintenance of experimental flies

To explore the hypotheses relating to longevity and reproduction we examined a set of three longevity selected lines (LS) and three control lines (C). Selection and control lines were established from the same base population in September 2002 (Bubliy and Loeschcke, 2005). Unselected control flies (C1, 2 and 4) were kept at a density of ~100 flies per 100 ml bottle with 35 ml standard oatmeal–sugar–yeast–agar (Leeds) medium at 25 °C (12:12 D:L). Following eclosion, flies were aged for five days prior to oviposition into new bottles with standard medium and live yeast to initiate the next generation. The longevity selected (LS1, 2 and 4) lines had been kept under the same conditions as C lines at ~40 flies per 35 ml vial with 4 ml medium, while being transferred to fresh vials without live yeast every second day until 50% cumulative mortality had occurred. The surviving individuals were used to propagate the next generation. Every generation of selection was followed by a generation without selection. This intervening generation allowed the population to recover and increase in numbers, avoiding possible cross-generation effects. During this selection free generation, flies were maintained under the same conditions as the C lines. Prior to the reproduction experiments LS lines had been selected for 47 generations. Stress assays, viability, developmental time and virgin period were assessed after 50 generations of selection and life span, body size and metabolic rate were examined after 52 generations of selection. All experimental flies, except when stated otherwise, were reared at a controlled density of ~40 larvae per vial with 7 ml Leeds medium at 25 °C and L:D 12:12. Density was maintained by counting eggs. For all experiments mated flies were used, unless stated otherwise.

2.2. Longevity assay

Experimental flies were collected during an 8-hour window post eclosion. One day after collecting the flies were sexed while sedated with CO_2 and placed at a density of 15 males and 15 females per vial containing 4 ml Leeds medium. Flies were transferred to new vials and mortality was scored every other day until all flies had died. For each of the six lines 20 replicate vials were assayed.

2.3. Female fecundity

Fecundity was examined for 45 females per line. One day prior to eclosion of the females two males from the same line were collected for every female. Experimental females eclosed during a 3-hour window and were collected while sedated with CO_2. Throughout their life flies were kept in clear plastic vials with a spoon filled with ~0.5 ml of Leeds medium without live yeast but with red food dye to facilitate egg counting. For the first four days oviposition was assessed every 3 h. From day 5 until their death oviposition was scored every 24 h.