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Laboratory selection for increased longevity in *Drosophila melanogaster* reduces field performance

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ABSTRACT

Drosophila melanogaster is frequently used in ageing studies to elucidate which mechanisms determine the onset and progress of senescence. Lines selected for increased longevity have often been shown to perform as well as or superior to control lines in life history, stress resistance and behavioural traits when tested in the laboratory. Functional senescence in longevity selected lines has also been shown to occur at a slower rate. However, it is known that performance in a controlled laboratory setting is not necessarily representative of performance in nature. In this study the effect of ageing, environmental temperature and longevity selection on performance in the field was tested. Flies from longevity selected and control lines of different ages (2, 5, 10 and 15 days) were released in an environment free of natural food sources. Control flies were tested at low, intermediate and high temperatures, while longevity selected flies were tested at the intermediate temperature only. The ability of flies to locate and reach a food source was tested. Flies of intermediate age were generally better at locating resources than both younger and older flies, where hot and cold environments accelerate the senescent decline in performance. Control lines were better able to locate a resource compared to longevity selected lines of the same age, suggesting that longevity comes at a cost in early life field fitness, supporting the antagonistic pleiotropy theory of ageing.

1. Introduction

For decades scientists have investigated ageing in *Drosophila melanogaster* and other model organisms in laboratory settings to elucidate the underlying mechanisms, as well as to understand how ageing has evolved. Ageing is defined by Zwaan (1999) 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". Many life history and physiological traits are correlated to the ageing process, illustrating its quantitative nature.

Life history traits are expected to trade off with one another as a result of the restriction of adaptation potential due to constraints posed by the evolutionary history as well as the physiology of organisms (Stearns, 1989, 1992). Many of the studies on trade-offs and correlated traits in *D. melanogaster* have focussed on lines selected for increased longevity (Bubliy and Loeschcke, 2005; Luckinbill et al., 1984; Rose, 1984) but the trade-off between longevity, reproduction and stress resistance has been studied in other lines, too (Chapman, 2001; Cordts and Partridge, 1996; Fowler and Partridge, 1989; Harshman et al., 1999; Hoffmann and Parsons, 1993a,b; Prowse and Partridge, 1997). Trade-off patterns have been shown to be conflicting both between (Chippindale et al., 1997; Force et al., 1995; Vermeulen and Bijlsma, 2006; Wit et al., 2013) as well as within (Arking et al., 2002; Leroi et al., 1994a,b) selection regimes.

In nature, selection for increased longevity could be limited due to the constraints posed by trade-offs in suboptimal environments (Partridge and Barton, 1993; Stearns, 1989). These constraints might be less significant in a laboratory environment with ample food, limited competition and likely fewer incidences of adverse biological interactions. In line with suggestions of laboratory selection leading to correlated responses unlike those expected in nature (Harshman and Hoffmann, 2000; Houle, 1991), lines selected for late life reproduction in our laboratory (Bubliy and Loeschcke, 2005; Sarup et al., 2011) have previously been shown to perform similar, if not superior, in a range of traits when compared to control lines in a standardized laboratory environment (Wit et al., 2013). Others, too, find that lines selected for increased longevity show similar or increased performance in a number of correlated traits (Arking and Wells, 1990; Chippindale et al., 1994, 1997; Force et al., 1995; Service et al., 1985) The most notable exception to this trend is early life reproduction (Luckinbill et al., 1984; Service, 1989; Zwaan et al., 1995, but see Arking et al., 2002; Leroi et al., 1994a; Vermeulen

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and Bijlsma, 2006) while developmental time, body size and starvation resistance have occasionally been shown to be negatively affected by selection for increased life span, too (Buck et al., 2000; Force et al., 1995; Vermeulen et al., 2006).

Another factor influencing correlated responses in experiments with laboratory selection lines is experimental setup. Kristensen et al. (2008a) showed that costs as well as strong benefits associated with cold acclimation were evident in a field-release setup but not in the laboratory. Inbreeding effects have also been shown to be stronger in field- rather than laboratory studies (Kristensen et al., 2008b), while *D. melanogaster* with a genetically modified heat-stress response also show more positive responses when tested in the laboratory compared to thermal assays in the field (Sørensen et al., 2008). Thus, it appears from these studies that negative effects of genetic manipulation, inbreeding, or thermal stress might be compensated for in a laboratory setting, while testing in a natural environment uncovers detrimental effects. Also, assay environment influences performance in laboratory settings. This is highlighted by a strong genotype-by-environment interaction for early life reproduction in flies selected for late life reproduction (Leroi et al., 1994b). Overall, these studies indicate that extrapolating results from different rearing, selection and test environments requires caution, and testing under natural or semi-natural conditions might help in elucidating robust and ecologically relevant correlated responses.

Laboratory studies on correlated traits often use relatively young flies, typically less than a week old (Harshman et al., 1999; Kellermann et al., 2007; Kristensen et al., 2008a; Sørensen et al., 2008; Vermeulen et al., 2005). When studying ageing, performance later in life can also give information about why some flies live longer than others. It has been shown that longevity selected lines keep performing better in behavioural traits as they age compared to control lines, i.e., their rate of functional senescence has decreased (Arking and Wells, 1990; Graves et al., 1988; Wit et al., 2013). Other studies, in non-selected lines, show a steady decline in performance over time for multiple types of stress resistance (Piazza et al., 2009; Sørensen and Loeschcke, 2002; Stratman and Markow, 1998, but see Magwere et al., 2006). Generally, the younger the fly, the better it performs in these traits (but see Service, 1987; Service et al., 1985).

To test if the fitness superiority of a set of longevity selected lines (Bubliy and Loeschcke, 2005) as assessed in Wit et al. (2013) holds in the field, a release–capture study was undertaken and the ability of flies to locate, and reach, food in an otherwise resource-free environment was tested. Further, the effect of temperature and age on this ability in control lines was assessed, as well as the performance over time of control versus longevity selected lines of *D. melanogaster* (Bubliy and Loeschcke, 2005). Based on laboratory results both control and longevity selected lines are hypothesized to have a lower performance as they age, with a less pronounced decline in the longevity selected lines. Longevity selected lines are expected to start out at similar, if not better performance than the control lines based on previous studies in the laboratory of e.g., stress resistance, and a stressful environment is hypothesized to enhance the detrimental effects of ageing.

2. Materials and methods

2.1. Maintenance and origin of experimental flies

Two sets of *D. melanogaster* lines were used for this study; control (C) and longevity selected (LS) lines. The longevity selected lines, LS1, LS2 and LS4, had been selected for increased mated longevity as described by Bubliy and Loeschcke (2005) for 52 generations, while the control lines, C1, C2 and C4, were established simultaneously and kept at a standard maintenance regime with a generation time of about two weeks. Both types of lines have been kept at 25 °C, 12:12 dark: light on a yeast–sugar–oatmeal–agar (Leeds) medium. For the study on the effects of ageing at low and high temperatures C1 was used. To assay the effect of longevity selection on capture patterns all 6 lines (C1, C2, C4, LS1, LS2, and LS4) were used. Two generations prior to the experiment the lines were transferred from 25 °C to 20 °C.

Experimental flies for the release assays were reared under standard laboratory conditions of 20 °C, Leeds medium and 12:12 dark:light, in 200 ml culture bottles. To control density ~10 parental pairs per bottle were allowed to lay eggs for 24 h. After hatching the flies were kept in 300 ml bottles at a density of 200 flies and tipped 3 times weekly until the experiment was performed. Flies used for the study on the effect of temperature on capture rates were 2, 5, 10 and, for releases at low temperature only, 15 days old. For the study on the effect of longevity selection flies were 5, 10 and 15 days old. The ages were chosen such that the time frame would allow for age to affect behaviour. Another reason for the age classes chosen was that natural life span estimates vary from a few days to a few weeks (Rosewell and Shorrocks, 1987; Turelli and Hoffmann, 1995), thus 2 to 15 days are ecologically relevant ages.

2.2. Longevity assay

The longevity assay was based on the setup used by Bubliy and Loeschcke (2005) and Wit et al. (2013). In short, experimental flies for the longevity assay were reared at a controlled density of ~40 larvae per vial with 7 ml Leeds medium at 20 °C and 12:12 dark:light. Eclosing flies were collected during an 8-hour window. One day after collecting they were sexed while sedated using CO_2 anaesthetics 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. Field release — effects of ageing at low and high temperatures

The general setup of the field release–capture is described in Kristensen et al. (2008b). The releases at high and low temperatures were performed on different days. Three release sites were set up under similar conditions in open woodland for each temperature. These releases will be referred to as Low 1, 2 and 3 and High 1, 2 and 3 for the releases at low and high temperatures, respectively (Table 1). Flies were transported to the release site at a density of 200 flies in 200 ml bottles. Temperature in the car during transportation was kept at 20 ± 1 °C.

Capture points were set up in a straight line from the release site in two directions every 5 m until 25 m, each consisting of 1.5 l buckets with yeasted mashed banana. Perpendicular to the direction in which the buckets were placed, two more buckets were placed 3 m from the original bucket on either side. At the release site, in the shade,

Table 1
Summary of the number of flies released (Released (#)) and percentage caught (Captured (%)) per release as well as temperatures during the releases. R = release. Selection = releases with control and longevity selected lines. High and Low = releases with control line at high and low temperatures, respectively. Rel = temperature at time of release.

R	Released (#)	Captured (%)	Temperature (°C)			
			Rel	Max	Min	Mean
Selection 1	12,000	30.7	22	26	17	22.6
Selection 2	12,000	16.5	23	24	16	20.1
Selection 3	12,000	15.4	23	26	16	22.5
Selection 4	12,000	7.9	20	25	16	21.6
Selection 5	12,000	31.1	20	26	16	20.9
Selection 6	12,000	21.5	20	25	17	21.0
High 1	9000	6.8	28	28	27	27.3
High 2	9000	24.3	28	28	26	26.8
High 3	9000	9.6	26.5	28	26	26.8
Low 1	12,000	10.8	14.5	18	15	16.7
Low 2	12,000	5.6	15	17	16	16.3
Low 3	12,000	11.7	15	19	15	17.6