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Changes in dopamine levels and locomotor activity in response to selection on virgin lifespan in *Drosophila melanogaster*

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Abstract

Among various other mechanisms, genetic differences in the production of reactive oxygen species are thought to underlie genetic variation for longevity. Here we report on possible changes in ROS production related processes in response to selection for divergent virgin lifespan in *Drosophila*. The selection lines were observed to differ significantly in dopamine levels and melanin pigmentation, which is associated with dopamine levels at eclosion. These findings confirm that variation in dopamine levels is associated with genetic variation for longevity. Dopamine has previously been implied in ROS production and in the occurrence of age-related neurodegenerative diseases. In addition, we propose a possible proximate mechanism by which dopamine levels affect longevity in *Drosophila*: We tested if increased dopamine levels were associated with a ''rate-of-living'' syndrome of increased activity and respiration levels, thus aggravating the level of oxidative stress. Findings on locomotor activity and oxygen consumption of short-lived flies were in line with expectations. However, the relation is not straightforward, as flies of the long-lived lines did not show any consistent differences in pigmentation or dopamine levels with respect to the control lines. Moreover, long-lived flies also had increased locomotor activity, but showed no consistent differences in respiration rate. This strongly suggests that the response for increased and decreased lifespan may be obtained by different mechanisms.

Keywords: *Drosophila melanogaster*; Lifespan; Artificial selection; Dopamine; Locomotor activity; Respiration rate

1. Introduction

Research on genetic variation for longevity provides useful insight into the ageing process. However, partly due to the fact that multiple mechanisms influence the trait, it has proven difficult to disentangle the complexity of the genetics of lifespan. Research on the genetic determination of lifespan can be greatly aided by considering ageing processes in model organisms, such as *Caenorhabditis elegans*, *Mus musculus* or *Drosophila melanogaster*. For example, in *Drosophila* several lines have been established that have been selected for increased age at reproduction (Rose and Charlesworth, 1981; Luckinbill et al., 1984; Partridge et al., 1999) or divergent virgin lifespan (Zwaan et al., 1995; Vermeulen and Bijlsma, 2006). These lines facilitate the study on proximate mechanisms of ageing because they allow assessment of genetic correlations in relevant life-history traits and physiological characters. The research described in this paper focuses on a unique set of selection lines of *D. melanogaster*, which has been successfully selected for divergent virgin lifespan and in the end differed on average 3 weeks in lifespan (Zwaan et al., 1995; Vermeulen and Bijlsma, 2006). Lines selected both for increased as well as decreased lifespan were established, which is an advantage of selection on virgin lifespan, and allows analysis of the full range of genetic variation for lifespan in *Drosophila*, this in contrast to selection for increased age at reproduction.

Several lines of evidence suggest resistance to oxidative damage to be a main determinant of the rate of ageing (Sohal et al., 2002; Bokov et al., 2004) and a number of mechanisms have been proposed that can affect the amount of oxidative damage sustained (Finkel and Holbrook, 2000). Some of these

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are mediated by dopamine levels and share common features with neurodegenerative diseases in man, such as Alzheimer's or Parkinson's disease (Troulinaki and Tavernakis, 2005). Although no causal link between dopamine metabolism and ageing has been established yet, there are several lines of evidence that make this an interesting candidate mechanism. For example, it has been shown that standing genetic variation at the Dopa decarboxylase (*Ddc*) locus, which codes for the enzyme that catalyses the final step in the synthesis of dopamine and serotonin, cosegregates with variation for longevity in *D. melanogaster* (De Luca et al., 2003). This suggests that variation in lifespan is associated with variation in the levels of dopamine. Furthermore, dopamine metabolism is known to affect longevity in humans, as it is involved in age-specific neurodegenerative diseases, e.g. Parkinson's disease (Zecca et al., 2003). There are two possible mechanisms in which dopamine may affect longevity, which are graphically depicted in Fig. 1. Dopamine metabolism may directly affect longevity, since it has been proposed to be a major source of reactive oxygen species (ROS) (Blum et al., 2001) and seems to exert its oxidative damage particularly in the central nervous system (CNS). In addition, there are several lines of evidence that dopamine levels can also indirectly affect longevity by modulating locomotor activity. This is due to the fact that dopamine serves as a neurotransmitter and neurohormone, and thus controls many aspects of behaviour, such as locomotor activity levels (Zhou and Palmiter, 1995; Yellman et al., 1997; Pendleton et al., 2000, 2002). Since energy requirements influence the metabolic rate, the level of activity also is an important determinant of the production of ROS, and thus possibly of longevity (Sohal et al., 1993; Yan and Sohal, 2000). This scenario involves a syndrome including altered dopamine, activity and respiration levels.

In this study, we assessed the possible involvement of dopamine physiology in lifespan determination by looking for a correlated response in dopamine levels in lines artificially selected for divergent lifespan, and attempted to uncover the mechanism by which this affects longevity. In order to establish this association of dopamine levels with genetic variation for lifespan, we assessed differences between selection lines in dopamine levels directly and also in pigmentation intensity. The motivation behind this is that in *Drosophila*, pigmentation is caused by the accumulation of cuticular melanin shortly after eclosion, which is derived from polymerisation of the quinone products of dopamine and L-DOPA (Wright, 1987; Wittkopp et al., 2002). If lines selected on lifespan experienced changes in their whole-body dopamine levels, changes in pigmentation intensity are also expected. Furthermore, in order to test if changes in dopamine levels elicited a syndrome with altered activity levels and metabolic rate, correlated responses in locomotor activity and oxygen consumption were assessed. Results of lines selected for increased and decreased lifespan showed significant differences in all characters investigated, but support for the hypothesis that dopamine affects longevity by modulating locomotor activity and respiration rate was only found in short-lived lines. These findings support the notion that the selection response for increased and decreased lifespan is accomplished by different physiological mechanisms.

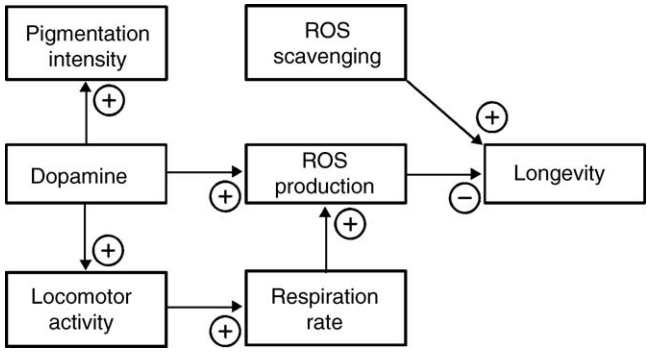

Fig. 1. Graphical depiction of the relationship between the characters assessed in this study and their possible relevance to longevity. Since dopamine is the precursor of melanin, dopamine levels are expected to be positively related to pigmentation. In addition dopamine levels can affect longevity by two different pathways. First, dopamine metabolism is a known source of reactive oxygen species (ROS) in the central nervous system, which has a negative effect on lifespan. Further, dopamine may modulate respiration rate through control of locomotor activity. This may also serve as a source of ROS. Finally, longevity is affected by the balance between ROS production and scavenging, which have antagonistic effects on survival.

2. Materials and methods

2.1. Stocks

Two sets of *D. melanogaster* lines selected for divergent virgin lifespan were used, denoted the primary and the derived set. Each set consisted of two replicated lines selected for either short (S) or long (L) lifespan and two control (C) lines. The primary set was the original set of selection lines, established by Zwaan et al. (1995). These lines were denoted S_1, S_2, L_1, L_2, C_1 and C_2 and each underwent six generations of longevity selection (see Zwaan et al., 1995 for procedure). The derived set was established from this primary set. After nine years of relaxation the lines still showed a considerable response. To possibly boost the differences in lifespan of short-lived and long-lived flies, the replicate lines within a selection regime of the primary set were crossed and given five generations to recombine, followed by five additional generations of selection. This procedure resulted in a new set of lines denoted S_A, S_B, L_A, L_B, C_A and C_B, with increased differences in lifespan between selection regimes. Details on the selection procedure and responses of those lines can be found in Vermeulen and Bijlsma (2006). Summarising, each selection procedure consisted of a design of family selection, whereby half of the family members was kept at 15 °C and the other half was tested for longevity at 29 °C. Full sibs of flies exhibiting the most extreme (both short and long) longevity phenotype at 29 °C were used to found the next generation. After establishment, lines were cultured in uncrowded quarter-pint bottles (30 ml standard medium: 26 g dead yeast, 54 g sugar, 17 g agar and 13 ml nipagine solution per litre). Selection was successful in both episodes of selection and resulted in pronounced differences in longevity between selection lines and their controls. At the most recent determination of lifespan, shortly after the last round of selection, S-lines had an average decrease in lifespan of 24% in both sets with respect to their control lines (sexes pooled), and L-lines showed an increase of 16% in the primary and 25% in the derived set. Control lines were shown to have average longevity values typical of outbred lines derived from the G83 base stock (approx. 40–45 days at 25 °C).

2.2. Rearing of flies

Eggs were collected from overnight egg-laying sessions and collected into vials containing 9 ml of standard medium and ampicillin (100 mg/litre). Experimental flies were grown in non-crowding conditions (100 eggs per vial) at 25 °C. At eclosion, flies were collected as virgins, sexed and kept in single-sex