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Lipid profiles as indicators of functional senescence in the medfly

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ABSTRACT

Changes associated with the age-related decline of physiological functions, and their relation with mortality rates, are thoroughly being investigated in the aging research field. We used the Mediterranean fruit fly *Ceratitis capitata*, largely studied by biodemographers, as a model for functional senescence studies. The aim of our work was to find novel combinatorial indicators able to reflect the functional state of adult insects, regardless of chronological age. We studied the profiles of neutral and polar lipids of head, thorax and abdomen of standard populations kept at 23 °C, at different ages. Lipid classes were separated by thin layer chromatography, and the quantitative values were used to find patterns of change using a multivariate principal component analysis approach. The lipid-dependent principal components obtained correlated with age, and differences between sexes were consistent with differences in the shape of the survival curves and the mortality parameters. These same components were able to discriminate populations with a behavioral decline due to a mild 28 °C thermal stress. Thus, young populations at 28 °C showed similar lipid profiles than old populations at 23 °C. The results indicated that the lipid-dependent components reflect the functional state of the flies, and so were named functional state components (FSCs). It is proposed that FSCs may be used as functional senescence indicators.

1. Introduction

Aging is a combination of external and internal factors acting upon the genetic background of an individual within a population, thus contributing to the overall senescence parameters of that population (Burger and Promislow, 2006; Kenyon, 2010). The aging process implies a progressive deterioration of the capacity to maintain homeostasis and, consequently, the physiological functions (Toroser et al., 2007). In the last years, the study of functional senescence, the intrinsic age-related decline in functional status, was able to identify key organ systems that fail with age, some of which might be directly involved in mortality (Grotewiel et al., 2005). Transcriptome analysis of *Drosophila melanogaster* using different body parts suggested that muscles might be particularly sensitive to aging (Girardot et al., 2006). Other works in *Drosophila* correlated behaviors that undergo age-related decline, such as locomotor activity, geotaxis, circadian rhythms and cognitive functions (Simon et al., 2006; Iliadi and Boulianne, 2010) with molecular-genetic determinants. For example, it has been proposed that functional senescence and age-dependent mortality are influenced by β-integrins (Goddeeris et al., 2003), whereas changes in dopamine levels have been associated with longevity and locomotor activity (Vermeulen et al., 2006). In many cases, traditional aging biomarker studies lack of a demographic and behavioral description of the populations used. Moreover, only one or few molecular parameters are eventually correlated with behavioral data.

The Mediterranean fruit fly *Ceratitis capitata* is a global orchard pest of great economic impact and one of the most studied species by biodemographers (Carey, 2011; Vaupel et al., 1998). However, most of the laboratory research in aging mechanisms has focused on genetically well-known organisms such as *D. melanogaster* (Helfand and Rogina, 2003) and *Caenorhabditis elegans* (Antebi, 2007). Pioneering studies in *C. capitata* described changes in main lipid classes during larval and adult stages (Madariaga et al., 1970). More recently, total lipid contents were shown to oscillate during *C. capitata* adult life, suggesting an endogenous regulation to maintain energetic balance (Nestel et al., 2005). In mosquitoes, cuticular lipids have been correlated with age and survival (Hugo et al., 2006). Age-dependent changes in dolichol levels in *Drosophila* showed interesting, though contradictory, results (Morris and Pullarkat, 1991; Parentini et al., 2005). These studies show the need to extend the analysis of changes in the overall lipid categories with age. Our aim was to condensate the quantitative changes in main categories of lipids during the adult life cycle in as few variables as possible. This was achieved using the multivariate approach called principal components analysis (PCA), that transforms data of correlated original variables into a smaller number of linear combinations called principal components (PCs) (Quinn and Keough,

Abbreviations: FSC, functional state component; PC, principal component; PCA, principal components analysis; TLC, thin layer chromatography.

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2002). In this analysis, most of the variation in the original variables is accounted for by the estimated first few new components (the PCs).

Several authors studied changes in mortality trajectories of fly populations subjected to environmental alterations, such as UV irradiation, hypergravity (Le Bourg, 1999), starvation, caloric restriction, density (Carey et al., 1995a; Gaskin et al., 2002), cold or heat stress (Helfand and Rogina, 2003), etc. Among environmental stressors, temperature has been associated with several traits such as longevity, body size, fecundity and fertility in *Drosophila* (Partridge et al., 1995; Norry and Loeschcke, 2002; Sisodia and Singh, 2002). *Drosophila* strains adapted to a given thermal regime showed lower longevity when reared under a different temperature (Partridge et al., 1995). Similar studies in *C. capitata* have shown a decrease in longevity when increasing the temperature from 25 to 30 °C (Shoukry and Hafez, 1979), and considerable differences in mortality have been reported among populations adapted to habitats with different mean temperature ranges and annual precipitations (Diamantidis et al., 2009).

In the present work we hypothesize that membrane and storage lipids reflect the functional state of an insect, and that they can be analyzed in a combinatorial way to statistically build novel indicators able to provide a grading score system to evaluate the age- and stress-dependent degree of senescence.

2. Materials and methods

2.1. Chemicals

1,2-dipalmitoyl-sn-glycerol and monomyristin were from Echelon Biosciences Inc. (Salt Lake City, UT, USA). Oleic acid, stearyl arachidate, methyl palmitate, cholesteryl palmitate, squalene, cardiolipin, 1-(3-*sn*-phosphatidyl)-*rac*-glycerol, L-α-phosphatidylcholine, L-α-phosphatidylinositol, L-α-phosphatidyl-L-serine, L-α-phosphatidylethanolamine, L-α-lisophosphatidylcholine, glyceryl trioleate and cholesterol were from Sigma-Aldrich Co. (St. Louis, MO, USA). Dolichol was from Avanti Polar Lipids Inc. (Alabaster, AL, USA). All solvents used were HPLC grade.

2.2. Insects

Standard populations of wild-type *C. capitata* (strain "Mendoza") larvae were reared in pumpkin-based medium (Pujol-Lereis et al., 2006). Flies were kept in a Conviron chamber CMP 3244, at 23 °C, 50–60% relative humidity, with a photoperiod of 16:8 light:dark. In all the experiments, adult flies less than 12 h old were collected, sexed under CO_2 and placed in flasks with free access to sucrose:dry yeast (3:1) and 1% agar as sources of food and water, respectively. Food and water were renewed every five days.

2.3. Lifespan assays

At the day of emergence (considered day 1), groups of 100 virgin flies, representing one laboratory population, were placed in 3.75 L flasks and maintained at 23 or 28 °C. Three replicas per sex were performed. Dead flies were counted and removed each day. We used the WinModest Program (Pletcher, 1999) to determine the mortality model that best fit the data, and to estimate the mortality parameters (see Section 2.7).

2.4. Spontaneous distribution of flies

We studied the spontaneous distribution of adult flies kept at 23 or 28 °C as a measure of their dispersal activity. Upper, middle and lower equal sections were marked in 0.5 L flasks (18 cm height) containing 40 flies (three replicas per sex). Flasks were placed at room temperature (21 °C) one hour before recording. The number of flies in each section of the flasks was counted at 10 AM of the days 2, 5, 10, 15, 20 and 30. We analyzed the age-dependent change in the proportion of flies in the lower section.

2.5. Negative geotaxis

Rapid iterative negative geotaxis (RING) assays were performed in our laboratory according to Gargano et al. (2005), and adapted to medfly. Flies of different ages (5, 15 and 30 days old) kept at 23 °C or 28 °C were tested to evaluate their ability to respond to a mechanical stimulus. Flies were placed in 0.5 L flasks, 40 individuals per flask and three flasks per sex. Ten hours before the trials, 10 flies per experimental unit were collected under CO_2 anesthesia, transferred to a 0.25 L test tube (28.5 cm height and 3.5 cm diameter) with 1% agar saturated with sucrose, and kept at room temperature overnight. This was done to let the flies recover from anesthesia. The trials were always started at 9 AM under the same illumination, at room temperature. Flies were forced to the bottom of the test tube by gentle tapping. As a consequence, the flies climbed up the side of the tube, and their position 10 s later was recorded with a digital camera (Sony DSC-W100). This was repeated 8 times for each sample, with

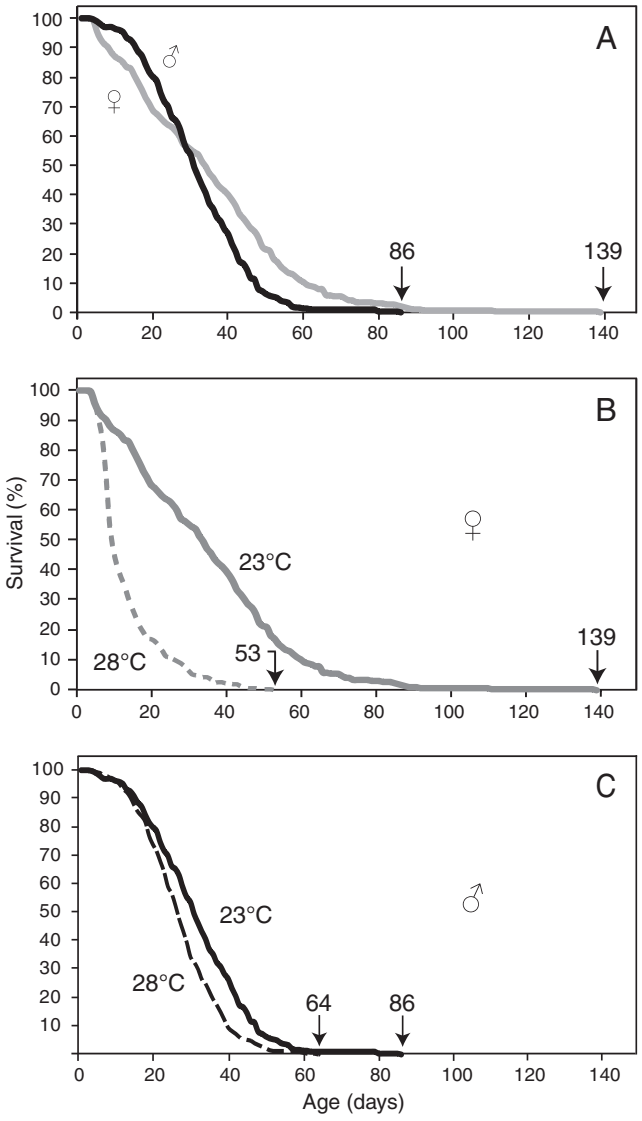

Fig. 1. Survival curves of *C. capitata* laboratory populations. (A) Females (gray) and males (black) at 23 °C. (B) Females under 23 °C (gray lines) and 28 °C (gray dashed lines). (C) Males under 23 °C (black lines) and 28 °C (black dashed lines). Arrows indicate maximum lifespan.