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Phospholipid fatty acid composition linking larval-density to lifespan of adult *Drosophila melanogaster*

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ABSTRACT

Pre-adult density-associated alterations in the composition of storage lipids may affect the cell membrane fatty acid profile (mainly phospholipids), membrane integrity, and cell function. The present study evaluated the impact of pre-adult density conditions, sex, and the selection regime on the composition of phospholipid fatty acids and lifespan of *Drosophila melanogaster*. The phospholipid profile of adult flies developed under larval crowding contained a higher proportion of polyunsaturated fatty acids, lower proportion of monounsaturated fatty acids, and greater risk of peroxidation. There was also a negative correlation between the peroxidation index (PI) and longevity. The longevity-selected females showed a lower PI compared with control lines under both densities. The present results indicate that pre-adult density may play a significant role in the lifespan of adult flies by altering the composition of phospholipids and shaping cell membrane bilayers with different susceptibilities to peroxidation.

1. Introduction

Environmental stressors may have positive effects on animals' lifespan by improving the functional ability of regulatory pathways and biological systems (Hoffmann and Parsons, 1991). The influence of any environmental stimulus on lifespan relies on its pattern of occurrence (Vermeulen and Loeschcke, 2007). Usually exposure of organisms to a mildly stressful environment has considerable impacts on fitness components and lifespan (Rattan, 2004; Rattan, 2005). One extensively studied example is the effects of suboptimal quantities and/or qualities of food without malnutrition on lifespan.

Extension of lifespan by nutritional stress was first observed in rodents (McCay et al., 1935) and thereafter in various model organisms, from yeast to mammals (Sohal et al., 2009; Fontana et al., 2010; Metaxakis and Partridge, 2013; Lan et al., 2015). The lifespan extension in response to caloric restriction or nutrient availability, is supporting the correlation between nutritional status and fitness-related traits (Merry, 2002; Magwere et al., 2004; Lee et al., 2008; Piper and Bartke, 2008; Sohal et al., 2009). This correlation is most apparent in holometabolous insects due to the tight association between their developmental stages (Borash et al., 2000). In these organisms the presence of a non-feeding pupal stage during development and the presence of larval-accumulated metabolites in newly eclosed flies highlight the role of larval accumulated resources on shaping the pattern of energy allocation and physiological properties during the adult stage (Aguila et al., 2007; Boggs, 2009; Aboagye-Antwi and Tripet, 2010). Therefore, even a mild variation in larval energy acquisition may have harmful or desirable effects on the physiological performance of adult flies.

The impact of pre-adult density on adult lifespan is a controversial issue due to inconsistent observations. Extended longevity in response to maintenance under a high larval density was reported in previous studies on *Drosophila* (Miller and Thomas, 1958; Lints and Lints, 1969; Barker and Podger, 1970; Zwaan et al., 1991; Sorensen and Loeschcke, 2001); although, these findings contradict other reports (Borash and Ho, 2001; Baldal et al., 2005). This discrepancy results from differences in the experimental approaches used to assess the influence of larval density on lifespan.

High-larval-density consequences may arise from food deprivation and/or high concentrations of toxic waste metabolites, such as ammonia (Mueller, 1997; Borash et al., 2000; Sorensen and Loeschcke, 2001). Borash et al. (1998), in a study on *Drosophila melanogaster*, observed that density-dependent natural selection shapes two different phenotypes in response to larval crowding. Early eclosed flies from crowded cultures had a high larval feeding rate and low egg-to-adult viability

Abbreviations: ACL, average chain length; C, control population; FAMEs, fatty acid methyl esters; L, longevity-selected population; PC, principal component; PCA, principal component analysis; PI, peroxidation index; ΣMUFAs, sum of monounsaturated fatty acids; ΣPUFAs, sum of polyunsaturated fatty acids; ΣSFAs, sum of saturated fatty acids; U/S, ratio of unsaturated to saturated fatty acids.

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Table 1
Results from the proportional hazard analysis.

Proportional hazard analysis			
	d.f.	χ^2	*P*
Selection	1	2230.04	<.0001
Sex	1	675.08	<.0001
Density	1	316.04	<.0001
Selection * sex	1	277.62	<.0001
Selection * density	1	250.08	<.0001
Sex * density	1	7.39	0.007
Selection * sex * density	1	41.83	<.0001
Biorep [selection]	4	90.92	<.0001

Biological replicates (biorep, fixed factor) were nested within selection regimes, followed by selection, sex, larval density (all as fixed factors), and their interactions.

while flies with a longer development time had a low larval feeding rate, and high egg-to-adult viability. These behavioral and physiological responses may improve the performance of a population to cope with nutritional stress and correlated damaging agents.

The larval efficiency at converting absorbed nutrients into storage metabolic substrates depends on the rearing environment (Santos et al., 1997). Density-related alterations in the amount of absorbed lipids by larvae and the related consequences on the storage lipid content of adult flies have previously been studied as a frequently proposed hypothesis (Zwaan et al., 1991; Borash and Ho, 2001; Baldal et al., 2005). Given the contribution of larval accumulated lipids in the formation of cell membrane lipid bilayers (Downer and Matthews, 1976), density-associated variation in the storage lipid composition/content may influence the physiochemical properties of cell membrane and optimal cell function (Spector and Yorek, 1985).

The present study aimed to address the impact of pre-adult density conditions in interaction with sex and the selection regime on the composition of the cell membrane phospholipid fatty acids and lifespan of *D. melanogaster*. The importance of cell membrane integrity in maximum lifespan is introduced under the "membrane pacemaker theory" of aging, which hypothesizes a tight association between the cell membrane's susceptibility to peroxidation and lifespan (Hulbert, 2005). Since larval crowding is a crucial component in natural populations of insects, the identification of density-associated mechanisms that preserve cellular homeostasis as an essential component of survival may provide new insights into the physiological pathways determining an insect's lifespan under semi-natural conditions (Imasheva and Bubliy, 2003).

2. Materials and methods

2.1. Stock population

The study was carried out on three replicates of longevity-selected (L) and control (C) lines of *D. melanogaster*. The origin of the population and the process of selection were previously described in detail (Bubliy and Loeschcke, 2005; Sarup et al., 2011). Briefly, a mass-bred population of *D. melanogaster* was initiated in September 2002 by mixing four geographically distinct populations (600–700 individuals from each population). After four generations, the L lines were selected for increased lifespan via gradual extension of generation time, resulting in a significantly longer lifespan compared with the C lines (≥1.5 times for both sexes). The flies were cultured on standard *Drosophila* oatmeal–agar–sugar–yeast medium seeded with live yeast under controlled densities. The stock population and experimental flies were developed in a climate-controlled room at 25 °C under a 12:12 light:dark cycle.

2.2. Larval density design

Prior to the experiment, both L and C lines were kept in a common garden environment for two generations to prevent maternal effects. During this step, the lines were maintained in 300 mL plastic bottles, with approximately 150 flies per bottle, 4 bottles per replicate, and 70 mL standard *Drosophila* medium supplemented with live yeast in each bottle. The offspring were allowed over a 3-h window to oviposit on small spoons filled with ~1 mL standard *Drosophila* medium seeded with live yeast. Eggs were collected at two exact densities including 10 (low density; LD: 70 vials per replicate) and 100 (high density; HD: 7 vials per replicate) eggs per vial and transferred into 35 mL plastic vials containing 7 mL standard *Drosophila* medium. In order for larvae and experimental flies to develop under equal nutritional conditions, live yeast was not added to medium from this step onwards. Within 24 h of the first eclosion, eclosed flies were collected, mixed, and distributed into 35 mL plastic vials (40 flies per vial) containing 3 mL standard *Drosophila* medium.

2.3. Longevity assay

At 3 days of age, flies were sorted into sexes under light CO_2 anesthesia. To evaluate the cost of mating on lifespan, the longevity of groups of 30 mated flies was assessed. Female and male flies were placed separately into 35 mL plastic vials (5–6 vials of each sex per replicate per density) containing 3 mL standard *Drosophila* medium without live yeast. The flies were transferred to new vials with fresh food every second day at 2 p.m. and dead flies were scored until all flies died.

2.4. Lipid characterization

2.4.1. Extraction

The 3-day-old flies were snap frozen in liquid N_2 and sorted into sexes in a cooled room (4 °C) to prevent oxidative damages to fatty acids. Prior to extraction, samples containing 10 female flies were assigned to three blocks, each comprising 24 samples (2 selection regimes × 2 sexes × 2 densities × 3 biological replicates). Phospholipid fatty acids were extracted from intact flies using a modified Bligh-Dyer single-phase method (Bligh and Dyer, 1959), with 2:1:1 (*v*/*v*/v) chloroform:methanol:phosphate buffer in two steps (Kates, 1986). Solid-phase extraction was performed using pre-packed silica columns (100 mg silicic acid; Isolute, Mid Glamorgan, UK) to isolate phospholipid fatty acids from other lipids with different polarities. The phospholipid fatty acid extract was eluted with 1.5 mL methanol and dried under a stream of N_2 before trans-methylation using the method of Dowling et al. (1986). Fatty acid methyl esters (FAMEs) were dissolved in 1.5 mL heptane and preserved at −18 °C for further analyses.

Table 2
Mean and maximum lifespans (mean ± s.e.) of males and females of longevity-selected and control lines at low (LD) and high (HD) density conditions.

	Longevity line				Control line			
	LD	HD	LD	HD	LD	HD	LD	HD
	Male		Female		Male		Female	
Mean of lifespan (day)	47.90 (0.50)^a	42.58 (0.69)^b	65.42(0.57)^c	66.67 (0.54)^c	36.21 (0.44)^c	27.51 (0.42)^d	40.37 (0.67)^e	28.16 (0.39)^d
Maximum of lifespan (day)	89	81	99	97	63	55	73	55
Number of samples	527	509	528	516	438	445	420	468

Different superscript letters show the significant differences ($P \leq 0.05$) between groups (low and high densities, longevity-selected and control lines, males and females).