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Social interactions regulate resource utilization in a Tephritidae fruit fly

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ABSTRACT

Previous studies have suggested that social interactions (e.g., the actions and reactions elicited by the interaction of co-specific individuals) induce individual fruit flies (Tephritidae) to ingest more food, especially protein-rich food. Changes in feeding behavior related to social interactions have been associated with reproduction (e.g., when different sexes are present), reproductive facilitation (e.g., when two females interact) and stress and aggression (e.g., flies of the same sex, or crowdedness). The present study investigated the effect of social interaction on the feeding, longevity and resource management of the Ethiopian fruit fly, *Dacus ciliatus*. Single flies and pairs of flies (of the same or different sexes) were confined to a small arena (the PUB system), in which we measured the amount of liquid food ingested daily by each fly. In addition, we sampled flies of different ages, extracted and quantified their lipid and protein contents, and related individual metabolic contents to the ingestion of a fructose and protein hydrolysate solution. Results showed that individual ingestion was significantly higher in flies maintained in pairs than in flies kept as solitary individuals. The highest intake rates were observed for the female–female pairs. In general, females ingested significantly greater volumes than males. Lipid contents tended to decrease progressively with age in flies kept as solitary individuals, especially in female flies, while lipid levels decreased and then increased in flies maintained in pairs. Protein trends were similar, although less pronounced than the patterns observed for the lipids. The flies kept as solitary individuals lived significantly longer than those kept in pairs. A resource-management analysis points to a decreased metabolic rate in flies kept as solitary individuals, as compared to paired flies. Results are discussed in view of theories of resource management and survival strategies.

1. Introduction

An organism's utilization of resources is modulated by both internal pre-established genetic mechanisms and environmental cues (Downer, 1981). Food-type and the pathways by which nutrients are metabolized are species-specific and genetically determined, while food-intake timing, selection of specific nutrients and activation or deactivation of biochemical pathways are regulated by the environmental conditions the organism encounters over its lifetime.

Resource acquisition and its effects upon reproduction, development and longevity have been extensively studied in Tephritidae ("true" fruit flies). Fruit flies' selection and consumption of food have been shown to be affected by life-cycle stage and age (Webster et al., 1979; Galun et al., 1981; Nestel et al., 1985; Landolt and Davis-Hernandez, 1993), physiological and nutritional state (Galun et al., 1981, 1985; Nestel et al., 1986; Cohen and Voet, 2002) and food quality and availability (Zucoloto, 1987; Cangussu and Zucoloto, 1992; Canato and Zucoloto, 1993; Fernandes-da-Silva and Zucoloto, 1993). Patterns of food acquisition have also been suggested to be of central importance in metabolic regulation (Nestel et al., 1985, 1986, 2004a; Jacome et al., 1995; Warburg and Yuval, 1996; Butov et al., 2003; Romanyukha et al., 2004; Levy et al., 2005; Nestel et al., 2005; Nestel and Nemny-Lavy, 2008), modulation of life-history strategies (Carey et al., 1998a, 2005), reproduction, senescence and longevity of fruit flies (Webster and Stoffolano, 1978; Blay and Yuval, 1997; Kaspi and Yuval, 2000; Aluja et al., 2001; Carey et al., 2002; Prabhu et al., 2008). However, the metabolic courses of nutrients and energy metabolites, and the environmental and physiological regulation of resource partitioning and allocation are poorly understood in this group of insects, and in other organisms as well. Enhanced understanding of these processes may shed light on fundamental processes of longevity, reproduction and senescence (Carey et al., 1998a; Novoseltev et al., 2004).

Diet has been shown to affect egg-laying patterns and longevity in fruit flies (Carey et al., 2002; Nestel et al., 2005), and it has been postulated that diet may be a key factor in the physiological

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response of an organism to its environment (Butov et al., 2003; Romanyukha et al., 2004). In this regard, Romanyukha et al. (2004) suggested that resource allocation and management in fruit flies, as observed during laboratory food manipulations, may explain differences in longevity and reproductive patterns. Based on the resource allocation model that they developed, Romanyukha et al. (2004) suggested that flies kept on a restricted diet consisting of only carbohydrates enter into a waiting mode, in which resources allocated for maintenance are systematically used up and replenished from reserves kept for future reproduction. This reproductive activity will occur when environmental conditions improve (e.g., when more protein becomes available), unless the fly dies before conditions become favorable for reproduction. Once a fly's resources have been depleted below a certain threshold level, it will die. If flies that are in a waiting-mode encounter protein-rich food, resources will be managed and reallocated to allow the flies to shift from a waiting mode to a reproductive mode, extending their life-span. In contrast, if protein-rich food is available early in adult life, resources will be allocated so that flies will tend to exploit their reproductive potential earlier in life, exhausting both maintenance and reproductive energy resources, so that these flies will die relatively young.

While the effect of food quantity and quality on longevity and reproduction has been extensively investigated, almost no information exists concerning the effects of social interactions (e.g., the actions and reactions elicited by the interaction of co-specific individuals) on the resource management, longevity and reproduction of fruit flies. Aluja et al. (2001) recently suggested that the simple presence of a male fruit fly may affect a female fly's food ingestion, even in the absence of any physical contact. The feeding behavior of these female flies will be significantly different from that observed when no other male or female fly is present in the environment surrounding an individual female fly. This hypothesis was tested further in *Anastrepha obliqua* fruit flies (Tephritidae) by Cresoni-Pereira and Zucoloto (2005), who found that not only the presence of males, but also their nutritional status (e.g., protein nourished), affected the protein intake of female flies. Moreover, Mangan (2003) showed that compulsory contact between male and female *A. ludens* during reproductive maturation influenced egg production more than the presence of the food itself. Social interactions have also been reported as beneficial in vinegar (*Drosophila*) flies: from an extended reviewing of studies, Lee et al. (2008) suggested than flies in cohabitation live longer than flies maintained singly. Additionally, Ruan and Wu (2008) showed that social interactions in a short-lived strain of *Drosophila* increased life-span, and improved stress resistance and motor ability.

The aim of the present study was to investigate the effects of social interactions (e.g., isolation vs. pairing) on the fruit fly's management of nutritional and energetic resources (e.g., food intake and metabolism). In order to achieve this, we conducted experiments using the PUB (Phagostimulation Unit Bioassay) system (Nestel et al., 2004b), which allowed us to measure daily individual net-intake of liquid food and to relate food intake to the age-dynamics of metabolic indicators (e.g., endogenous lipid and protein contents). The PUB system also allowed us to keep the flies in relatively small areas, restricting their locomotion. In this manner, we were able to investigate food and energy resource management in a low-energy expenditure scenario (in the case of isolated organisms), as well as the effects of social interactions, and probably stress, created by the pairing of flies (different types of pairing) in relatively small areas. The study was conducted with the Ethiopian fruit fly (*Dacus ciliatus*), which is an oligophagous fly that develops on cucurbits. Our study used only adult flies. We hypothesized that the presence of a second fly in the PUB and the sex of that second fly (e.g., same sex or different sex) would induce social interactions that would lead the flies to manage both their food intake and their energy resources differently. We also hypothesized that the type of social interaction, or lack of interaction, might affect longevity patterns and possibly metabolic regulation as well, shedding light on the relationships between reproduction, metabolism and survival.

2. Materials and methods

2.1. Source of insects

Adult flies (*D. ciliatus*) were obtained from a laboratory colony maintained in the quarantine facilities of the Plant Protection and Inspection Services of the Ministry of Agriculture, Israel. This colony had been reared on zucchini fruit for approximately 48 generations. Eggs are laid by adults on zucchini fruit, where larvae developed until pupation. Adult diet consists of a mixture of sucrose: yeast hydrolysate enzymatic (3:1) (MP Biomedicals, LLC) provided as a solid cake to the flies. Pupae for the experiment were transferred to an emerging cage with *ad libitum* access to water. Within the first 6 h after emergence, flies to be used in the study were individually relocated to the PUB system (“experimental arena”) and sorted for the different experiments.

2.2. Experimental arena: the PUB system

Flies were placed, individually or in pairs, within the PUB system (Nestel et al., 2004b), which functions as a small cage. The PUB system consists of an inverted 50-ml polypropylene plastic tube (Greiner Bio-one, Frickenhausen, Germany), in which an inverted cap of a 0.5-ml thermo-tube (ABgene, Survey, UK) is placed (see Nestel et al., 2004b for a schematic description). Ventilation was provided by creating holes in the plastic of the propylene tube. Humidity was increased by placing a wet cotton wick in the base of the 50-ml plastic tube. A set quantity of liquid food (usually 40 μl) is applied to the concave surface of the inner side of the thermo-tube, which serves as a “serving” cup. Due to the small size of the inverted thermo-tube vessel, flies have free access to the food through the extension of their proboscis, without sinking on the small pond of food solution. Food intake is measured after 24 h, or the desired time, by removing the solution remaining in the vessel with a micro-capillary tube and calculating the ingested volume, after subtraction of the evaporation control (evaporation is quantified by keeping control PUB arenas without flies). The food supply can then be replenished, and the fly feeding and activity can continue for another measuring period. When two flies were kept in the same PUB, we added a second cup. When two flies were kept together in the same PUB, we divided the total ingested volume equally between the two flies.

The food consisted of a solution of 5% fructose (Sigma) and 1% yeast hydrolysate enzymatic (MP Biomedicals, LLC). The composition of the food solution was determined following a preliminary study in large cages (20 cm × 20 cm × 20 cm), in which 100 flies were maintained on different solutions containing different proportions of the two nutrients (D. Nestel and T. Zur, unpublished results; Zur, 2008). The selection of the experimental diet was based on the previously observed results concerning longevity and egg production.

2.3. Experimental setting

Sets of PUB vials containing three types of social treatments were simultaneously managed on a single experiment. To increase sample size, we conducted two consecutive experiments. Social treatments included: (a) individual males or females; (b) different-sex pairs (a female and a male in the same PUB vial); and (c) same-

sex pairs (two males or two females in the same PUB vial). Several PUB vials were used for each social treatment, in each of the two separate experiments. For the individual-male treatment, a total of 72 units were used; for the individual-female treatment, a total of 72 units were used; for the different-sex pairing, a total of 40 units were used; and for the same-sex pairing, a total of 40 units were used for each of the two combinations (male with male and female with female). The amounts of food consumed in each of the PUB cages were recorded daily through the end of the experiment. In this way, we were able to obtain specific daily intake information for each one of the units and link it later to the lipid and protein contents of the specific resident fly, or pair of flies, at the time of sampling. We also registered mortality as a function of age. In the pairing treatments, as soon as one of the flies was found dead, the sex of the dead specimen was recorded and that PUB vial was removed from the experiment. We also sampled, without substitution, subsets of living flies at different ages, in order to analyze their lipid and protein contents, and relate their metabolite contents to the amount of calories that were ingested by the time of sacrifice. Sampling ages were as follows: for the flies kept as solitary individuals, we sampled 5 units (flies) per age at ages 3, 5, 7 and 11 days old. For the flies maintained in pairs, we sampled 3–4 units (pairs) per age, at ages 3, 6, 8 and 9 (sampling after age 9 was not possible due to the shorter life-spans of the paired flies, as compared to flies maintained individually). Sampling of pairs was adjusted while carrying out the experiment and slightly differed from the sampling ages of solitary flies. The adjustment throughout the experiment responded to the higher, and earlier, mortality encountered in pairs. Thus, in order to secure enough samples of paired flies at appropriate ages, we decided to hold sampling of pairs for an extra day (instead of age 5, age 6, and instead of age 7, age 8). For treatments in which flies were maintained individually, five flies per sampling date were processed for chemical analysis; while in the case of the different-sex pairs, we processed three flies per sex per sampling date. For the same-sex pairs, we processed 6–8 flies per sampling date. Teneral levels of lipids and proteins were estimated from 20 individual males and females sampled from the emergence cage at the time of the establishment of the experiments. These teneral levels were common to all treatments. We continued the experiments until the early-reproductive adult age of 10 days, when the number of running PUB vials was low due to mortality and sampling (under laboratory conditions, flies start reproducing at age 8–10 days and peak at around 15 days of age). PUB vials were kept inside a humidity chamber (100 cm × 100 cm × 50 cm) that was covered to keep the humidity level around 60% and the temperature at 26 °C.

2.4. Lipid and protein quantification

Protein and lipids were extracted from the same individual fly, following the procedure described by Yuval et al. (1998), with some modifications. After measuring wing length and determining fresh weight, flies were individually homogenized in 1 ml of phosphate buffered saline (PBS). Homogenates were centrifuged (10,000 rev min^{-1}) and an aliquot (25 μl) was reacted with 200 μl Bradford reagent (Bio-Rad Laboratories) for 10 min on an ELISA plate. The amount of protein was determined at 595 nm in an ELISA reader (EL311SX Bio-Tek), using bovine serum albumin (Sigma) as a reference. Lipids were extracted from a 100 μl aliquot of the homogenate and precipitate, which was dissolved in 900 μl of a chloroform: methanol (1:2) solution. After centrifugation, 250 μl were transferred to a new vial, dried on a heat-block, and reacted with 250 μl of H_2SO_4 at 95 °C for 10 min. Lipids were developed using the vanillin reagent (Yuval et al., 1998), and quantified at 530 nm using triolein (Sigma) as a standard.

2.5. Data analysis

The amount of solution consumed in each of the PUB vials hosting two flies was divided equally between the two flies. The cumulative and individual ingestion of pairs of flies thus refers to the average volume ingested by a fly in the PUB vial, without discerning the actual amount ingested by each one of the flies. For the PUB vials hosting only one fly, ingestion refers to the actual volume ingested by that single fly. Differences in the cumulative amounts of solution ingested by the flies in the different social treatments were investigated at age 7 days using a one-way ANOVA (Sokal and Rohlf, 1981). Means were separated using an LSD test ($P < 0.05$). Individual daily intake volumes as affected by social treatment are graphically displayed using event history diagrams (Carey et al., 1998b). Lipid and protein contents of individual flies were weighted by wing length (e.g., μg/mm). Differences in metabolite contents as affected by social treatment, age and the interaction between the two variables were inferred using general linear models (GLM; Stagraphics Plus 5, 2000). Means were separated using an LSD. Kaplan–Meier estimators of survival were calculated for each social treatment. Pair-wise comparisons were conducted using the log-rank (Mantel–Cox) test (Collet, 2003).

In order to graphically model the relationship between feeding ("caloric income") and the dynamics of metabolic reserves (lipids and proteins) in each of the social treatments, we converted solution intake into calories (approximate values based on the protein and fructose contents of the nutrient solution). The caloric intake derived from protein in the solution was estimated from the protein content of the yeast hydrolysate enzymatic (60%) and the caloric value of protein, which has been reported to be 4 kcal/g (Mair et al., 2005). Fructose caloric content was estimated as 4 kcal/g (Mair et al., 2005). Thus, cumulative caloric intake represents the amount eaten by a single fly throughout its stay in the PUB cage, until it was sampled for lipid and protein analyses. Levels of lipids and protein at the time of fly sampling were differentiated (e.g., Δ) by subtracting their respective values from the average teneral levels (initial group average levels of lipids and protein at the time of emergence). Thus, content levels exceeding the teneral level suggest the anabolism of metabolites, while content levels below the teneral level suggest the catabolism of metabolites.

3. Results

3.1. Effect of social interaction on food intake

Individuals in female–female pairs and individuals in female–male pairs ingested significantly more solution throughout the 7-day test periods than individuals in male–male couples, single females and single males ($F = 48.1$; d.f. 4, 95; $P < 0.01$; Fig. 1). Flies kept in the PUB as solitary individuals ingested significantly less solution than those kept in pairs (Fig. 1); males kept as solitary individuals ingested the smallest amounts of solution. The daily feeding patterns of individual flies maintained in the different social treatments are shown in Fig. 2. The event history chart (Carey et al., 1998b) shows that some flies in female–female pairs started ingesting relatively large volumes of solution as early as the first day of adult life. In this group, as well as in the other pair treatments, feeding was relatively intensive from day 4 through the rest of the fly's life. Few females maintained as solitary individuals showed patterns of intensive feeding during the first and second days. Most females displayed low–intermediate intake activity during these first days, as well as afterwards. Males kept as solitary individuals displayed relatively low levels of intake activity throughout the study.

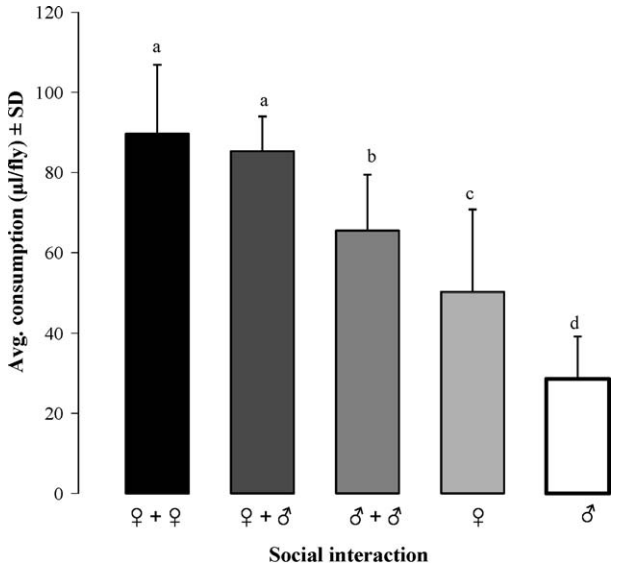

Fig. 1. Cumulative ingestion of food solution (5% fructose + 1% yeast hydrolysate enzymatic) until day 7 of adult life in *D. ciliatus* maintained in the PUB system. Social treatments include single flies and different pairings of flies.

3.2. Effect of social interaction on lipid and protein contents

Lipid contents were significantly affected by the social treatments ($F = 3.6$; d.f. 5, 217; $P < 0.01$) and by age ($F = 13.9$; d.f. 4, 217; $P < 0.01$). There was a significant interaction between social treatment and age ($F = 5.7$; d.f. 20, 217; $P < 0.01$), due to the different age-patterns in the lipid contents of the single flies, as compared to the paired flies. The lipid levels of single females (Fig. 3a) tended to progressively, although not significantly, decreased with age. Male's lipid levels (Fig. 3b) remained almost constant throughout the period of the study. In contrast, the lipid levels of females (Fig. 3a) and males (Fig. 3b) kept in pairs initially

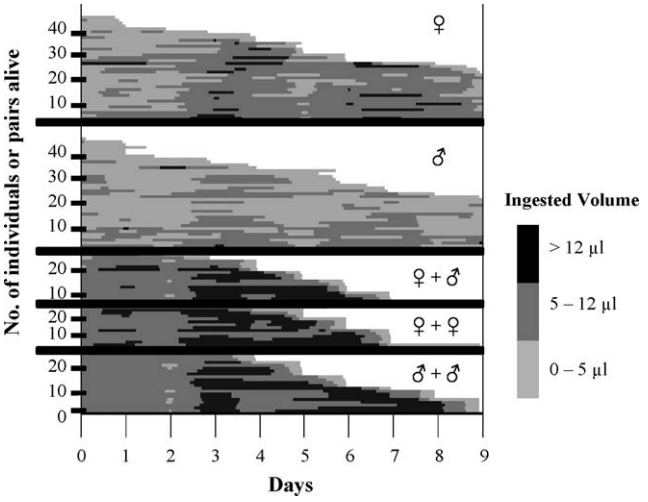

Fig. 2. Event history chart (Carey et al., 1998b) showing the range of daily food intake among individual *D. ciliatus* maintained in the PUB system throughout their adult lives. Intake is classified according to three different intensity levels. Social treatments included flies that were kept as individuals and flies kept in different types of pairings. The chart shows the data for all of the flies in the experiments. Horizontal lines describe single flies' food consumption throughout their lives. Flies are grouped by type of social treatment and are horizontally ordered within each group, from those flies that died early in the experiment to those that survived until the experiment was discontinued. Groups of flies are separated by horizontal lines and the type of social treatment is indicated by the sexual symbol or pair of symbols.

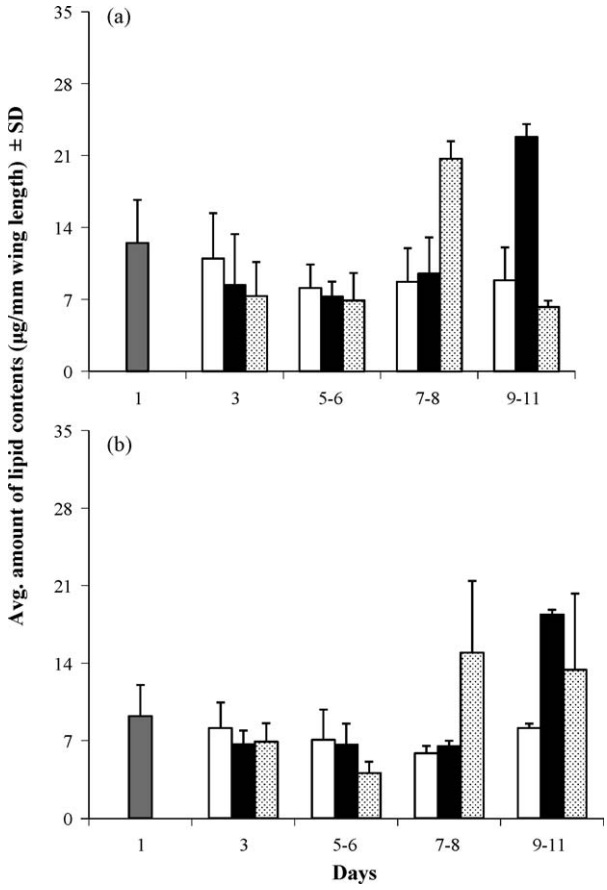

Fig. 3. Average levels of somatic lipids throughout the experimental period for female (a) and male (b) *D. ciliatus* adults maintained in the PUB system. The protein and lipid levels at day 1 are common to all of the treatment groups. White bars represent female or male flies kept as solitary individuals in the PUB, while black bars indicate the lipid levels of flies paired with flies of the opposite sex. The dotted bars indicate the lipid levels of flies kept in same-sex pairs.

decreased below the teneral levels, but then increased above teneral levels after the age of 5–6 days.

Protein contents were significantly affected by social treatment ($F = 3.0$; d.f. 5, 217; $P < 0.05$) and by age ($F = 12.8$; d.f. 4, 217; $P < 0.01$). The interaction between social treatment and age was not significant ($F = 1.4$; d.f. 20, 217; $P = 0.12$), suggesting parallel oscillations in protein levels over time. The protein levels of solitary females (Fig. 4a) and solitary males (Fig. 4b) decreased slightly below the teneral levels through day 5–6, and then increased back to the teneral levels by age 9–11 days. The protein levels of flies kept in pairs also decreased initially, but recovered to teneral levels after age 5–6 days. Both female–female pairs (Fig. 4a) and male–male pairs (Fig. 4b) showed a new decrease in protein levels after age 7–8 days. The changes in protein levels observed over time in paired flies were more dramatic than those observed in flies kept as solitary individuals.

3.3. Effect of social treatments on survival trends

Flies maintained as solitary individuals survived significantly longer than flies kept in pairs (log-rank test, $\chi^2 = 12.1$; d.f. 2; $P < 0.01$ for females and $\chi^2 = 8.1$; d.f. 2; $P < 0.05$ for males; Fig. 5). No significant differences were found between females and males kept as single individuals (log-rank test, $\chi^2 = 0.03$; d.f. 1; $P = 0.97$). Flies in male–male pairs survived significantly longer than those in female–female pairs (log-rank test, $\chi^2 = 2.1$; d.f. 1; $P < 0.05$). No differences in survival were observed between the males and

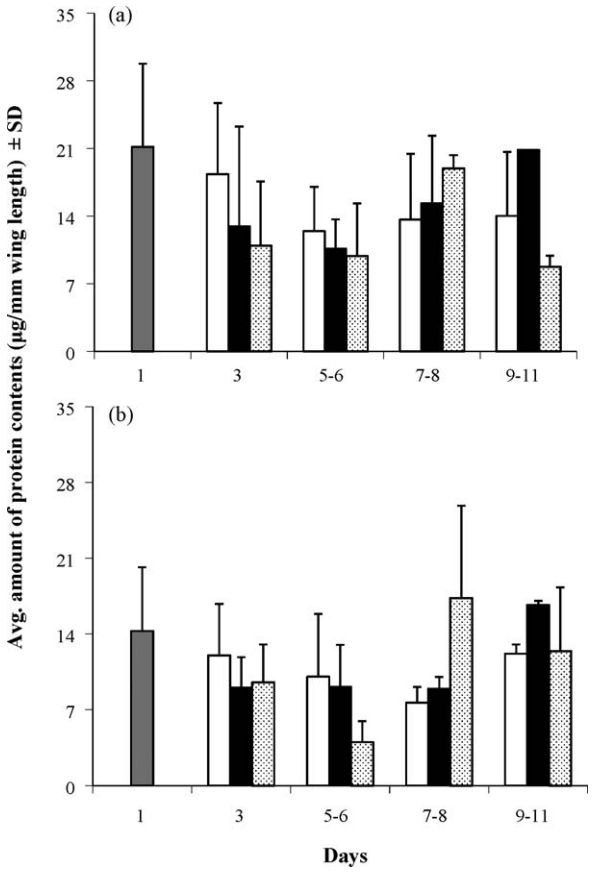

Fig. 4. Average levels of somatic protein throughout the experiment in female (a) and male (b) *D. ciliatus* adults maintained in the PUB system. The level at day 1 is common to all of the treatment groups. White bars stand for female or male flies kept as solitary individuals in the PUB. Black bars describe the levels of lipids in flies paired with flies of the opposite sex. The dotted bars indicate the levels of somatic protein in flies kept in same-sex pairs.

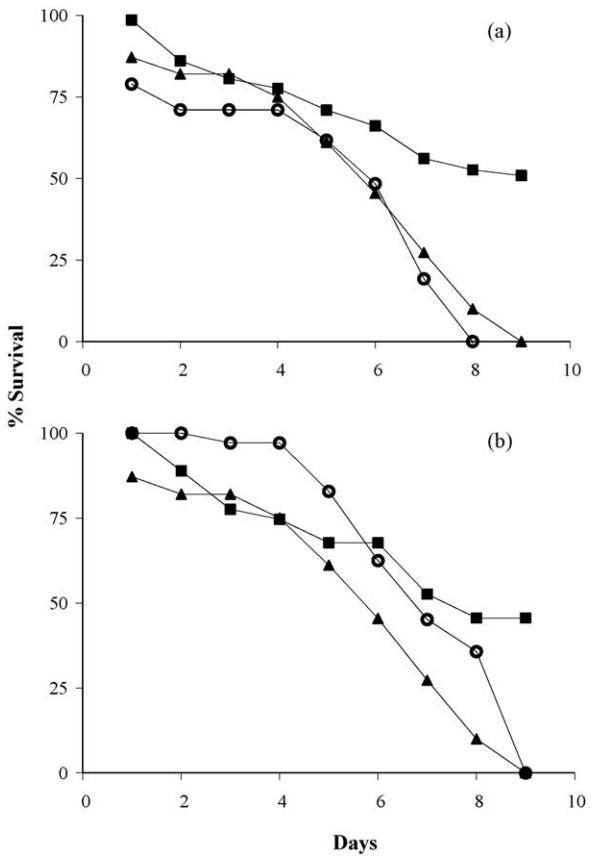

Fig. 5. Survival of female (a) and male (b) *D. ciliatus* adults maintained in the PUB system as affected by social treatment: single flies (■), flies in different-sex pairs (▲) and flies in same-sex pairs (○).

females maintained in male–female pairs (log-rank test, $\chi^2 = 1.2$; d.f. 1; $P = 0.20$).

3.4. Effect of social interaction on the management of resources

Female *D. ciliatus* maintained in the PUB system as individuals ingested a relatively small cumulative amount of calories over the study period, in contrast to flies maintained in pairs (less than 50 calories in 9–11 days; Fig. 6a–f). Similarly, male flies kept as single individuals ingested relatively small cumulative amounts of calories, as compared to both males kept in pairs and females maintained as single individuals (Fig. 7a–f). Female and male flies kept in pairs (either same-sex pairs or different-sex pairs) ingested larger cumulative amounts of calories over the study period (more than 100 calories in 9 days). Lipid and protein contents of both female and male flies kept as single individuals tended to decrease below the teneral level with age (Figs. 6a–b and 7a–b). In contrast, the lipid and protein contents of female and male flies maintained in pairs varied greatly by age and caloric intake (Figs. 6c–f and 7c–f). Lipid contents of female flies kept in same-sex or different-sex pairs decreased below teneral levels during the first days of adult life, and then increased afterwards (Fig. 6c and e). Similarly, male flies kept in pairs also showed a small decrease in lipid levels (below teneral levels) early on, followed by an increase in lipid content after 8 days (Fig. 7c and e). In both male and female flies kept in pairs, lipid anabolism and an increase in lipid content above teneral levels occurred after flies were able to ingest at least 50 calories of food. On the other hand, protein levels in females kept in pairs were below teneral levels throughout the study (Fig. 6d and f). In contrast, the protein contents of males kept in pairs showed a dynamic pattern, dropping below teneral levels early in life, and then increasing later on (Fig. 7d and f). In contrast to the other treatments, the lipid and protein levels in male–male couples showed peculiar short-term oscillations above and below the teneral levels (Fig. 7e and f).

4. Discussion

The presence of a second fly in the PUB system fundamentally affected the feeding and metabolic behavior of the Ethiopian fruit flies. The results suggest that flies kept alone in the PUB seem to adopt a reproductive waiting-mode condition (Carey et al., 1998a), in which energy income is significantly reduced, metabolism is slowed and longevity is extended. So, similar to the reported effect of protein-food availability on the reproductive and longevity strategies of Tephritidae (Carey et al., 1998a, 2002, 2005; Aluja et al., 2001; Butov et al., 2003; Romanyukha et al., 2004; Nestel et al., 2005), social interactions, or the lack thereof, seem to induce 'dual modes of aging' in *D. ciliatus*.

Previous studies have shown the effect of caged fly-density on reproduction and longevity. Aluja et al. (2001) observed that ovarian development in *Anastrepha* fruit flies was facilitated by the presence of a second female in the cage, thus strengthening the notion of social facilitation in fruit flies (Prokopy and Reynolds, 1998). Aluja et al. (2001) have also suggested that social facilitation may be indirectly linked to an increase in food consumption and/or may have a direct effect upon the endocrine system of the fly. The results of this study provide some evidence concerning the physiological mechanism underlying social facilitation. Although

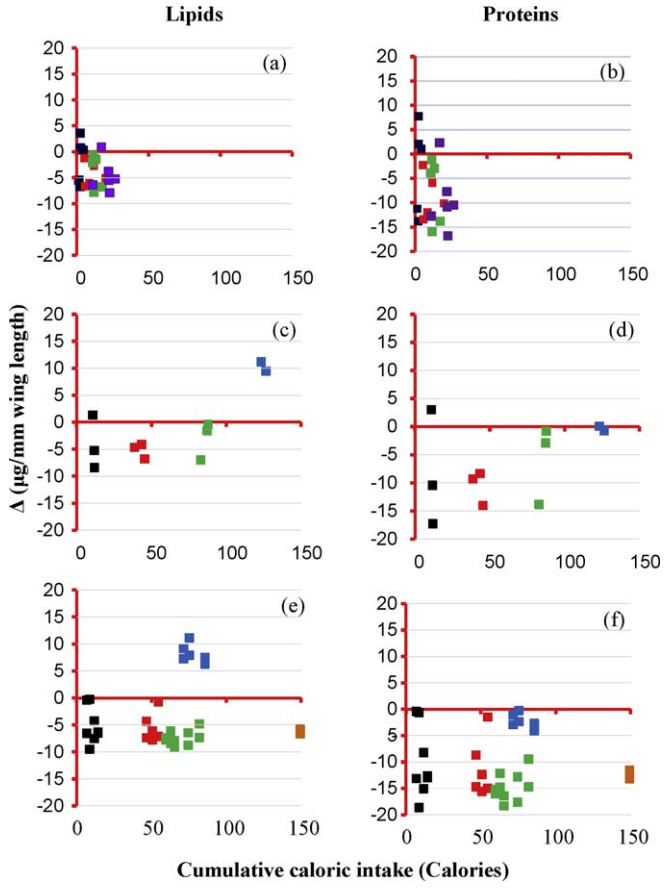

Fig. 6. Relationship between the cumulative amounts of food calories (fructose + protein) ingested by individual *D. ciliatus* females and the relative lipid and protein contents of females maintained in different social treatments in the PUB system: flies kept in solitude (a and b), kept in different-sex pairs (c and d) and kept in same-sex pairs (e and f). The *y*-axis describes the difference between the average metabolite contents at the time of emergence (calculated from a group of 20 flies) and the levels in the individual at the time of sampling. Thus, levels above zero indicate contents greater than the teneral level, while levels below zero indicate contents less than the average teneral level. Colored marks indicate the ages of the flies at the time of sampling: 3 days old (black), 6–5 days old (red), 7 days old (green), 8 days old (blue), 9 days old (orange) and 11 days old (purple). The flies sampled at age 11 days (purple color) had all been kept as individuals (paired flies died more quickly). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

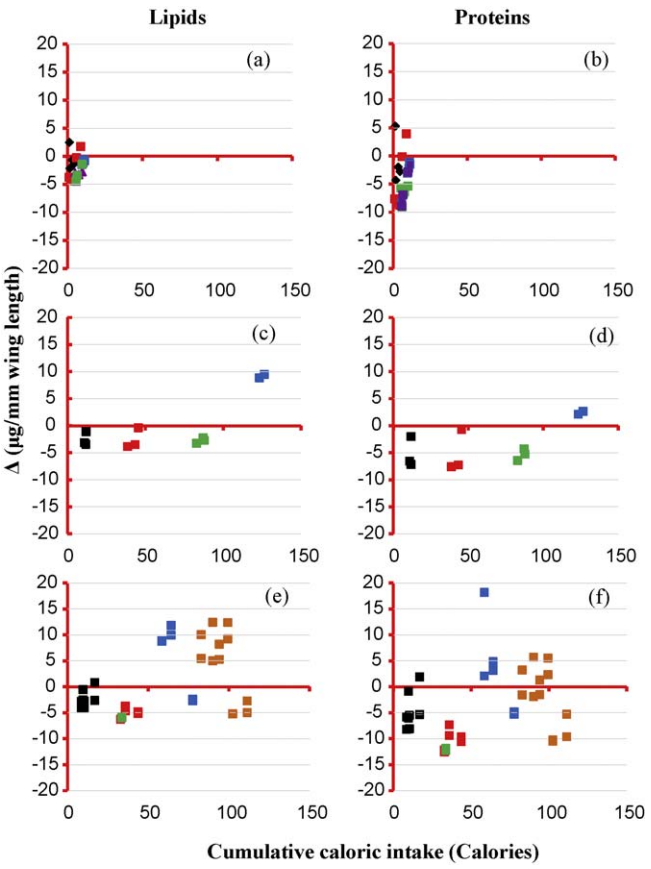

Fig. 7. Relationship between the cumulative amounts of food calories (fructose + protein) ingested by individual *D. ciliatus* males and the relative lipid and protein contents of males maintained in different social treatments in the PUB system: individuals (a and b), different-sex pairs (c and d) and same-sex pairs (e and f). The *y*-axis shows the difference between the average metabolite content at the time of emergence (calculated from a group of 20 flies) and the metabolite content of the individual at the time of sampling. Thus, levels above zero indicate contents exceeding the teneral level, while levels below zero indicate content levels below than the average teneral level. Colored marks indicate the ages of the flies at the time of sampling: 3 days old (black), 6–5 days old (red), 7 days old (green), 8 days old (blue), 9 days old (orange) and 11 days old (purple). The flies sampled at age 11 days (purple color) had all been kept as individuals (paired flies died more quickly). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

we did not follow egg-maturation or egg-laying patterns, this study clearly shows that flies reduce caloric income under solitary confinement and significantly increase their food intake when paired with another fly in the same PUB cage. Moreover, the study also shows clear signs of differential regulation of lipid and protein reserves in solitary flies, in contrast to that observed in paired flies. The fact that the increase in the amount of food consumed and the differences in metabolic regulation were observed in all of the types of pairings, regardless of the type of social interaction, is also noteworthy. Larger caloric intakes were consistently observed in PUB systems hosting females, while lower intakes were observed in those hosting males. Larger caloric intakes in PUB systems hosting a male and a female may be related to reproductive behavior and reproductive needs. The larger intakes observed in the female–female system may be related to 'social facilitation', or to stress and/or aggression created by the presence of two females within a restricted area (Papadopoulos et al., in press).

If solitary confinement did in fact induced a waiting-mode condition in *D. ciliatus*, our results may shed some light on the management of resources in relation to different reproductive and longevity strategies. In contrast to flies kept in pairs, solitary flies slowly consumed part of their reserves over the study period. On the other hand, paired flies expressed a recovery of lipids and protein levels already starting from age 5. Thus, a waiting mode in *D. ciliatus* appears to lead to a slow catabolism of reserves (at least of lipid reserves) and reduced caloric intake, which may have been responsible for the utilization of reserves without replenishment in this group of flies. In this sense, the patterns of resource utilization observed in this study may follow the resource allocation model proposed by Romanyukha et al. (2004). In this model, when flies are fed a restricted, carbohydrate-only diet, maintenance resources are progressively utilized until death occurs, leaving most of the resources targeted for reproduction (e.g., protein) intact. On the other hand, and reminiscent of the resource allocation model presented by Romanyukha et al. (2004) for flies fed protein, pairing induced an active "reproductive" or "stress" condition, in which resources for reproduction and maintenance were more actively utilized and replenished.

Caloric restriction has been linked to increased longevity in many organisms, including fruit flies (Butov et al., 2003; Lee et al., 2008). Our results concerning the caloric intakes of solitary flies

support this view. Caloric restriction has usually been referred to as the dietary restriction of protein food. Thus, organisms that eat both protein and carbohydrates have been shown to die more quickly than organisms that are fed only carbohydrates, probably as a result of a trade-off between longevity and reproduction (Carey et al., 1998a). In this study, we did not restrict protein intake, but the solitary flies significantly "self-restricted" their caloric intake by reducing their food intakes. This self-restriction of caloric intake resulted in a significant increase in longevity. The ability of individual *Drosophila* flies to regulate caloric intake was recently shown by Lee et al. (2008). It has been claimed that caloric restriction (in the traditional sense of energy content of the diet) is not responsible for extending the life-span of these flies, but that, paradoxically, increased protein intake is the cause of premature death (Lee et al., 2008). In this sense, a fly living without the stimulation of a partner-fly may extend its life-span by reducing its intake of protein, through the decreased consumption of food solution. Moreover, the theory that protein consumption in and of itself may be detrimental (Mair et al., 2005; Min et al., 2007; Lee et al., 2008) is strengthened by our own unpublished results (T. Zur and D. Nestel). In a preliminary study, we observed very low mortality rates during the first 10 days of adult life in *D. ciliatus* kept as solitary individuals in PUB systems, in which they were fed only carbohydrates (e.g., only about 10% of flies kept in solitude and fed 5% sucrose or fructose died within the first 10 days of adult life, in contrast to the 40–50% mortality rate observed in the present study for individual flies fed the protein–fructose diet; Zur, 2008).

It has been suggested that caloric restriction may influence bioenergetics and metabolic rate (Hunt et al., 2006). Some studies (reviewed by Hunt et al., 2006) have shown that organisms tend to show transient or periodic decreases in their metabolic rates following caloric restriction. Although we did not directly measure respiration or O_2 consumption, the slow, progressive utilization of lipids and proteins observed in solitary flies, in contrast to the relatively large fluctuations in the metabolite contents of paired flies, may indicate a reduced metabolic rate in these flies. If this is the case, it means that the waiting-mode condition not only affects the fly's energy income (through the self-regulation of caloric intake), but may also affect the fly's expenditure of energy. Low-energy income and low metabolism in individual *D. ciliatus*, which seem to express a reproductive 'waiting-mode' condition, may thus be a physiological mechanism that promotes longevity in this fly, and probably in other organisms as well. This aspect requires further research, and systems like the PUB may be suitable experimental system for testing different hypotheses.

Uncertainty concerning the availability of food (especially nitrogen availability) in nature may be an environmental cue that elicits a reproductive waiting-mode strategy in many organisms (Carey et al., 1998a). As far as we know, no previous study has shown or suggested that solitude may also induce a reproductive waiting mode in fruit flies. Although not reported earlier, in certain natural situations, individual fruit flies may find themselves alone, such as when the fly population is starting to build-up after hibernation, aestivation or diapause, and/or when hosts are temporarily unavailable. The observed ability of the fly to reduce its energy income, and possibly its metabolic rate, and enter into a waiting mode when the probability of encountering a mate, or lek, in the environments is low, is thus an adaptation that may increase the fitness of the fly by extending its life until an appropriate mate can be found.

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