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ORIGINAL ARTICLE

Fitness traits of *Drosophila melanogaster* (Diptera: Drosophilidae) after long-term laboratory rearing on different diets

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Abstract. Nutrition is one of the most important environmental factors that influence the development and growth in *Drosophila*. The food composition strongly affects their reproduction, welfare and survival, so it is necessary for flies to search for a mixture of macronutrients that maximizes their fitness. We have five *D. melanogaster* strains, which were reared for 13 years on five different substrates: standard cornmeal-agar-sugar-yeast medium and four substrates modified by adding tomato, banana, carrot and apple. This study was aimed at determining how such long-term rearing of flies on substrates with different protein content affects fitness traits (dynamics of eclosion, developmental time and egg-to-adult survival). Further, we determined how transferring flies reared on fruit/vegetable substrates to a standard laboratory diet affected their fitness. Results indicate that strains reared on the diet with the lowest content of protein and the highest C/N ratio had the slowest eclosion and developmental time, and lowest egg-to-adult survival (apple diet). The flies reared on the diet with the highest protein content and the lowest C/N ratio had the highest survival (tomato diet). Flies reared on the carrot diet, which is quite similar in protein content and C/N ratio to the standard cornmeal diet, had the fastest development. Transferring flies to the standard cornmeal diet accelerate eclosion and developmental time, but did not affect survival.

INTRODUCTION

Food is essential for the survival of all organisms, as it provides energy for different biological functions. During the course of a lifetime there is a requirement for specific nutrients for optimal body growth. However, in nature animals are often exposed to changes in the quality and quantity of food, and in its availability. Consequently, there are changes in their resistance to stress, life-history traits and reproduction (Djawdan et al., 1998; Bross et al., 2005; Broughton et al., 2005; Carsten et al., 2005; Burger et al., 2007; Sisodia & Singh, 2012; Reddiex et al., 2013; Abed-Vieillard et al., 2014; Rodrigues et al., 2015; Kristensen et al., 2016).

D. melanogaster is one of the most frequently used model organisms in a variety of nutritional studies. Consuming food of different qualities is widely evaluated in ethanol-tolerance studies (McKechnie & Geer, 1993), mobility and cardiac physiology (Bazzell et al., 2013), developmental and metabolic studies (Kolss et al., 2009; Matzkin et al., 2011), ageing (Piper & Partridge, 2007), morphological investigations (Kristensen et al., 2011; Güler et al., 2015) including fluctuating asymmetry studies (Vijendravarma et al., 2011), learning and memory (Kolss & Kawecki, 2008; Wright, 2011), cuticular chemistry research (Fedina et al., 2012; Pavković-Lučić et al., 2016) and sexual selection studies (Fricke et al., 2008; Abed-Vieilliard & Cortot, 2016). So far, nutrigenomic studies on *D. melanogaster* have been used to further our understanding of human nutrigenomics, as metabolism is evolutionarily conserved (Ruden & Lu, 2006), and a promising way of developing strategies for dealing with metabolic diseases (Matzkin et al., 2011).

Most of the nutritional studies involving *D. melanogaster* focus on adults, in spite of the fact that larvae could be a better choice for studying this species nutritional requirements (Scherer et al., 2003; Schwarz et al., 2014). Depending on the nutritional composition of the diet, larvae increase in body mass (Sang, 1956, 1978), but are sensitive to the amount of simple sugars and yeast in the diet (Durisko & Dukas, 2013; Neuser et al., 2005).

D. melanogaster is a generalist that uses different fruits and vegetables for both feeding and reproduction (Shorrocks, 1972; Markow, 2015). Certain amounts of protein, carbohydrate, lipid, vitamins and minerals are essential for growth and survival of juveniles (Simpson & Raubenheimer, 1993; Simpson et al., 2004). The amount of protein and carbohydrate in the larval food of *D. melanogaster* affects their developmental time, egg-to-adult survival and lifespan (Chippindale et al., 1998; Heilbronn & Ravussin, 2005; Fanson et al., 2009; Andersen et al., 2010; Kristensen et al., 2011; Merkey et al., 2011; Rodrigues et al., 2015; Reis, 2016). However, it is only protein that determines body and tissue growth in larvae (Britton & Edgar, 1998; Colombani et al., 2003). Larval nutrition further affects resistance of adults to heat and cold, starvation and desiccation (Andersen et al., 2010; Kristensen et al., 2016). Effects of nutrition can be sex specific (Lee & Micchelli, 2013; Reddiex et al., 2013; Nazario-Yepiz et al., 2017). Thus, for females of *D. melanogaster* the protein to carbohydrate (P : C) ratio affects longevity, egg laying rate and lifetime egg production (Lee et al., 2008; Fanson et al., 2009; Rodrigues et al., 2015).

To our knowledge, there are insufficient studies on the fitness consequences of long-term culturing of insects (over decades), including *D. melanogaster*, on diets of different qualities. In most nutritional studies on *D. melanogaster*, the quality of the diet is manipulated by altering the concentrations and ratios of yeast and sugar (Kristensen et al., 2011; Matzkin et al., 2011; Fanson et al., 2012; Güller et al., 2015), or by modifying the food by using different species of yeast (Anagnostou et al., 2010), dietary carbohydrates (Lushchak et al., 2014), lipids, vitamins (Reis, 2016) and food additives (Neethu et al., 2014). However, this study involved using diets that are modifications of this flies' natural food (tomato, banana, carrot and apple), prepared without adding sugar and yeast. Tomatoes are hosts for many insects: *D. melanogaster* is frequently recorded infesting tomato crops, which in the past resulted in a great deal of damage (De Camargo & Phaff, 1957; Lange & Bronson, 1981). One of the most attractive blends for *D. melanogaster* is produced by banana (Schubert et al., 2014), which was often used as the diet for maintaining cultures of flies and in different experiments (Jaenike, 1983; Demerc & Kaufman, 1996; Svilpe & Matjuškova, 2010; Stamps et al., 2012; Chhabra et al., 2013; Ho et al., 2013; Prakash & Krishna, 2015). Carrot is included in *D. melanogaster* diets as it is a rich source of carotenoids. It is reported that *D. melanogaster* larvae need large amounts of dietary carotenoids for the biosynthesis of visual pigments (Giovannucci & Stephenson, 1999). Further, carotenoids influence insect multitrophic interactions and affect the evolutionary outcomes (Heath et al., 2013). Apples are also highly attractive and commonly used for trapping fruit flies, and experiments on habitat selection and life-history traits (Jaenike, 1983; Hoffmann et al., 1984; Hoffmann, 1985; Pavković-Lučić et al., 2012; Kristensen et al., 2016). It is reported that apple polyphenols, which are a rich source of antioxidants, extend the lifespan of *D. melanogaster* (Peng et al., 2011).

Using the aforementioned *D. melanogaster* strains, we set out to determine (1) the extent to which the long-term rearing of flies on different fruit/vegetable foods affects their fitness traits and (2) whether these traits are affected by transferring eggs to a standard laboratory diet, assuming that plastic adaptation to the new nutritive environment may have fitness consequences.

In nature, *D. melanogaster* lives, feeds, and breeds in the same place (Reaume & Sokolowski, 2006), so the ability to adapt to a new diet is important. *D. melanogaster* can detect the nutritional quality of a particular food and induce an adaptive plastic response (Partridge et al., 2005). Phenotypic plasticity, defined as "the ability of individual genotypes to produce different phenotypes when exposed to different environmental conditions" (Pigliucci et al., 2006) can influence fitness directly, if the ability to be plastic is adaptive (Sultan & Spencer, 2002; Crispo, 2008; Stomp et al., 2008), or indirectly, if the plastic response results in the development of an adaptive phenotype (Via et al., 1995). During their lifetime, individuals may adapt by means of developmental plasticity, since they may experience environments in early life that are associated with particular conditions they will experience later in life (Monaghan, 2008; Pilakouta et al., 2015). Selection for feeding on different foods can result in trade-offs associated with the adaptation, which could be manifested in terms of larval development and survival (Kolss et al., 2009). If there is a diet-induced developmental plasticity then our strains should differ in their efficiency to utilize the carbohydrates and proteins in the diet. The shift to a standard laboratory food, prepared with yeast and sugar as an important protein and energy sources, could affect their fitness.

MATERIAL AND METHODS

Chemical analysis of substrates

Total dry weight of samples of substrate was determined by oven drying to constant weight at 105°C to (6 h + 2 h, depending on the sample) (Bradley, 2010). For the analysis of carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) content a Vario EL III CHNS/O Elemental Analyzer was used. Content of crude protein was calculated from the nitrogen content by multiplying it by the factor 6.25 (AOAC, 1995).

Fly strains and substrates

D. melanogaster flies were collected from a natural population and reared over 13 years (more than 300 generations) on five different diets, in 20 replicated lines for each diet group. Flies were reared on the standard cornmeal-sugar-agar-yeast diet (St), and four diets modified by adding tomato (T), banana (B), carrot (C) and apple (A) (Fig. 1). Only the standard diet was prepared with additional sugar and yeast, which were not added to the fruit/vegetable diets (Table 1, Kekić & Pavković-Lučić, 2003). Flies were reared in 250 ml glass bottles filled with 50 ml of food (20 bottles per substrate), under standard laboratory conditions (temperature of ~ 25°C, relative humidity of 60%, 300 lux of illumination, and 12L : 12D cycle). Large population, of about 2000 individuals per strain, were maintained from generation to generation in density controlled, low competition conditions (about 100 individuals per bottle) in order to reduce genetic drift.

Fig. 1. Bottles containing *D. melanogaster* flies reared on standard, tomato, banana, carrot and apple diets.

Experimental groups

Two experimental groups (Fig. 2) were set up and scored for fitness components. In experimental group I, fitness components were scored for flies reared on the diet they had been reared on for 13 years ("native" diet). In experimental group II, flies laid eggs on their native diet, which were then transferred to the St diet, on which their fitness components were determined.

Experimental procedure

To estimate the fitness components, thirty to fifty 4–5 days old fertilized females were transferred to egg laying vials. They were left to oviposit in 60 ø mm Petri dishes for 12 h. Petri dishes were filled with the native substrate for every strain. Eggs were collected and transferred in groups of 60 to new vials filled with 30 ml of the native diet for every strain (for experimental group I) or filled with 30 ml of standard diet (for experimental group II). There were 5–7 replicates per strain and per experimental group. The number of flies that emerged was counted daily until no further flies emerged.

Assessment of fitness components

We measured the following fitness components: dynamics of eclosion, developmental time, and egg-to-adult survival. Dynamics of eclosion is the percentage of flies the emerged per day. Developmental time (Dt) was calculated as the average time weighted by the number of adults that emerged. It was determined using the following formula: $Dt = (\Sigma n_d \times d) / \Sigma n_d$, where n_d is the number of adults that emerged per each day, and d is day of hatching. Since there were no differences between the replicates the results for each strain were pooled. Egg-to-adult survival was expressed as the ratio of the number of flies that emerged and the number of eggs placed in a vial.

Table 1. Composition of the five diets used for long-term culturing of *D. melanogaster* strains (according to Kekić & Pavković-Lučić, 2003). Abbreviations: N – Nipagin, E – ethanol.

Ingredients	Standard diet	Tomato diet	Banana, Carrot, Apple diets
Distilled water	1100 ml	200 ml	680 ml
Quantity of fruits/vegetables	/	900 g	600 g
Cornmeal	104 g	60 g	20 g
Sugar	94 g	/	/
Yeast	20 g	/	/
Agar	7 g	12 g	10 g
Fungicide	2.5g N/ 30ml E	2g N/ 20ml E	2g N/ 20ml E

*Amount is sufficient for 20 (250 ml) glass bottles.

Statistical analysis

The assumption of normality of variances and homoscedasticity was confirmed using Kolmogorov-Smirnov and Levene's tests for both developmental time and survival. One-Way ANOVA was used to analyse developmental time and egg-to-adult survival, depending on the diets. Mean developmental time and mean egg-to-adult survival were calculated for each vial, and these values were used as "units" in ANOVA. Further, a Post hoc Fisher's LSD test was used. Spearman's rank test was used to analyse correlations between protein content and developmental time and egg-to-adult survival. All statistical analyses were performed in STATISTICA®, ver. 5.0 (StatSoft).

RESULTS

Chemical analysis of substrates

Percentage of nitrogen (N), carbon (C), hydrogen (H) and sulphur (S) in the five diets are presented in Table 2. Considering the nature of these diets, the expected amount of lipid in these substrates is unlikely to exceed a few percent (USDA Food Composition Databases). C/N ratio indicates the proportion of protein relative to the total content of organic carbon, which in this case accurately reflects

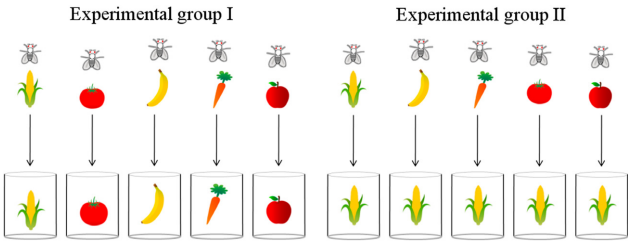

Fig. 2. Scheme of the experimental design.

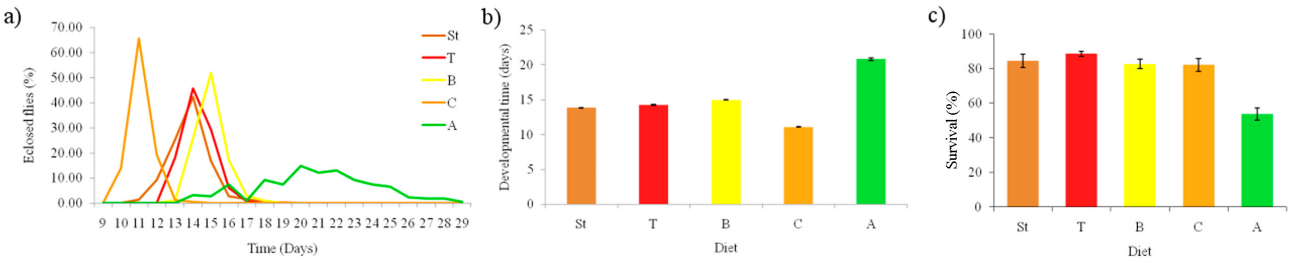

Fig. 3. Dynamics of eclosion (a), developmental time (b) and egg-to-adult survival (c) of *D. melanogaster* strains reared on five different diets for 13 years.

the protein: carbohydrate (P : C) ratio. Protein content was highest in the tomato diet and lowest in the apple diet. Consequently, the C/N ratio was the highest in the apple diet, and the lowest in the tomato diet (Table 2).

Experimental group I

Dynamics of eclosion and developmental time

Dynamics of eclosion and mean developmental time (± S.E.) of flies reared on their native diets are presented in Fig. 3a and b, respectively. St flies emerged from the 11th to 19th day, with the largest number emerging on day 14. Their mean developmental time was 13.82 ± 0.07 days. Eclosion of both T and B flies started on the 13th day and that of T flies ended on the 17th day and of B flies on the 18th day. The largest number of T flies emerged on day 14 and of B flies on day 15. For the T and B flies development lasted, on average, 14.25 ± 0.05 and 14.97 ± 0.05 days, respectively. Eclosion of C flies started on day 10 and ceased on day 14. The highest percentage of adults of C strain emerged on the 11th day, and mean developmental time was 11.08 ± 0.04 days. On the other hand, A flies started emerging on day 14 and the last emerged on day 29, and the largest number of flies emerged on day 20. Mean developmental time of A flies was 20.82 ± 0.22 days. One-Way ANOVA indicates that the strains significantly differed in developmental time ($F = 66.240$, $df = 4$, error $df = 25$, $p < 0.001$). Post hoc LSD test revealed that C flies developed the fastest ($p < 0.001$) and A flies the slowest ($p < 0.001$). Spearman's rank test revealed no significant correlations between developmental time and protein content ($r_s = –0.600$, $p > 0.05$).

Egg-to-adult survival

Mean egg-to-adult survival (± S.E.) in Experimental group I is presented in Fig. 3c. One-Way ANOVA revealed significant difference in egg-to-adult survival among strains ($F = 22.342$, $df = 4$, error $df = 25$, $p < 0.001$). LSD Post hoc analysis indicates that the egg-to-adult survival of T flies was the highest (88.61% ± 1.41; $p < 0.001$) and that of A flies the lowest (53.71% ± 3.48; $p < 0.001$). The egg-to-adult viabilities of St, B and C flies were 84.44% ± 3.82, 82.67% ± 2.70 and 82.22% ± 3.71, respectively. The Spearmen's rank coefficient revealed a significant correlation between protein content and egg-to-adult survival ($r_s = 0.900$, $p < 0.05$).

Table 2. Content of macronutrients (g/100g of dry matter of substrate). Abbreviations: N – Nitrogen, C – Carbon, H – Hydrogen, S – Sulphur.

Diet	N %	C %	H %	S %	Crude protein %, N × 6.25	C/N ratio
Standard diet	1.29	42.41	6.35	0.65	8.06	32.87
Tomato diet	2.21	46.30	6.48	0.53	13.81	20.95
Banana diet	0.72	42.61	6.00	0.50	4.47	59.59
Carrot diet	1.22	41.91	5.92	0.48	7.63	34.35
Apple diet	0.25	41.34	5.94	0.44	1.53	168.73

Experimental group II

Dynamics of eclosion and developmental time

Dynamics of eclosion and mean developmental time (± S.E.) in Experimental group II is presented in Fig. 4a and b, respectively. After transferring to the St diet, flies of all strains started to emerge on day 10, except T-to-St flies, which started on day 12. The largest number of flies emerged on days 12 and 13. The duration of emergence varied among diets: that of C-to-St flies lasted 14 days and of A-to-St flies 20 days. One-Way ANOVA detected significant difference in developmental time among flies transferred from their native to the St diet ($F = 3.734$, $df = 4$, error $df = 20$, $p < 0.05$). LSD test revealed that both the C-to-St flies (in days: 11.94 ± 0.06; $p < 0.01$) and B-to-St flies (in days: 11.61 ± 0.08; $p < 0.01$) developed significantly faster than the St flies (in days: 13.82 ± 0.07; $p < 0.01$) and B-to-St flies than the A-to-St flies (in days: 12.64 ± 0.11; $p < 0.05$). Mean developmental time of T-to-St flies was 12.83 ± 0.07 days.

Egg-to-adult survival

Mean egg-to-adult survival (± S.E.) of strains reared on fruit and vegetable diets and transferred to the St diet is presented in Fig. 4c. One-Way ANOVA confirmed significant differences in egg-to-adult survival among the strains transferred to the St diet ($F = 4.082$, $df = 4$, error $df = 20$, $p < 0.05$). LSD test indicated that the egg-to-adult survival of A-to-St flies (65.15% ± 5.52) was significantly lower than that of the C-to-St flies (84.17% ± 3.22, $p < 0.05$), T-to-St flies (86.94% ± 0.96, $p < 0.01$) and St flies (84.44% ± 3.82, $p < 0.01$). Egg-to-adult survival of B-to-St flies was 79.17% ± 7.77.

Experimental group I vs. Experimental group II

Dynamics of eclosion and developmental time

The beginning of hatching and the largest number of T flies emerging was recorded 1 day earlier, when T flies were transferred to the St diet. Also, the hatching of B flies started 2 days earlier and the largest number of flies emerged 3 days earlier on the St diet than on their native diet. The largest change in dynamics of eclosion was re-

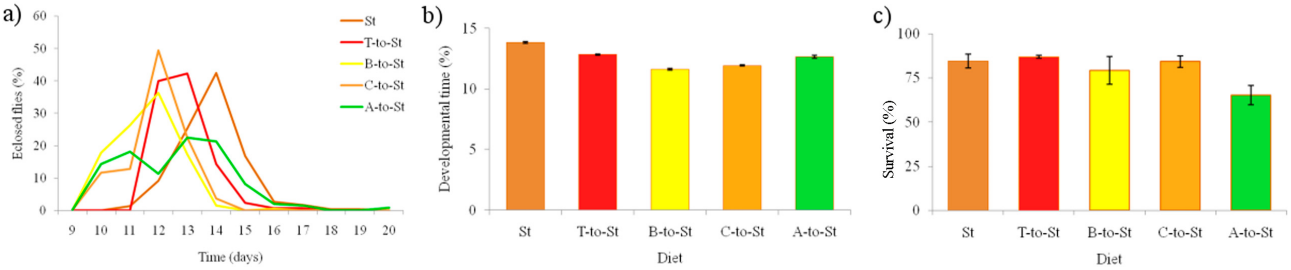

Fig. 4. Dynamics of eclosion (a), developmental time (b) and egg-to-adult survival (c) of *D. melanogaster* strains reared on different diets for 13 years, after transfer to the standard diet.

corded for A flies. Emergence of A flies started 4 days earlier and ended 9 days earlier on the St diet than on their native diet. Further, the largest number of flies emerged 7 days earlier on the St diet. Flies of T, B and A strains developed significantly faster on the St diet than on their native diets (T strain: $F = 10.48$, $df = 1$, error $df = 7$, $p < 0.05$; B strain: $F = 65.099$, error $df = 7$, $df = 1$, $p < 0.001$; A strain: $F = 66.030$, $df = 1$, error $df = 12$, $p < 0.001$). On the other hand, dynamics of eclosion of C flies remained the same on the St and C diets, while the largest number of flies emerged 1 day earlier on the St diet. Based on the One-Way ANOVA, there was no difference in the developmental times C flies reared on their native diet and the St diet ($F = 5.06$, $df = 1$, error $df = 9$, $p > 0.05$).

Egg-to-adult survival

Egg-to-adult survival of flies transferred to the St diet did not differ significantly from that recorded on their native diets (T flies: $F = 0.1390$, $df = 1$, error $df = 7$, $p > 0.05$; B flies: $F = 0.1904$, $df = 1$, error $df = 7$, $p > 0.05$; C flies: $F = 0.328$, $df = 1$, error $df = 9$, $p > 0.05$; A flies: $F = 3.1634$, $df = 1$, error $df = 12$, $p > 0.05$).

DISCUSSION

Diet affects many biological processes, starting from cellular metabolism up to behaviour. Quality and amount of nutritive resources, as well as the balance of macronutrients in food, have a strong effect on the life-history traits of *D. melanogaster* (Lee et al., 2008; Kolss et al., 2009; Kristensen et al., 2011; Schwarz et al., 2014; May et al., 2015; Rodrigues et al., 2015; Simpson et al., 2015; Abed-Vieillard & Cortot, 2016). Nutritive demands may change during the course of life and may be sex-specific, if the sexes maximize fitness in different ways (Lee et al., 2008; Maklakov et al., 2008; Lihoreau et al., 2016).

D. melanogaster larvae feed and live on rotting fruit, acquiring most of their protein from the yeasts present on fruit when it is decomposing (Begon, 1982). It is recorded that *D. melanogaster* reared on a protein rich diet are more viable, heavier and larger, and have more ovarioles (Rodrigues et al., 2015). On the other hand, a low level of protein in the diet results in prolonged development, delay in emergence and decrease in egg-to-adult survival, fecundity and growth in *D. melanogaster* (Wang & Clark, 1995; Tu & Tatar, 2003; Kolss et al., 2009; Rodrigues et al., 2015). Results obtained in this study confirm the results of the above mentioned studies. The strain reared on the diet with the highest percentage of protein, and the lowest C/N ratio (tomato diet, C/N ratio = 20.95), had the highest egg-to-adult survival. On the diet with the lowest percentage of protein and highest C/N ratio (apple diet, C/N ratio = 168.73) the emergence of adults was delayed and development prolonged, and the lowest egg-to-adult survival recorded. However, the banana diet also has a high C/N ratio (C/N ratio = 59.59), but the fitness components did not differ significantly from those recorded for the strain reared on the standard diet, or on the tomato diet (which had the smallest C/N ratio). As eggs and larvae are more sessile than adult flies, it is expected that their oviposition behaviour and selection of pupation sites has been strongly selected for (Markow, 2015). However, Jaenike (1983), reports that females lay more eggs on apple than on tomato, although our study indicates that the apple diet had "the lowest" quality in terms of both larval development and egg-to-adult survival. In nature, a prolonged developmental time increases the risk of running out of food and not completing their development. In such condition, selection against very slow development may be strong. Thus, relatively fast larval development in nature is an important aspect of the adaptation to larval nutrition (Kolss et al., 2009).

D. melanogaster larvae have a natural propensity to balance their diet. Flies fed on a diet deficient in proteins or carbohydrates later preferred the diet containing the nutrients they required. Also, when larvae are offered a choice between a balanced, protein rich or carbohydrate rich diets, they chose the balanced diet (Schwarz et al., 2014). Recently, Rodrigues et al. (2015) report that the shortest developmental time is recorded for flies fed on a diet with an intermediate P : C ratio (ratio of about 1 : 2–1 : 4). Also, egg-laying rate and lifetime egg production is maximized when fed on diets with a P : C ratio 1 : 2 and 1 : 4, respectively (Lee et al., 2008). Krijger et al. (2001) report that among neotropical *Drosophila* species, those with a short development had a competitive advantage over those with a long development. In our study, flies reared on the carrot diet had a shorter development and dynamics of eclosion than the other four strains. Although the carrot diet and standard diet did not differ in protein content and C/N ratio (carrot diet, C/N ratio = 34.35; standard diet, C/N ratio = 32.87), it is possible that these two diets may differ in the quality of the protein (i.e., in amino acid composition).

The standard cornmeal-sugar-agar-yeast medium is commonly used for maintaining cultures of *D. melanogaster* flies under laboratory conditions. In that sense, fitness components of the flies reared on the standard diet could be the control for all other diets (Kolss et al., 2009). Changes in fitness components after transferring eggs from their native fruit/vegetable diets to the standard diet could be the result of plastic responses to the different nutrition. After transferring eggs to the standard diet, the developmental time of almost all the strains changed, but egg-to-adult survival was not affected. Thus, it is possible that regulation of the developmental time buffered survival, because the developmental time was not correlated with protein content, while egg-to-adult survival was.

D. melanogaster has a genetic potential to adapt to different nutritional conditions based on transcriptional dynamics (Kolss et al., 2009; Whitaker et al., 2014). Developmental plasticity provides developing individuals with multiple phenotypes each expressed under different nutritional regimes (Xie et al., 2015). This is an adaptive process, which may result in changes manifested at physiological, morphological and behavioural levels (Monaghan, 2008; Kolss et al., 2009; Shingleton et al., 2009; Beldade et al., 2011; Kristensen et al., 2011; Trajković et al., 2013; Güller et al., 2015; Rodrigues et al., 2015). Phenotypic plasticity might allow individuals to "jump" from one fitness peak to another, without crossing fitness valleys (Price et al., 2003; Crispo, 2007). This study reveals that such a scenario is possible in just one generation.

Results of this study demonstrate that different fitness components do not respond similarly to the different protein compositions and C/N ratios of the larval diet, and indicate a plastic response when *D. melanogaster* is exposed to different nutritional environments. In that sense, differences in fitness traits recorded in the strains used is a challenge for future studies, which could include establishing relationships with morphological characteristics (e.g. body size), physiological properties (e.g. tolerance of stress) and different behavioural traits. Adaptive significance of phenotypic plasticity, possible trade-offs between larval and adult traits, as well as underlying molecular mechanisms should also be further investigated.

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