These substances also effected several morphological changes as indicated. Pre-adult stages of *D. melanogaster* are particularly susceptible as this is the time of active growth and development. Nutri-C showed the least effect on morphology. This was not the case for Sari-C and Eve exposed flies, which showed more abnormal phenotypes. Wing and abdominal defects were the morphological defects observed in F_1 flies. Haq *et al*. (2012) observed morphological changes to wings, abdomen, and color when *D. melanogaster* larvae were fed with lead acetate. Wing alterations were also observed when *D. melanogaster* was exposed to ethidium bromide (Ouchi *et al*., 2011). Some flies exposed to Eve were also seen to have orange colored abdomen probably indicating indigestion.

These substances thus have the ability to affect development in pre-adult stages and induce detrimental changes to the eclosed adult. The toxicological effect of Eve may be due to its much higher concentration and its composition of dyes (Sunset yellow) and artificial sweeteners (Aspartame). Sayed *et al*. (2012) demonstrated the mutagenic action of sunset yellow also showing an increase of morphological abnormalities in spermatozoids of mice.

The toxicological effect of Nutri-C and Sari-C may also be due to its composition of aspartame, tartrazine, colorings, and acesulfame-K in Sari-C. Gomes *et al*. (2013) found that tartrazine yellow dye has anti-proliferative activity action and potential to cause cellular aberrations using the *Allium cepa* test.

Conclusion

This study demonstrated signs of toxicity of the orange flavored drinks on *D. melanogaster*. A reduction in survival of parent flies as well as morphological changes in F_1 progenies was observed. At the very least, this has shown that these substances can affect some aspects of the biology of fruit flies. Further research should be carried out to determine the mode of action of these substances on *D. melanogaster* and on mammalian test systems.

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How nutritive conditions determine life-history traits in *Drosophila melanogaster*?

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Drosophila melanogaster uses various fruits and vegetables in different manners: as a food, egg-laying sites, or for reproduction (Shorrocks, 1972). Since flies are often exposed to different quality, quantity, and availability of nutritional resources, adjustment to new nutritional environment induces adaptive plastic responses, which include changes in morphological, physiological, life-history, and behavioral traits (Djawdan

et al., 1998; Bross *et al*., 2005; Broughton *et al*., 2005; Carsten *et al*., 2005; Partridge *et al*., 2005; Burger *et al*., 2007; Sisodia and Singh, 2012; Reddiex *et al*., 2013; Trajković *et al*., 2013, 2017a, 2017b; Abed-Vieillard *et al*., 2014; Rodrigues *et al*., 2015; Kristensen *et al*., 2016). Adaptation of individuals to different environmental conditions by developmental plasticity could be manifested, among other things, through the larval developmental time and viability (Kolss *et al*., 2009).

Our previous research confirmed that long term rearing of fruit flies on different diets (standard corn-meal substrates, as well as tomato, banana, carrot and apple diets) resulted in significant differences in developmental time, eclosion dynamic, and viability (Trajković *et al*., 2017a). Namely, flies maintained on carrot diet for more than 300 generations had the fastest developmental time, while flies reared on apple diet expressed the slowest development (Trajković *et al*., 2017a). In this respect, the purpose of this research was to explore and quantify potential changes in certain life-history traits, when flies grown on carrot diet were transferred to apple diet and *vice versa*.

In this experiment, we used *D. melanogaster* flies which were reared for more than 300 generations on carrot (C) and apple (A) diets. Media were prepared according to recipes published by Kekić and Pavković-Lučić (2003). Over the years, flies were maintained in 250 ml glass bottles (about 100 individuals *per* bottle), in optimal laboratory conditions (temperature of ~ 25^{o}C, relative humidity of 60%, 300 lux of illumination, and 12 h: 12 h light: dark cycle).

For experimental purposes, flies maintained on carrot diet (C flies), which had the fastest developmental time, were transferred to the apple diet (C-to-A flies), and flies maintained on apple diet (A flies), which were previously characterized by the slowest developmental time (Trajković *et al*., 2017a), were transferred to the carrot diet (A-to-C flies) (Figure 1). After that, three life history traits were scored: developmental time, dynamics of eclosion, and viability.

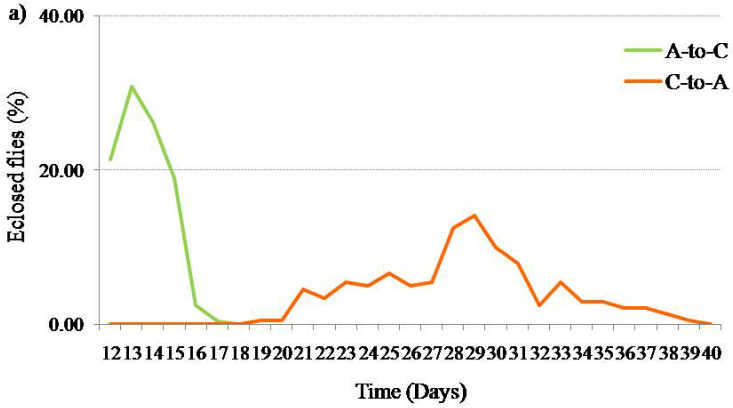
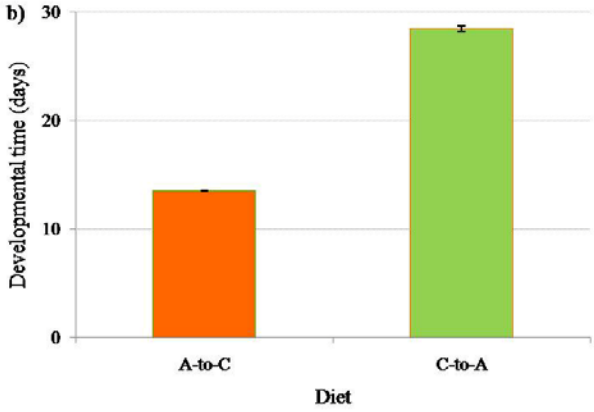
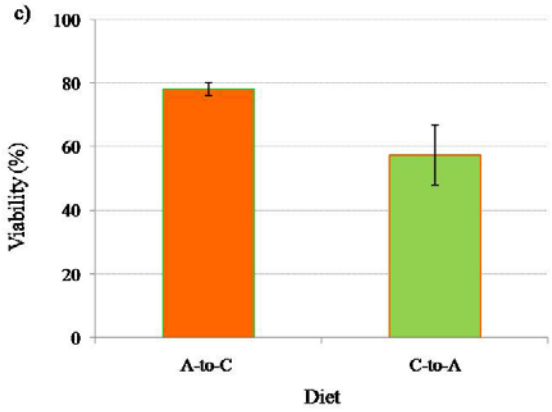


Figure 1. The scheme of experimental design.





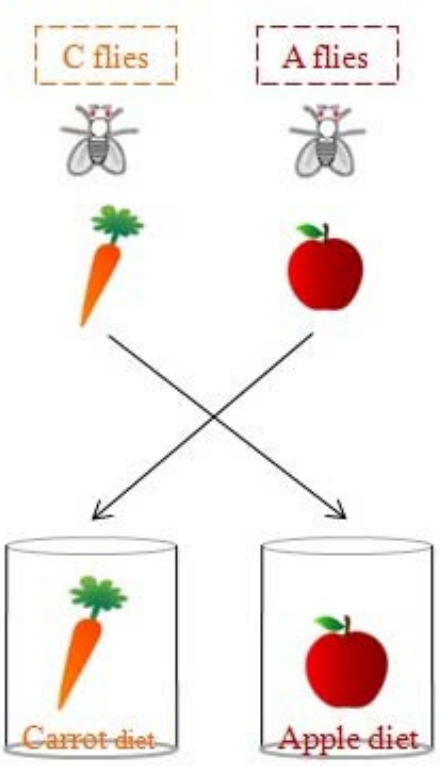


Figure 1 (continued).

Thirty to fifty fertilized females of C and A strains were transferred to the egg laying vials filled with their "native" substrates. Further, sixty eggs were collected and transferred to the new vials in the manner shown in Figure 1, and 5-7 such replicates were obtained *per* both combinations. Life-history traits were determined according to Trajković *et al*. (2017a). Dynamics of eclosion were presented as percentage of flies emerged *per* day, while developmental time was calculated as the average time weighted by the number of adults emerged. Egg-to-adult viability was expressed as the ratio of emerged flies and eggs placed in the vial.

Dynamic of eclosion, mean developmental time, and egg-to-adult viability are presented in Figure 2.

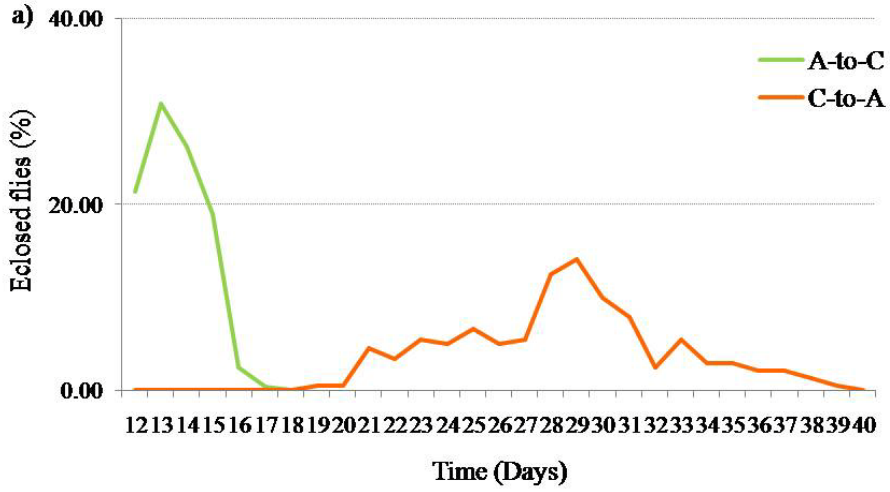
A-to-C flies hatched from the 12th to 17th day, and the largest number of eclosed flies was recorded on day 13 (Figure 2a). Emergence of C-to-A flies started at day 19 and lasted until day 39 (Figure 2a). The largest number of emerged C-to-A flies was recorded at 29th day (Figure 2a).

A-to-C flies have significantly shorter development (mean developmental time: 13.51 ± 0.06 days; Figure 2b) in comparison with C-to-A flies (mean developmental time: 28.44 ± 0.27 days; Figure 2b) ($F = 192.211$, $df = 1$, $p < 0.001$). Also, development of A-to-C flies lasted considerably shorter compared with development of A flies ($F = 74.323$, $df = 1$, $p < 0.001$). On the other hand, developmental time of C-to-A flies was significantly prolonged in comparison with C flies ($F = 235.829$, $df = 1$, $p < 0.001$).

After transferring eggs from the apple to the carrot diet, egg-to-adult viability significantly increased, from 53.71% (Trajković *et al*., 2017a) up to 78.10% ($F = 36.568$, $df = 1$, $p < 0.001$) (Figure 2c). In the reverse situation, when eggs from the carrot diet were transferred to the apple diet, egg-to-adult viability decreased from 82.22% (Trajković *et al*., 2017a) to 57.38% ($F = 32.941$, $df = 1$, $p < 0.001$) (Figure 2c). Further, A-to-C flies manifested significantly higher egg-to-adult viability than C-to-A flies ($F = 13.173$, $df = 1$, $p < 0.01$).

Under natural conditions, it is very important for *D. melanogaster* to adjust to the diverse environmental variations (including nutritional), which is mostly achieved by metabolic and physiological adaptations. Numerous studies pointed out that *Drosophila* life-history traits depend on both quality and amount of nutritive resources (Rodrigues *et al*., 2015; Abed-Vieillard and Cortot, 2016; Kolss *et al*., 2009; Kristensen *et al*., 2011; Lee *et al*., 2008; Schwarz *et al*., 2014).

To our knowledge, only one experimental evolutionary study on *D. melanogaster* confirmed the existence of adaptations to malnutrition. Namely, flies reared on poor larval nutrition showed a higher egg-to-adult viability and faster development on poor in comparison to the standard medium (Kolss *et al*., 2009). In our study, dynamics of eclosion, developmental time, and egg-to-adult viability were considerably dependent on the diet type. Previously, chemical analysis of diets used for growing flies in our laboratory revealed differences in protein/carbohydrate (P: C) ratio (Trajković *et al*., 2017a). Apple diet contains very low amounts of proteins, and flies maintained for many years on this diet type exhibited the slowest developmental time and the lowest viability (Trajković *et al*., 2017a). When those flies were transferred to standard (Trajković *et al*., 2017a) or, as in this research, to carrot diets, which contain higher protein amounts, they developed faster and expressed higher viability. In the opposite situation, when C flies which exhibited the fastest development and higher viability were transferred to the apple diet, their development was prolonged and viability reduced.



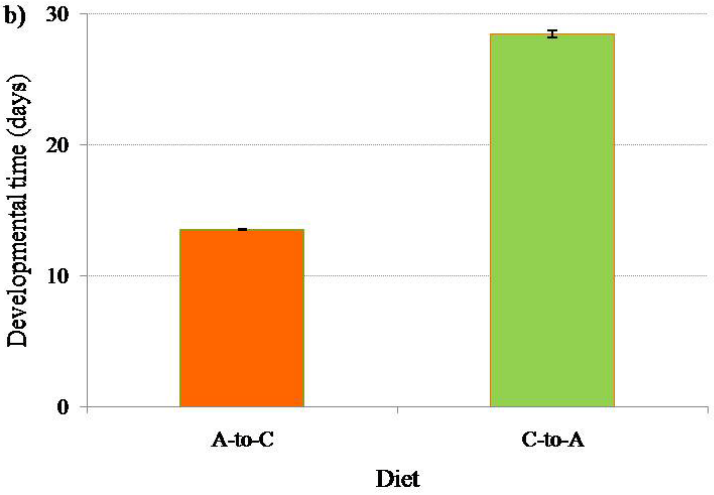


Figure 2. Dynamics of eclosion (a), mean developmental time (b), and mean egg-to-adult viability (c) of *D. melanogaster* A-to-C and C-to-A flies.

Results of this research confirmed the existence of developmental plasticity when *D. melanogaster* flies were exposed to different nutritional environments, and that developmental time is not deeply channeled. Furthermore, presence of developmental plasticity gives flies the possibility to adjust to highly variable environmental conditions.

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White eye mutation in *Drosophila melanogaster* does not affect fitness – a support for a neutral theory of molecular evolution.

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Abstract

White eye mutation in *Drosophila melanogaster* resulted in significant reduction in pre-adult development time. However, this reduction in pre-adult development time was accompanied by non-significant reduction in adult dry weight, life-time oviposition, and longevity lending a fortuitous support to the 'neutral theory of molecular evolution'. Key words: white eye, life history, oviposition, longevity, lipid content

Introduction

Mutations are an important source of heritable variation. They are acted upon by evolutionary forces such as natural selection and genetic drift in a population. New mutations could arise from DNA replication and repair infidelity, spontaneous point mutations, transposable elements, and a variety of other sources (reviewed in Mackay, 2010).

White eye, the first mutant phenotype identified in *Drosophila* by Morgan in 1910, is due to a mutation in an ABC transporter gene (Sullivan *et al.*, 1974; Mackenzie *et al.*, 1999). White functions with products of either scarlet or brown genes as paired heterodimers for transport of pigment precursors, tryptophan and guanine, respectively, into the eye of the fly (Ewart and Howells, 1998). The red pigments- drosopterins, and the brown pigments- ommochromes, are synthesized from guanine and tryptophan, respectively (Summers *et al.*, 1982). The repercussion of inefficient transport of pigment precursors is correlated to defective vision in the mutant flies at different wavelengths of light (Cosens and Briscoe, 1972), partially attributable to the inability to screen stray light due to the lack of optical insulation provided by the pigments (Hengstenberg and Götz, 1967). This also results in 'dazzling' the flies because of over-flow in daylight conditions (Krstic *et al.*, 2013). The white eye flies are positively phototactic but may completely lack optomotor responses (Kalmus, 1943) and have abnormal electroretinograms (Wu and Wong, 1977). Due to the low levels of expression of White, molecular studies are often difficult to conduct and hence characterizing expression in tissues other than the eye is problematic, though its expression in the CNS has been established and expected to express in the PNS too (Krstic *et al.*, 2013).

Furthermore, the white gene has been implicated in a plethora of complex processes such as mating behavior in males, transport of biogenic amines involved in memory formation (Sitaraman *et al.*, 2008), and