C. Starcke, C. Riisager Sørensen, and M. Fristrup Schou 2016, Insect Science 23: 771–779; Lee, K.P., S.J. Simpson, F.J. Clissold, R. Brooks, J.W.O. Ballard, P.W. Taylor, N. Soran, and D. Raubenheimer 2008, PNAS 105: 2498–2503; Reddiex, A.J., T.P. Gosden, R. Bonduriansky, and S.F. Chenoweth 2013, The American Naturalist 182: 91-102; Rodrigues, M.A., N.E. Martins, L.F. Balance, L.N. Broom, A.J.S. Dias, A.S.D. Fernandes, F. Rodrigues, E. Sucena, and C.K. Mirth 2015, Journal of Insect Physiology 81: 69–80; Schwarz, S., Z. Durisko, and R. Dukas 2014, Naturwissenschaften 101: 61–68; Shorrocks, B., 1972, Ginn, London, pp. 144; Sisodia, S., and B.N. Singh 2012, PLoS ONE 7: e46131; Trajković, J., S. Pavković-Lučić, and T. Savić 2013, Behaviour 150: 1431-1448; Trajković, J., V. Vujić, D. Miličić, G. Gojgić-Cvijović, S. Pavković-Lučić, and T. Savić 2017a, European Journal of Entomology 114: 222–229; Trajković, J., D. Miličić, T. Savić, and S. Pavković-Lučić 2017b, Behavioural Processes 140: 81-86.

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

cGMP transport in the malpighian tubules (Evans *et al.*, 2008). Varying transport of biogenic amine precursors by White and its ligands, leads to concomitant diminished accumulation and differential release of the neuromodulators histamine, dopamine, and serotonin in mutants of *white*, *scarlet*, and *brown* genes (Borycz *et al.*, 2005, 2008). The effects of reduced serotonin and dopamine have been correlated with the differences in memory performance of the white eye mutants (Sitaraman *et al.*, 2008).

Studies have demonstrated differences in behavior due to varied neuronal action on exposure to volatile general anesthetics (Campbell and Nash, 2001), and recorded reduction in spatial learning (Diegelmann *et al.*, 2006), olfactory learning (Anaka *et al.*, 2008), and suppression of phototactic personality (Kain *et al.*, 2012) amongst white mutants and wild type. White mutants have been observed to court both decapitated males and females in the dark unselectively, or form chains in daylight conditions when placed in groups of males (Zhang and Odenwald, 1995; Hing and Carlson, 1996). It has also been reported that both the mis-expression of *mini-white* or mis-localization of the same and the lack of extra-retinal White can cause alteration in the sexual behavior of male flies (Zhang and Odenwald, 1995; Anaka *et al.*, 2008; Krstic *et al.*, 2013). Further, it is also argued that deficit of White function heightens the sexual arousal of males in general, indirectly leading to male-male courtship (Krstic *et al.*, 2013).

Pre-reproductive and reproductive activities are costly as they make high energetic demands. This is typically manifested as increased death rate of non-virgin female flies as opposed to virgin female flies (Partridge *et al.*, 1986; Partridge and Fowler, 1990), or the high mortality of frequent maters compared to less frequently mating females (Fowler and Partridge, 1989), due to large amount of seminal fluid molecules transferred by males (Chapman *et al.*, 1995). Male *D. melanogaster* flies, too, invest heavily in mating (Cordts and Partridge, 1996). Significant behavior and growth related trade-offs have been seen as a consequence of increased male reproductive effort in *Poecilia reticulate* (Jordan and Brooks, 2010). Cost of reproduction significantly affects life history evolution including key traits such as future survival and age-specific oviposition schedules in animals that reproduce sexually (Partridge and Farquhar, 1983; Partridge *et al.*, 1985). In addition to energetic costs of reproduction, courtship itself carries a cost. Courtship harassment by males is shown to affect the fitness of females in *Drosophila melanogaster* (Partridge and Fowler, 1990). Taken together, the white eye mutants are expected to impose higher cost due to heightened arousal and thus be less fit compared to their wild type relatives.

In this paper we have evaluated the fitness and fitness related traits of white eye mutant and discussed their implication to understanding the evolutionary concept of 'the neutral theory of molecular evolution' (Kimura, 1968, 1983, 1991) that suggests no affect/effect of mutations on fitness of the organism bearing them. We have assessed the effect of white eye mutation on important life history traits namely, pre-adult development time, egg to adult viability, adult weight, lipid content in adult fly, adult longevity, and life time egg production. The results from this study clearly are in support of the 'neutral theory of molecular evolution'.

Materials and Methods

Drosophila melanogaster is well known for its extensive use in research due to its numerous advantages as a model organism. It is a holometabolous insect with life cycle inclusive of four discrete stages-egg, larva, pupa, and adult (imago) form. Wild type (JB) and white eye (WE) populations were used in this study. Ancestry of the wild populations used in this study can be traced back to the IV population (Ives, 1970). They are maintained on a 21 day egg-to-egg discrete generation cycle under standard laboratory conditions of 25±1°C temperature, 24:0 LD cycles and 75±5% relative humidity (Mishra *et al.*, 2017; Chandrashekara and Shakarad, 2011). Adults in cages are given yeast supplement 3 days prior to (on day 18) the egg collection (day 21) for starting the subsequent generation. A fresh uncontaminated food plate is then provided and flies are allowed to lay eggs on it for 18 hours. Eggs from this plate are transferred to 6 mL media vials at approximate densities of 40-60 eggs. Forty such vials were maintained per line. All adults emerging from the 40 vials were transferred to pre-labelled Plexiglass cages on day 12 and provided with fresh food plates every alternate day until day 18. The white eye mutant was a natural mutant isolated from the wild (JB) population and bred to build a complete population by standard back-cross procedure. The white eye mutant population was also maintained on a 21 day egg-to-egg discrete generation cycle. The population maintenance was

identical to the wild type JB populations. The breeding population sizes in both the wild and mutant populations, was 1600-1800 each. The wild JB populations had been through 311 generations, while the white population had been through 40 generations of maintenance in the laboratory at the time of assaying their life history traits.

Fly food: Composition and Preparation

A fine paste of 205 g banana, 35 g jaggery, 25 g barley, and 36 g yeast (Prestige) ground in 180 mL water was added to 12.4 g agar dissolved in 1L water. The mixture was brought to a boil on low flame and cooled down to ~37°C using a cold waterbath. Following this, 2.4 g methyl paraben (Fisher Scientific) dissolved in 45 mL of absolute ethanol (Changshu Yangyuan Chemicals China) was added to the cooled media and mixed thoroughly. 2 mL and 6 mL of media were poured into 8 dram vials for oviposition and developmental time assays, respectively.

Egg to adult Development time Assay

Flies were provided with fresh uncontaminated food plate and allowed to lay eggs for 1 hour. The plates were taken out and eggs were harvested using moist, fine tip camel hair brush. Exactly 50 eggs were counted under stereo zoom microscope and dispensed into 6 mL food vials. 10 vials each were set up for the wild and mutant populations. The vials were incubated under SLC, on pupation the vials were inspected twice daily for eye spots in pupae. On observation of eyespots, vigil checks were conducted every four hours and emerging flies were collected into pre-labelled empty dry vials, sorted based on gender under light CO_2 anaesthesia and transferred to holding vials with fresh media till used in further experiments.

Mid-point of egg laying window was considered to be the 0^{th} hour and mid-points of two successive 4 hourly checks considered as the time of emergence. The 4 hourly checks were carried out until no fly emerged from any of the vials. Finally, the pupae on the walls and those on the bottom of the vials were scored to assess pupation rate.

Adult Dry weight and Lipid Content

Virgin males and females from both mutant and wild populations, from the development time assay were over-etherized using diethyl ether and transferred to pre-labelled, clean glass vials in batches of 10 flies each, and dried at 70°C in oven for 36 hours and weighed to obtain dry weights. The flies were then put into pre-labelled 1.5 mL centrifuge tube to which 1.3 mL diethyl ether (≥99.5 purity, Merck) was added and placed on a gel rocker set to 2000 rpm and rocked for 36 hours at room temperature with a change of ether every 12 hours. The flies were then transferred to new centrifuge tubes and given 1 wash with diethyl ether, dried at 40°C in oven for 1 hour and weighed again to obtain lipid free weights. Three replicate vials per fly type per gender were set up.

Longevity and Lifetime Oviposition Assay

Flies transferred to holding vials were segregated into 40 pairs (one male + one female) and transferred to 2 mL media vials. Flies were subsequently transferred to fresh media vials every 24 hours and eggs laid during the preceding 24 hours were counted under the microscope and recorded. The death of every fly was recorded to calculate longevity. The assay was terminated following the death of all the flies.

Statistical analyses

The egg to adult development time, pupation percentage, egg-to-adult viability, and average longevity data were subjected to two-sample t-test. The dry weight and lipid content data were subjected to mixed model analyses of variance (ANOVA), treating replicate vials as a random factor and fly type and gender as fixed factors crossed with vial. In all cases, the vial means were used as the units of analysis and, therefore, only fixed-factor effects and their interactions can be tested for significance (Prasad *et al.*, 2001). Further, the gender ratio data were analysed using 2×2 contingency χ^2 test. The difference in the adult survival probability curves was analysed by Kaplan-Meier log-rank test (Fisher and Van, 1993).

Results

Egg-To-Adult Development Time

There was a significant effect of population type on egg-to-adult development time (t_{18} = 3.27, p < 0.005). The white eye mutant flies emerged as adults (226.577 h) nearly 3 hours earlier compared to their wild type ancestors (229.53 h). The significant difference in the egg-to-adult development time was largely due the reduction in development time of the males (t_{18} = 3.410, p < 0.005) as compared to the females (t_{18} = 2.653, p < 0.05). The male flies from the mutant population emerged in approximately 9 days and 11 h (227.316 h) compared to 9 days and 15 h (230.794 h) of the ancestral wild populations, and the female mutant flies emerged in nearly 9 days and 10 h (225.963 h) as compared to 9 days and 12 h (228.348 h) of the wild population, respectively (Figure 1).

Figure 1. Mean (±se) of development time for wild and white eye populations.

Dry Weight and Lipid Content

The mutant and their ancestral wild relatives had non-significantly different dry weights ($F_{1,2}$ = 6.23, p = 0.129, Figure 2). The gender of the fly had significant effect on its dry weight ($F_{1,2}$ = 4635.843, p = 0.0002). Average dry weight of male flies from mutant and wild type populations were found to be 257.23 µg and 269.57 µg, whereas those of the females were 364.97 µg and 389.93 µg, respectively. The fly type × gender interaction was not significant ($F_{1,2}$ = 7.467, p = 0.112).

Lipid content of the flies was not affected by the fly type ($F_{1,2}$ = 7.332, p = 0.114, Figure 3). The gender of the flies had significant effect on its lipid content ($F_{1,2}$ = 280.957, p = 0.004). Fly type × gender ($F_{1,2}$ = 0.121, p = 0.761) interaction was not significant. Both male and female wild type flies had non-significantly higher lipid content with 50.17 µg and 67.2 µg than their WE mutant counterparts with 41.43 µg and 60.7 µg, respectively.

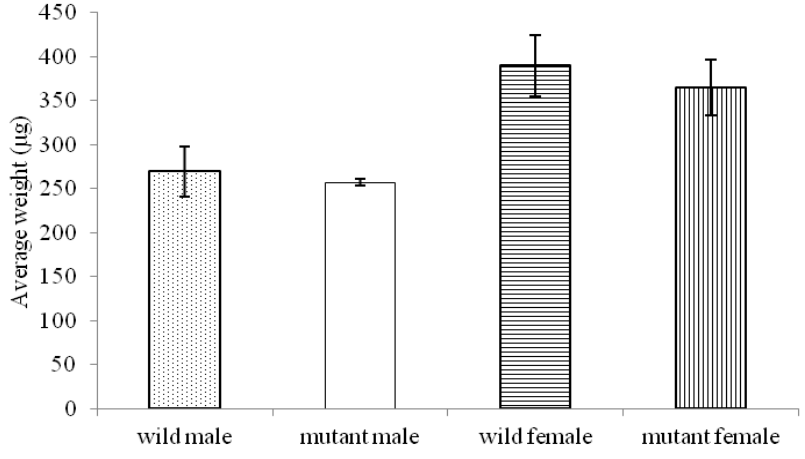

Figure 2. Mean (±se) dry weight of male and female flies from the wild and white eye populations.

Pupation, Viability and Gender Ratio

There was no significant effect of mutation on percentage pupae formed (t_{18} = 0.709, p > 0.05; Figure 4) as well as egg to adult viability (t_{18} = 0.795, p > 0.05; Figure 5). The mean pupation and viability for wild and WE flies was found to be 87% and 88.8%, and 84.2% and 86.6%, respectively. There was no significant effect of fly type on the sex ratio of the emerging flies (χ^2 = 0.101, p > 0.05, Figure 6).

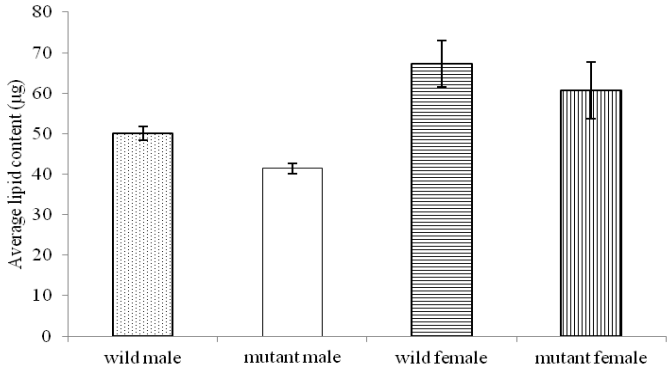

Figure 3. Mean (±se) lipid content in wild and white eye populations.

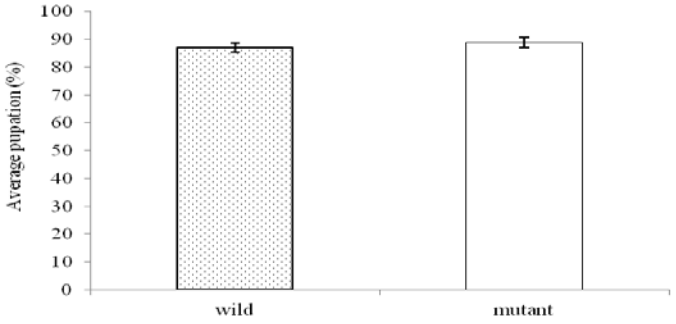

Figure 4. Mean (±se) percentage pupation for wild and white eye populations.

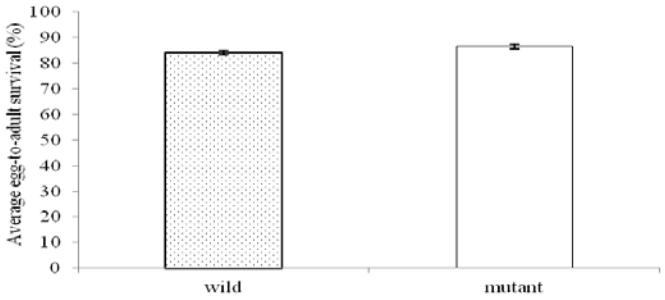

Figure 5. Mean (±se) of egg-to-adult viability of wild and white eye populations.

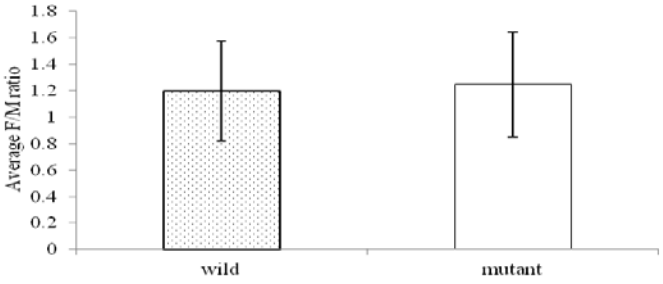

Figure 6. Mean (±se) of sex ratio of the wild and white eye populations.

Longevity and Survival Probability

Average longevities of the white eye and wild-type flies pooled across genders were not significantly different ($t_{74} = 0.529$, $p > 0.05$; Figure 7), even though mutant population lived ~ 2 days longer. Overall, there was a significant effect of gender on the average longevity ($t_{74} = 2.923$, $p < 0.01$). The average longevity of male flies pooled across population types was 44.57 d as against 36.34 d in females. Average lifespan of adult wild type males was 42.73 days as compared to 46.31 days of the mutants, whereas that of the wild-type and

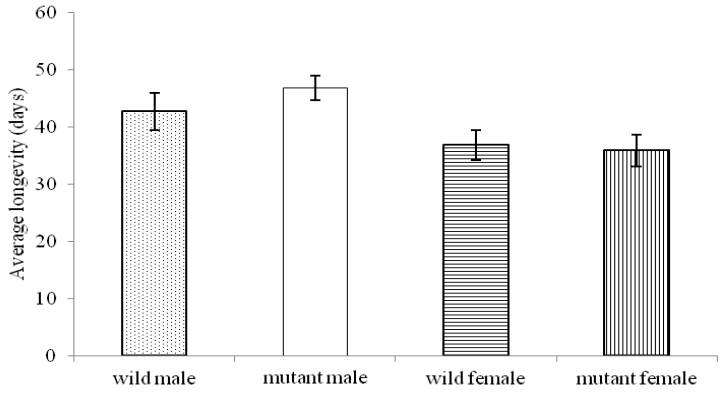

Figure 7. Mean (±se) longevity of wild and white eye populations.

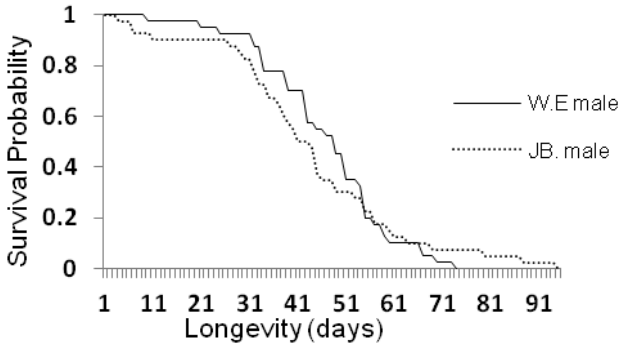

Figure 8. Kaplan-Meir survival curves for mutant and wild type males.

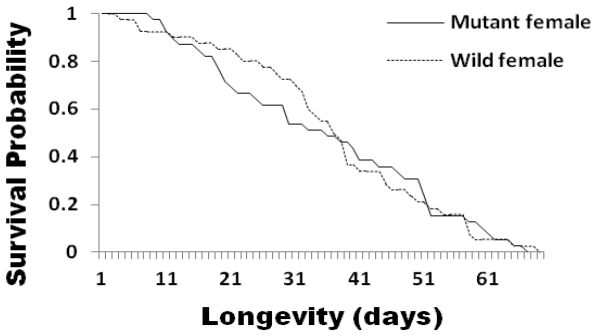

Figure 9. Kaplan-Meir survival curves for mutant (WE) and wild type female flies.

mutant females was 36.78 days and 35.92 days, respectively. The average is only a descriptive statistic that does not provide an insight into the biological process(es), thus necessitating comparison of the survival probabilities (Chandrashekara and Shakarad, 2011; Chandrashekara *et al.,* 2014). The survival probability of the white eye mutant and their ancestral wild type flies were significantly different for males ($\chi^2 = 24.19962$, $p < 0.001$; Figure 8) as well as females ($\chi^2 = 35.95932$, $p < 0.001$; Figure 9).

Lifetime Oviposition

Although an average WE mutant fly laid fewer eggs (400.05) compared to its wild relative (435.82), the differences were not statistically significant ($t_{37} = 1.074$, $p > 0.05$; Figure 10).

Discussion

Pre-adult development time is an important trait that has a direct impact on the life-history in all holometabolous insect species, especially those that thrive on ephemeral habitat, such as *Drosophila melanogaster*. The significant reduction in the white eye mutant development time compared to their wild type ancestors could be due to founder effect as single male fly was identified and expanded into a full population by crossing with 100 virgin female siblings. Unlike other studies that showed significant reduction in adult body size as a correlated response (Zwaan *et al.*, 1995; Nunney, 1996; Chippindale *et al.*, 1997; Prasad *et al.*, 2000, 2001; Handa *et al.*, 2014), the reduction in body size was only marginal in the present study. Further, the other associated traits, namely lipid content, longevity, viability, pupation, gender ratio, and

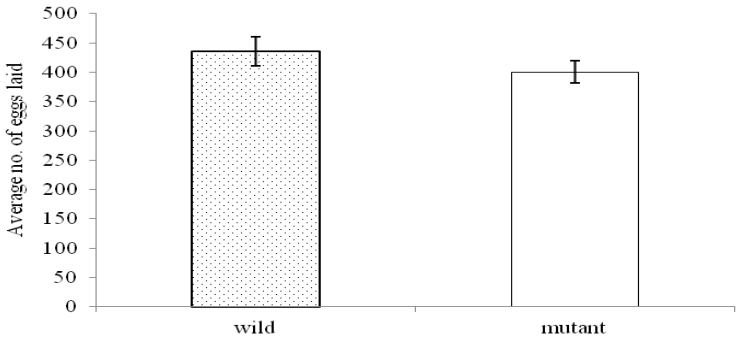

Figure 10. Mean (±se) life-time oviposition for female flies from wild and white eye populations.

oviposition did not show any significant difference between the white eye and wild-type populations. The non-significant decrease in the body size of the white eye flies was accompanied by non-significant decrease in the life-time oviposition, which in turn was accompanied by non-significant increase in the longevity. The relationship between fecundity and female size is shown to be close to 1 in many insect species (Honek, 1993). In addition, several studies have reported a trade-off between reproduction and longevity (Chippindale *et al*., 1993; Chapman and Partridge, 1996; Tu and Tatar, 2003). The reduction in life-time oviposition of white eye mutants in the present study could either be due to courtship harassement by males (Partridge and Fowler, 1990) or due to reduced courtship, a result of lowered sexual arousal exhibited by the white eye mutant males (Krstic *et al*., 2013). Partridge and Fowler (1990) also reported a reduction in longevity of females as a result of exposure to courting males. However, the non-significant increase in the longevity of the white eye mutants in our study suggests that the males are perhaps in a lowered arousal state (Krstic *et al*., 2013).

Reproductive life-span is an important fitness trait for all iteroparous organisms. Reproductive life-span is a composite variable trait influenced by the genotype for longevity and reproduction, both of which are highly influenced by the environment. The variability in any biological trait is due to mutations and shuffling of genome due to sexual reproduction. Our results show that white eye mutation does not significantly affect the fitness of the organisms, yet has the potential to alter the course of evolution through drift. This is the first study that provides experimental support to neutral theory of molecular evolution (Kimura, 1968, 1983, 1991).

References: Anaka, M., C.D. MacDonald, E. Barkova, K. Simon, R. Rostom, R.A. Godoy, A.J. Haigh, I.A. Meinertzhagen, and V. Lloyd 2008, J. Neurogenet. 22: 243–276; Borycz, J.A., J. Borycz, A. Kubów, R. Kostyleva, and I.A. Meinertzhagen 2005, J. Neurophysiol. 93: 1611-1619; Borycz, J., J.A. Borycz, A. Kubów, V. Lloyd, and I.A. Meinertzhagen 2008, J. Exp. Biol. 211(Pt 21): 3454-66; Campbell, J.L., and H.A. Nash 2001, J. Neurobiol. 49: 339–349; Mishra, N., N. Chauhan, G. Sageena, H. Chhabra, and M.N. Shakarad 2017, Journal of Entomology and Zoology Studies 5(4): 1877-1881; Chandrashekara, K.T., and M.N. Shakarad 2011, J. Gerontol. A Biol. Sci. Med. Sci. 66A (9): 965–971; Chandrashekara, K.T., S. Popli, and M.N. Shakarad 2014, Age (Dordr.) 36(5): 9702; Chapman, T., and L. Partridge 1996, Proc. R. Soc. Lond. B 263: 755-759; Chapman, T., L.F. Liddle, J.M. Kalb, M.F. Wolfner, and L. Partridge 1995, Nature 373: 241-244; Chippindale, A.K., A.M. Leroi, S.B. Kim, and M.R. Rose 1993, J. Evol. Biol. 6: 171–193; Chippindale, A.K., J.A. Alipaz, H.-W. Chen, and M.R. Rose 1997, Evolution 51(5): 1536-1551; Cordts, R., and L. Partridge 1996, Animal Behaviour 52: 269-178; Cosens, D., and D. Briscoe 1972, J. Insect Physiol. 18: 627-632; Diegelmann, S., M. Zars, and T. Zars 2006, Learn Mem. 13: 72–83; Evans, J.M., J.P. Day, P. Cabrero, J.A.T. Dow, and S.A. Davies 2008, J. Exp. Biol. 211: 890-899; Ewart, G.D., and A.J. Howells 1998, Meth. Enzymol. 292: 213-224; Fisher, L.D., and B.G. Van 1993, *Biostatistics: A Methodology for the Health Sciences*. New York: Wiley. pp 786–843; Fowler, K., and L. Partridge 1989, Nature, Lond. 338: 760-761; Handa, J., K.T. Chandrashekhara, K. Kashyap, G. Sageena, and M.N. Shakarad 2014, J. Biosci. 39(4): 609-20; Hengstenberg, R., and K.G. Götz 1967, Kybernetik 3(6): 276-285; Hing, A.L., and J.R. Carlson 1996, J. Neurobiol. 30: 454-464; Honek, A., 1993, OIKOS 66: 483-492; Ives, P.T., 1970, Evolution 24: 507-518; Jordan, L.A., and R.C. Brooks 2010, J. Evol. Biol. 23(11): 2403-9; Kain, J.S., C. Stokes, and B.L. de Bivort 2012, Proc. Natl. Acad. Sci. USA 109: 19834-19839; Kalmus, H., 1943, J. Genet. 45: 206–213; Kimura, M., 1968, Nature 217: 624-626; Kimura, M., 1983, *The Neutral Theory of Molecular Evolution*. Cambridge University Press, New York; Kimura, M., 1991, Proc. Natl. Acad. Sci. USA. 88: 5969-5973; Krstic, D., W.

Boll, and M. Noll 2013, PLoS One 8: e77904; Mackay, T.F.C., 2010, Phil. Trans. R. Soc. Lond., B, Biol. Sci. 365(1544): 1229-1239; Mackenzie, S.M., M.R. Brooker, T.R. Gill, G.B. Cox, A.J. Howells, and G.D. Ewart 1999, Biochim. Biophys. Acta Biomembranes 1419: 173-185; Morgan, T.H., 1910, Science 32(812): 120-122; Nunney, L., 1996, Biol. J. Linn. Soc. Lond. 59(4): 411-425; Partridge, L., and K. Fowler 1990, J. Insect Physiol. 36: 419-425; Partridge, L., and M. Farquhar 1983, Anim. Behav. 31: 871–877; Partridge, L., K. Fowler, S. Trevitt, and W. Sharp 1986, J. Insect Physiol. 32: 925-929; Partridge, L., T.F.C. Mackay, and S. Aitken 1985, Genet. Res. 46: 279–285; Prasad, N.G., M. Shakarad, V.M. Gohil, V. Sheeba, M. Rajamani, and A. Joshi 2000, Genet. Res. 76: 249-259; Prasad, N.G., M. Shakarad, D. Anitha, M. Rajamani, and A. Joshi 2001, Evolution 55: 1363–1372; Sitaraman, D., M. Zars, H. LaFerriere, Y.C. Chen, A. Sable-Smith, T. Kitamoto, G.E. Rottinghaus, and T. Zars 2008, Proc. Natl. Acad. Sci. USA 105: 5579–5584; Sullivan, D.T., S.L. Grillo, and R.J. Kitos 1974, J. Exp. Zool. 188: 225–234; Summers, K.M., A.J. Howells, and N.A. Pyliotis 1982, Adv. in Insect Phys. 16: 119-166; Tu, M.P., and M. Tatar 2003, Aging Cell 2(6): 327-333; Wu, C.F., and F. Wong 1977, J. Gen. Physiol. 69: 705–724; Zhang, S.D., and W.F. Odenwald 1995, Proc. Natl. Acad. Sci. 92(12): 5525-9; Zwaan, B., R. Bijlsma, and R.F. Hoekstra 1995, Evolution 49(4): 635–648.

Genetic composition of seven microsatellite DNA loci of females and males from a natural population of *Drosophila mediopunctata* collected in a highland Araucaria forest fragment of Brazil.

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Introduction

As evolutionary processes primarily act shaping the genetic variability of a population, studies on genetic variability of natural populations are extremely important to understand the evolutionary history of a species. *Drosophila mediopunctata* belongs to the *tripunctata* group (Frota-Pessoa, 1954), which is endemic of the Neotropical region and includes 64 species that inhabit forest fragments (Vilela and Bächli, 2000). It is the second largest group of *Drosophila* in this region, and the largest of Neotropical forests (Klaczko, 2006). *Drosophila mediopunctata* is a strictly forest dwelling species that is not associated to human habitats, it has a wide geographic distribution and can be found in Brazil and El Salvador (Val *et al.*, 1981). It is very abundant in some areas, particularly in the south of its distribution or in high altitudes during the coldest months of the year (Saavedra *et al.*, 1995).

Laborda *et al.* (2009) described 134 microsatellite DNA loci for *D. mediopunctata*, and Cavasini *et al.* (2015) establish the chromosomal location of seventeen of these loci, one from each of the five major linkage groups previously published (Laborda *et al.*, 2012), and twelve new loci. So, a very important and well-described genetic marker is available for this species. Thus, the objective of this work was to perform a preliminary analysis of the genetic composition of seven microsatellite DNA loci of naturally collected females of *D. mediopunctata* and their offspring (from which we were able to infer the parental male composition), obtained from a highland Araucaria forest fragment in the South of Brazil.

Material and Methods

This work was performed in a fragment of highland Araucaria Forest phytophysiognomy (Mixed Ombrophylous Forest) of the Atlantic Forest biome, named Parque Municipal das Araucárias (25°23'36" S, 51°27'19" W), where *Araucaria angustifolia* (Coniferae: Araucariaceae) is the predominant vegetal species. This fragment is located in the third plateau of the State of Parana, Brazil, in the subregion named as Plateau of Guarapuava by Maack (1981). According to the climatic classification of Köeppen, this subregion has humid