Conclusion

Based on biochemical investigations, we have found that dietary feeding of Curcumin to *Drosophila* for a short duration has the propensity to attenuate paraquat induced oxidative stress owing to its antioxidative nature and its ability to modulate the activities of antioxidant defenses, such as reduced GSH and antioxidant defenses. Additional evidence, *viz*., lower incidence of paraquat induced mortality and higher resistance to paraquat among flies pretreated with Curcumin, clearly support such a mechanism. Further, its antioxidant property was clearly evident by its ability to significantly abrogate paraquat induced oxidative stress, by depleting the lipidperoxidation product malanoldialdehyde.

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***Drosophila polymorpha* life cycle.**

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Introduction

Many studies on the subject of the *Drosophila* life history have revealed that much of the observed interspecific variability can be explained by genetic interaction and ecological traits (Markow and O'Grady, 2006; Prasad and Joshi, 2003). The life cycle is one of the most important factors that determine the *Drosophila* life history.

There is considerable interspecific variation in each of the *Drosophila*'s life cycle stages, making this type of fly a quite versatile model for life history studies (Jennings, 2011). Thorough knowledge of each developmental stage of *Drosophila* could clarify some evolutionary questions, such as the mechanisms underlying morphological differentiation, and also the ecological results during the speciation process. Our study expands upon the *Drosophila polymorpha* life cycle, from egg to adult. Furthermore, a comparison is made with other species of *Drosophila*, an important factor that leads to a better understanding of evolution within the genus.

Increasing numbers of studies on sexual isolation of *Drosophila* have ensured that there are many inter-specific differences in the reproductive biology for this group that contribute to the speciation process (Coyne *et al*., 1994).

The elucidating life cycle of *Drosophila polymorpha,* from egg to adult, and its age of sexual maturity in particular, are important aspects to be explored, once it can be shown that they have valuable roles involving specific ecological traits. The investigation of these topics also incites good maintenance of the flies stock for its studies.

D. polymorpha belongs to the cardini group, and, as for most of Neotropical species, there are not many studies regarding their reproductive biology and life cycle. Therefore, so far, these topics remain poorly understood.

Material and Methods

Seven different samples of *D. polymorpha* were obtained from two conserved areas in the central and southern regions of Rainforest in the state of Santa Catarina, Brazil: the Parque Estadual da Serra do Tabuleiro (27°48’20"S; 48°33’50"O), and the Reserva Biológica Estadual do Aguaí (27º16'49.55"S; 49º8'31.7"O).

In order to guarantee that all flies were raised under identical conditions, the vials containing a potato flake medium (Bizzo *et al*., 2012) were maintained at 22ºC in a 12-hr light/12-hr dark photoperiodic cycle. With the purpose of facilitating observation of both eggs and larvae, the medium was colored with blue food dye. These conditions were maintained throughout the experiment.

The life cycle was tested using 15 females and 5 males, all of the test objects with a lifespan of one week. They were isolated in a vial containing medium for one hour with the aim of achieving copulation and oviposition. Subsequently the adults were removed, thus ensuring that all eggs were laid at a similar time. During the 12 h of light, the vials were inspected under a stereoscopic microscope every three hours, in order to check the larval development. The experiment was repeated four times, and the oviposition action was considered as the starting point of the cycle.

In the second experiment, 20 couples of flies were collected on the day of hatching and individualized in vials containing a culture medium. During the daylight period, the couples were observed every hour for 15 minutes. Male sexual maturity was considered to be reached once 80% of male flies showed courtship behavior or copulation. The evidence of sexual maturity for females was based on either at least 80% of females allowing copulation or the presence of eggs (Markow and O’Grady, 2006).

Results

During the life cycle experiment of *D. polymorpha*, on the first and second day of the experiment, no larval instars were detected; only eggs were observed. On the third day, the first instar larvae were found in all vials. On the fifth day, second instar larvae were observed, and on the sixth day, third instar larvae. On the seventh day the pupal stage took place, and finally, on the 14th day, adult flies hatched. Thus, the *D. polymorpha* developmental time, from egg to adult, is achieved in 14 days. The whole life cycle is represented in Figure 1.

In the second test, only 6 vials containing the fly couples remained alive until the end of the experiment. We observed two males displaying courtship on the fourth day, three on the fifth day, and six on the sixth day. Regarding the females, one showed receptivity on the fifth day and another on the sixth day. Interesting data showed intraspecific variation: At 8:40 am on the fourth day of experiment, the first male demonstrated courtship behavior, and the first copulation was recorded

only on the next day at the same time. Although these are preliminary results, the age of male sexual maturity is clearly reached on the fifth day after hatching, for females on the sixth day.

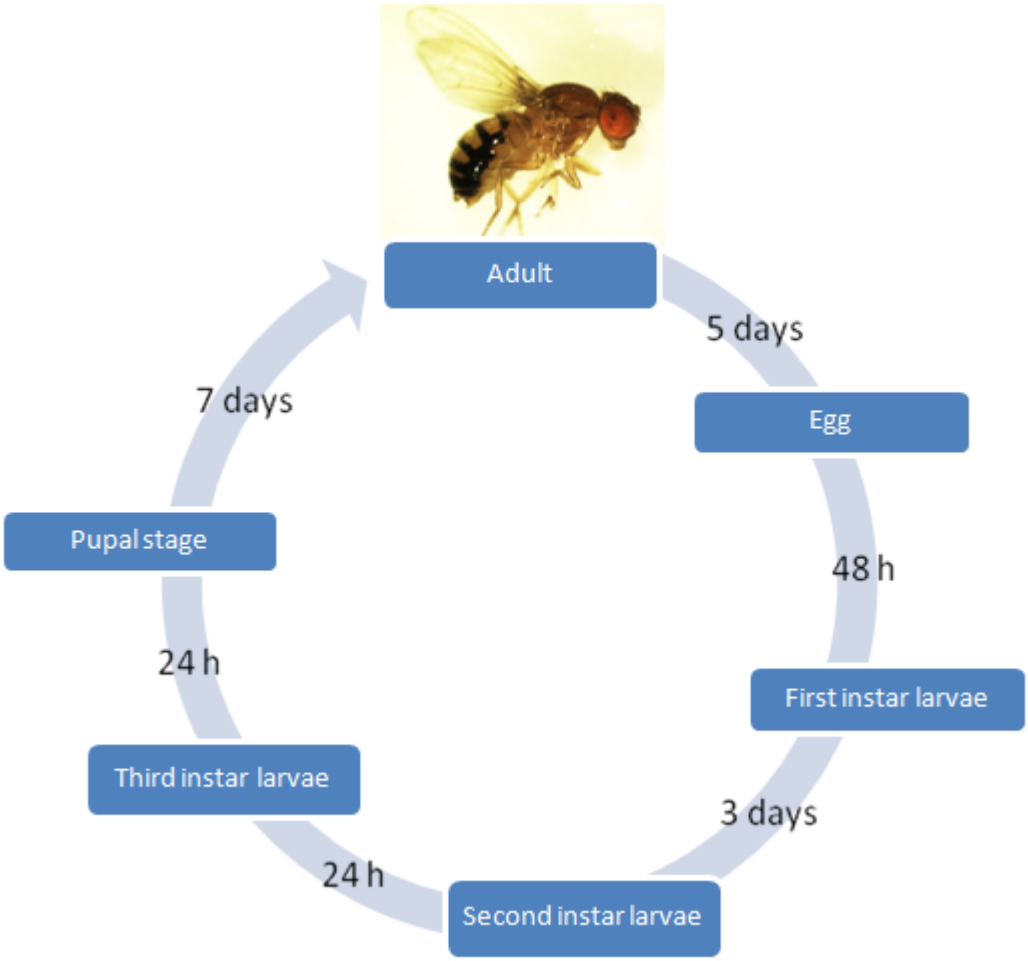

Figure 1. *Drosophila polymorpha* life cycle.

Discussion

The average life cycle of *Drosophila polymorpha* reported on here was 14 days in duration, close to the 15 days of lines at the Tucson *Drosophila* Stock Center under similar temperature conditions (Markow and O'Grady, 2006). It is very difficult to explain variations; however, a number of factors could provide some important insight. The first is culture medium variation, since we use a different recipe compared to Tucson. Bizzo *et al*. (2012) also reported that *D. polymorpha* presented an average 30 days life cycle in corn medium. A second factor is intraspecific genetic variation, since the lines of the Stock Center are from Central America, while ours are from southern South America. A third influential factor is endogamy: Stock Center lines have been maintained for decades in artificial laboratory conditions. The fly lines that were used during this investigation were established in March 2013, which might suggest that the results presented here are in closer resemblance of natural conditions.

Table 1. Egg to adult development time of the *cardini* group at the Tucson *Drosophila* Stock Center.

Species	Days	Temperature (ºC)
subgroup *dunni*		
D. dunni	15	18
D.nigrodunni	15.5	18
subgroup *cardini*		
D. cardinoides	15	18
D. neocardini	15	18
D. parthenogenetica	15	18
D. polymorpha	15	18
D. procardinoides	15	18

Table adapted from Markow & O'Grady, 2006

In any case, the differences were small and were within the expected length for the *cardini* group, as shown in Table 1. The fact that in this experiment the flies were kept at higher temperatures may be the reason for acceleration of the cycle. Also, the San Diego *Drosophila* Stock Center maintains its flies of the *cardini* group at temperatures between 18-25°C, and reports life cycles between 12-16 days, depending on the species and the temperature used. Still, this study enriches the literature, recounting the life cycle in more detail.

For the majority of species, freshly hatched adults are not sexually mature (Markow, 1996), and *D. polymorpha* keep this pattern. In fact, sexual maturity may require up to several weeks, depending on the species (Markow and O'Grady, 2006). While males of some species mature earlier than females, most males mature later than females (Markow and O'Grady, 2008). The results of our work indicate that this species belongs to the first case, similar to *D. melanogaster* that requires 4 days for females to mature and 2 days for males. Equally they are unlike *D. mojavensis* that requires 3 days for females to mature and 7 for males. Furthermore, it can be seen that there is much variation in time between the three species mentioned, a fact that can be justified by the phylogenetic distance between them.

Aiming to map the start of sexual maturity for South Brazilian non-inbreeding lines, more experiments will need to be performed using this species, especially owing to the high mortality rate. Also, the flies' courtship behavior should not necessarily be considered a fully decisive indication of sexual maturity. This is demonstrated by the fact that immature males can achieve copulation without releasing sperm, and females can become sexually receptive before they in fact present mature eggs (Markow, 1996). Furthermore, because metabolic waste from males, present in the culture medium, can change the age at which sexual maturity is reached (Joshi *et al*., 1998), new isolines have been collected in order to reinforce the data obtained from this study.

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Drosophila **collections in the Arc of Deforestation, Brazil.**

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Introduction

The region known as "Arc of Deforestation" covers a massive Amazonian frontier (Figure 1) and shows alarming rates of clearcutting. Close to half of the world's tropical deforestation occurs in