plates. Hsu (1949) mentions that the anal plate is fused with genital arch at the upper half and the number of teeth present on the primary clasper region is 5-6 and they are not similar in size. Whereas the present species has anal plate fused with genital arch at the center and lower side and the number of teeth present on the primary clasper is 4-5 and they are equal in their size (Figure 2b). The collected *D. polychaeta* is very much similar to the characters mentioned by Watabe *et al*. (1990). Species belonging to *polychaeta* species group are rarely reported by *Drosophila* taxonomists across India. *D. daruma* was first reported by Vaidya and Godbole (1976) from Poona and surrounding regions. Similarly, *D. latifshahi* was reported by Gupta and Raychaudhuri (1970) from Chakia forest in North India, but they categorised this species under subgenus *Scaptodrosophila*. Later Toda and Peng (1989) reclassified this species under *polychaeta* species group. These species were reported for the first time from South India by Srinath and Shivanna (2012, 2014) from Dharwad and surrounding areas. *D. polychaeta* is reported for the first time from the Indian subcontinent. Hence this species is the third addition to the present list of species under *polychaeta* species group reported from India.

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References: Bachli, G., 2014, Taxodros: The database on taxonomy of Drosophilidae. URL: http:// www.taxodros.unizh.cw; Bock, I.R., and M.R. Wheeler 1972, Univ. Texas Publ. 7213: 1–102; Fartyal, R.S., M. Saraswat, N. Lhamo, P.C. Sati, and Asha 2014, Dros. Inf. Serv. 97: 119–123; Gupta, J.P., and S.P. Raychaudhuri 1970, Proc. R. Entomol. Soc. (B) Taxonomy 39(5-6): 57-72; Gupta, J.P., 1974, Indian Biologist 5: 7–30; Hegde, S.N., V. Vasudev, and M.S. Krishna 2001, In: *Trends in Wildlife Biodiversity Conservation and Management* (Hosetti, B.B., and M. Venkateswarulu, eds.). Daya Publishers, New Delhi, pp. 55-68; Hsu, T.C., 1949, Univ. Texas Publ. 4920: 80–142; Kandpal, M.C., and B.K. Singh 2010a, Dros. Inf. Serv. 93: 11–20; Markow, T.A., and P.M. O'Grady 2006, Drosophila, *A Guide to Species Identification and Use*. Academic Press, London. 247 pp.; Okada, T., 1956, Systematic Study of Drosophilidae and Allied Families of Japan. Gihodo Co. Ltd., Tokyo, Japan. 183 pp.; Srinath, B.S., and N. Shivanna 2012, Biosystematica 6(1): 39-42; Srinath, B.S., and N. Shivanna 2013, Dros. Inf. Serv. 96: 61–64; Srinath, B.S., and N. Shivanna 2014, Dros. Inf. Serv. 97: 59–63; Toda, M.J., and T.X. Peng 1989, Zool. Sci. 6(1): 155-166; Vaidya, V.G., and N.N. Godbole 1976, J. Univ. Poona 48: 85-92; Watabe, H., X.C. Liang, and W.X. Zhang 1990, Zool. Sci. 7: 459–467.

Interaction of chlorophyllin with radiation-induced autosomal recessive lethals.

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Abstract

Chlorophyllin (SCC), sodium copper chlorophyll, presents protective action against damage induced by different physical and chemical agents. In *Drosophila melanogaster*, this effect has been reported for somatic cells. However, on germ cells for sex chromosomes, the inhibitory effect was not found. We are interested in the lethal induced effect on the second chromosome of this species. Canton-S males were given a 24-hour pre-treatment with and without 69 mM of SCC and later exposed or not to 40 Gy of gamma radiation. Those males were screened with the Cy L /Pm technique for detection of recessive lethal genes. Results showed the SCC pre-treatment did not produce significant changes in frequency for recessive lethal genes in the second chromosome, due to 40 Gy gamma radiation. In order to evaluate the effects of chlorophyllin on damage done by radiation, we considered the presence of autosomal lethals and semi-lethals. We observed that, even with radiation, the frequency of semi-lethals did decrease when chlorophyllin is applied but not significantly. For lethals, either with or without radiation, the frequency slightly increased. Keywords: chlorophyllin, radiation, lethal genes, *Drosophila*

Introduction

For almost three decades, research towards evaluating the modifying action of sodium copper chlorophyll (SCC) on ionizing radiation mutagenicity has been conducted at Instituto Nacional de Investigaciones Nucleares (ININ) *Drosophila* laboratory. SCC is a compound with strong protective action against damage induced by different physical and chemical agents, and it has been evaluated on different biological systems. We are interested in the effect it has on several *Drosophila melanogaster* tests. Graf *et al.* (1984) evaluated its effect through the mutation and somatic recombination test. Zimmering *et al.* (1990) found that, when applied at a concentration of 69 mM as a pre-treatment, the frequency of gamma radiation induced mutation and chromosomal break would significantly decrease. The same concentration for a 24-hour pre-treatment during larval stage was able to prevent damage from gamma radiation (Pimentel *et al.*, 1999).

Based on the fact that X-rays or gamma rays induce somatic recombination with a quadratic dose-response relationship or a two-strike phenomenon (Merriam and Fyffe, 1972) and that, theoretically, this should be valid for all somatic cells, with the results of this research, SCC is suggested to inhibit, on a lower grade, events related to ruptures in comparison with the ones related to mutations (Pimentel *et al.*, 2000).

Regarding SCC's action against damage induced by direct chemical agents, it was found to protect against damage induced by chromium trioxide (Olvera *et al.*, 1997) only during the first two days; however, the effect is reversed and mutation frequency increases (Cruces *et al.*, 2003). The same result was returned against damaged induced by N-ethyl-N-nitrosourea (ENU) (Pimentel *et al*., 2003) and damage induced by dimethylhydrazine; after that time it behaved as a damage promoter (Guerrero, 2004). In addition, it is important to point out that there are very few tests regarding CCS's inhibition or promotion of induced genetic damage on germ cells. Of course, the significance of damage induced on this kind of cells is greater, since it can be transferred through generations. In this regard, studies have been made to detect SCC's effect on tests with and without radiation by sex-linked recessive lethals screening (Pimentel and collaborators, unpublished data), who did not see any protective effect from SCC against radiation. With this background, we set out to achieve determining SCC's effect against gamma radiation keeping in mind the damage it causes to *D. melanogaster*'s second chromosome.

Materials and Methods

Two strains of *D. melanogaster* were used, the Canton-S that acted as treated strain and the Cy L /Pm; H /Sb as the marker one.

Groups of Canton-S males from 3 to 5 days of age were previously treated for 24 hours as follows: two control groups, one fed with 5% sucrose and another one with a 69 mM chlorophyllin solution and two experimental groups that received the same pre-treatment but were then subject to gamma radiation with a 40 Gy dose provided at the Institute's Gamma Cell.

Both the chlorophyllin and the sucrose were administered orally. The study was done by several stages since the work overload impedes the simultaneous analysis on all four treatments. After treatment, males were crossed individually, according to the technique described by (Wallace, 1956) for this species' second chromosome recessive lethals detection, with 2-3 virgin females of strain Cy L /Pm; H /Sb 2-5 days old. From this cross, a Cy L /+ male was extracted from each culture and back-crossed with 2-3 virgin females of mother strain Cy L /Pm; H /Sb.

At time when the offspring of this back-cross emerged, five virgin females were selected per culture, since with these crosses there are also Cy L /Pm and +/+ individuals, and five Cy L /+ males, which were interbred. This series of crosses allows us to track down a chromosome II of each treated male, represented on cross like +/+.

When the offspring of this last crossbreed was born, each culture's counts were done. Since these are Mendelian crosses, it is expected for the progeny to be two kinds of individuals: Cy L /+ (curved wings and partially reduced eyes) and +/+ (wild ones) with a 2:1 proportion. Cultures with this proportion are considered normal, meaning those whose chromosome did not suffer any damage, since deviations from this proportion indicate the presence of damaged genes.

When +/+ individuals do not show, there is a lethal-gene carried in the chromosome and if there are +/+ flies in a frequency about 10% of individuals, with wild +/+ phenotype and genotype, then it carries a semi-lethal gene.

All crosses were conducted at 25 ± 1° C and 60% relative humidity. The supplied medium was the common use one at the lab, made from corn flour, sugar, beer yeast, and agar. Data obtained was screened with an X^2 test to identify differences.

Results

A total of 949 second chromosomes were analyzed, 232 of them were treated only with sucrose, 217 with chlorophyllin, 213 with sucrose and radiation, and 287 with chlorophyllin and radiation; as shown on Table 1, which also indicates the number and frequency of each category of genes: normal, semi-lethal, and lethal ones, calculated from each culture's counts. If we consider the observed frequencies on the treatment with sucrose as a basis, we see that in three out of four possible comparisons –that we are interested in– chlorophyllin itself decreases the frequency of semi-lethals. On the other hand, the comparison that did not decrease was the frequency of lethals when treated without radiation; in this case it slightly increased.

Nevertheless, our goal is to know how significant these differences are, but a direct observation on the relative frequency of the different categories shows that differences are so small that they do not require a statistical analysis because of their similarity.

Discussion

Chlorophyllin's inhibition or promotion effect against radiation varies according to the system used. Among the different systems using *Drosophila*, the one carried out by Zimmering *et al.* (1990) stands out. They obtained evidence of action as radio-protective substance and Pimentel *et al.* (1999) confirm its persistence as radio-protective agent as well as indicate evidence of action as inhibition or promotion agent (Pimentel *et al.*, 2000).

Meanwhile, Olvera *et al.* (1997) consider it an anti-mutagen agent. It is also considered a genetic-damage promotor or inhibitor agent when its effect has been seen against chemical agents such as CrO_3 (Cruces *et al.*, 2003) or against 1,2-dimethylhydrazine (Guerrero, 2004). All of the previous cases dealt with somatic effects since, fundamentally, the assays were carried out via the mutation and somatic recombination test on *Drosophila*'s wing. When dealing with germ cells, the regular test is the sex-linked recessive lethals screening and, in this case, Pimentel and collaborators (on unpublished data) informed us about not having found a promotion or inhibition effect of SCC against radiation when performing this test.

The fact that no inhibition effect was observed on the sex-linked recessive lethals screening Pimentel and collaborators (unpublished) led us to analyze the effect of SCC on damage produced by gamma radiation via this species' second chromosome recessive lethals test.

Table 1. Relative frequency of normal, semi-lethal and lethal genes obtained through sucrose, SCC with and without 40 Gy of gamma radiation treatments.

	Sucrose	Chlorophyllin	Sucrose +40 Gy	Chlorophyllin + 40 Gy
Normal	212; 91.3%	209; 95%	188; 88.3%	256; 89.2%
Semi-lethal	17; 7.3%	6; 2.7%	15; 7%	17; 5.9%
Lethal	3; 1.3%	5; 2.3%	10; 4.7%	14; 4.9%
n	232	220	213	287

The results shown on Table 1 indicate on the semi-lethal gene category when they are a product exclusively of the sucrose or SCC pre-treatment, the effect of the latest is to inhibit the appearance of semi-lethals. In this case, there is a significant difference $P \leq 0.01$. However, when comparing both pre-treatments plus the radiation, this effect did not show up.

Nevertheless, in the case of SCC treatment alone, the frequency of observed lethal frequency did not decrease; it actually increased slightly. However, when besides the SCC there was exposure to radiation, a slight increase was obtained on the frequency of this kind of genes. On the remaining comparisons, to see the effect of chlorophyllin there are no possible significant differences.

The fact that there was no inhibition effect seen on the previously obtained sex-linked recessive lethals test matches with the effect observed by us on the second chromosome, as a result of this assay and with the one observed on the third chromosome on somatic cells. All the above show a similar action of SCC among sex and autosomal chromosomes against gamma radiation effect.

In virtue of the above data, we can conclude that chlorophyllin's action as pre-treatment against gamma radiation has no response that benefits a protective or inhibiting action as it also happens on sex chromosomes, which indicates chlorophyllin does not work on sex nor autosomal chromosomes against gamma radiation.

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References: Cruces, M.P., E. Pimentel, and S. Zimmering 2003, Mutation Res. 536: 139-144; Graf, U., E. Wurgler, and A. Katz 1984, Environ. Mutagen. 6: 153-188; Guerrero, L., 2004, (Thesis), Benemérita Universidad Autónoma de Puebla; Merriam, J., and W. Fyffe 1972, Mutation Res. 14: 309-314; Olvera, O, S. Zimmerimg, M.P. Cruces, E. Pimentel, C. Arceo, J. Guzmán, and M. De la Rosa 1997, In: *Food Factors for Cancer Prevention*. (Ohigasshi, Osawa T. Terao. J. Watanabe S. y Yoshikawa T., eds.). Springer-Verla, Tokio. 567-571; Pimentel, E., M.P. Cruces, and S. Zimmering 1999, Mutation Res. 446: 189-192; Pimentel, E., M.P. Cruces, and S. Zimmering 2000, Mutation Res. 472: 71-74; Wallace, B., 1956, J. Genet. 54: 280-293; Zimmering, S., O. Olvera, M.E. Hernández, M.P. Cruces, C. Arceo, and E. Pimentel 1990, Mutation. Res. 245: 47-49.

What happens when exotic species arrive in a new area? The case of drosophilids in the Brazilian Savanna.

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Abstract

Drosophilids have been widely used as models in scientific research, including the area of biological invasions. In the past two decades, three exotic species of these flies have arrived in the Neotropics. *Zaprionus indianus* was first detected in 1999, and after two years it was widely distributed throughout the Neotropics. Currently, it dominates drosophilid assemblages under specific environmental conditions. *Drosophila suzukii* and *D. nasuta* were recorded in southern Brazil in 2013 and 2015, respectively, and have rapidly expanded their distribution northward. In this paper, we describe the temporal variations in these two recently introduced species in the Brazilian Savanna, a two million-km^2 biome located in the center of South America, where we have been regularly collecting drosophilids since 1998. *Drosophila nasuta* and *D. suzukii* were first detected in the Brazilian Savanna in December 2013. Two years after their arrival, *D. nasuta* seems to have succeeded in colonizing forests, reaching peak abundance in the rainy season, whereas *D. suzukii* remains rare. We conclude that these exotic species differ in their ability to establish viable populations in natural environments and recommend monitoring them to understand the dynamics of the early stages of biological invasion.