Saccharomyces cerevisiae found in the crop of a Neotropical *Drosophila* species fly collected in a natural forest remnant – comments on Hoang, Kopp & Chandler (2015).

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Abstract

Background. Hoang, Kopp & Chandler (2015) questioned the use of commercial *Saccharomyces cerevisiae* as a model for investigating *Drosophila* – yeast association, since this approach "may not be fully representative of host-microbe interactions as they operate in nature". They also claimed: "*S. cerevisiae* is rarely found with natural populations of *D. melanogaster* or other *Drosophila* species". Indeed, previous choice experiments found that *Sophophora* subgenus flies (including invasive species *D. melanogaster*) are more attracted to banana baits inoculated with apiculate yeasts such as *Hanseniaspora uvarum* over *S. cerevisiae* inoculated baits. Yet, the *forest interior dwelling species* (FIDS) *D. tripunctata* group flies choose preferentially *S. cerevisiae* inoculated baits over *H. uvarum* in a natural forest environment.

Aim and Methods. Our objective was to carry out a pilot experiment to examine yeast species associated with *Drosophila* in a natural Atlantic Rainforest fragment, especially examining, the yeast found with FIDS of the *D. tripunctata* group. We sampled *Drosophila* in a natural population from a Neotropical forest fragment. Males were dissected for isolating yeast colonies from their crops and to use their genitalia for species identification. Yeast species were identified by sequencing the D1/D2 domains of the 26S rRNA gene.

Results and Conclusion. We isolated five yeast species from crops of *Drosophila* species of *tripunctata* group, including one strain of *S. cerevisiae* (from *D. paraguayensis*), confirming a previous record of *S. cerevisiae* isolates from a few *tripunctata* group species. Thus, their contention that "the results from *D. melanogaster*–*S. cerevisiae* laboratory experiments may not be fully representative of host–microbe interactions in nature" is probably right, but because *D. melanogaster* is an invasive species that is preferentially attracted in forests to apiculate yeasts, yet *S. cerevisiae* may be associated with FIDS *Drosophila* such as *D. paraguayensis*.

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Introduction

The symbiotic association between yeast and *Drosophila* in natural environments has long been assessed with experiments investigating *Drosophila* species attraction to baits inoculated with different yeast species as well as isolating yeasts from *Drosophila* crops (Dobzhansky & Da Cunha, 1955; Powell, 1997; Buser et al., 2014). A number of differential attractivity experiments have used baits inoculated with various yeast species isolated from *Drosophila* crops and also commercial *Saccharomyces cerevisiae*, as a control treatment (*e.g.*: Da Cunha, Dobzhansky & Sokoloff, 1951; Klaczko, Powell & Taylor, 1983; Becher et al., 2012).

Hoang, Kopp & Chandler (2015) criticized this approach, first, claiming that: "*S. cerevisiae* is rarely found with natural populations of *D. melanogaster* or other *Drosophila* species". To explain the finding of *D. simulans* associated with *S. cerevisiae* in a single study from New Zealand, they argued that it could be due to the unnatural environment (vineyard) where the flies were collected. Furthermore, they carried out a feeding preference experiment in the laboratory with *D. melanogaster*, when they allowed flies to choose between *S. cerevisiae* and another species taken from five natural yeast species. In no case, did the flies prefer *S. cerevisiae* over the other species. Finally, they questioned the overuse of *S. cerevisiae* as a model for studying the fly-yeast relationship, since it "may not be fully representative of host-microbe interactions as they operate in nature."

We collected specimens of *Drosophila tripunctata* species group within an Atlantic Rainforest fragment. This group encompasses 80 species (Bächli, 2016) and is widely distributed over the Neotropical region (Val, Vilela & Marques, 1981; Hatadani et al., 2009). Several species that belong to *D. tripunctata* group are *forest interior dwelling species* (FIDS) of flies and use

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naturally-occurring fruits for feeding and breeding (Mata, Valadão & Tidon, 2015; Machado, Gottschalk & Robe, 2016).

Our objective was to carry out a pilot experiment to examine yeast species associated with *Drosophila* species in a natural Atlantic Rainforest fragment, especially examining, the yeast found with FIDS of the *D. tripunctata* group.

Materials & Methods

We sampled yeast of *Drosophila* crops from an Atlantic Rainforest fragment located at Itatiba, SP, Brazil (23º 00.073' S, 46º 52.917' W; altitude = 740 m) on June 29, 2015. We collected drosophilids by sweeping entomological nets over baits of mashed banana inoculated with commercial *S. cerevisiae* and covered with sterile tulle cloth. Flies were brought to the laboratory and dissected within one hour as suggested by Phaff et al. (1956). Wild males were identified by their external morphology and genitalia (Breuer & Rocha, 1971; Vilela & Bächli, 1990).

Before dissected in a drop of *Drosophila* Ringer's solution, flies were immersed in distilled water and in alcohol 70%, following the procedures described by Hamby et al. (2012). Next, crops were streaked in formulated YM medium (1.0% glucose, 0.5% peptone A, 0.3% yeast extract, 0.3% malt extract, 2.0% agar with Chloramphenicol 1.0%) and incubated at 30°C for 48 hours. Then, genomic DNA of the colonies was extracted as described by Rosa et al. (2009). Regions ITS-D1/D2 of the 26S rRNA gene sequences were amplified according to PCR conditions and protocol described in Rosa et al. (2009).Yeast species were identified submitting the sequences to GenBank database and comparing them to entries for yeast.

Results

Twenty males of different *Drosophila* species had their crop dissected, but only five yeast strains were isolated from five fly specimens sampled of the Itatiba population (Table 1). From two different *D. mediopunctata* males two *Candida* sp. strains were isolated (top BLAST identity was 97% to *Candida sake* strain K2.6.1 and 96% to *Candida sake* strain NRRL Y-1622). A not yet identified yeast species was isolated from *D. frotapessoai*; from *D. unipunctata* a *Starmerella bacillaris* strain was identified with 100% identity to reference strain CBS 13663. Finally, from *D. paraguayensis* crop, *Saccharomyces cerevisiae* was isolated and identified with 100% identity to reference strain NRRL Y-12632.

Table 1 – Yeast strains isolated from crops of *Drosophila* species belonging to the *tripunctata* group, yeast species with top identity compared to sequences submitted in BLAST, with identity and percentage identity to reference accession number.

Yeast strains	*Drosophila* species	Yeast species –BLAST top identity (identity – % identity to reference)
BTC-L1	*Drosophila frotapessoai*	Not identified
BTC-L2	*Drosophila paraguayensis*	*Saccharomyces cerevisiae* (499/499 – 100% to NG042623)
BTD-L1	*Drosophila mediopunctata*	*Candida* sp. (467/483 – 97% to KC485459)
BTD-L2	*Drosophila unipunctata*	*Starmerella bacillaris* (405/405 – 100% to KP346913)
BTD-L3	*Drosophila mediopunctata*	*Candida* sp. (460/478 – 96% to U45728)

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Discussion & Conclusion

Several reports show the diversity of substrates where *Saccharomyces cerevisiae, Starmerella bacillaris* and *Candida sake* have already been found. Particularly, they were found in fruits, grains and in the soil of natural environments (ARS, 2016). Barbosa et al. (2016) reported the occurrence of natural populations of *S. cerevisiae* associated with bark trees in several Brazilian forest ecosystems, including Atlantic Rainforest. The results of this work show that yeast populations of this species are available to *Drosophila* in these ecosystems. Moreover, *Drosophila paraguayensis*, *D. mediopunctata* and its cryptic sibling species *D. unipunctata* have been collected repeatedly in the interior of forests, and adults have emerged from naturally collected fruits (Mata, Valadão & Tidon, 2015; Machado, Gottschalk & Robe, 2016). These are good evidences that they occur naturally within the forest environment.

Experiments of differential attractiveness in the field are important for characterizing the feeding habit differentiation of *Drosophila* species. For example, Klaczko, Powell & Taylor (1983) collected *Drosophila* over baits inoculated with *S. cerevisiae, Kloeckera apiculata* (=*Hanseniaspora uvarum*) and other yeasts in James Reserve, San Jacinto Mountains, USA. They collected fewer specimens of *D. obscura* group and *D. melanogaster* group over baits inoculated with *S. cerevisiae* than *K. apiculata* over baits (796 to 1243 respectively). Yet, flies from subgenus *Drosophila*, such as *D. occidentalis*, were more collected over *S. cerevisiae* baits (295 over 194).

We found a similar pattern in the Itatiba population (Batista et al., 2015). More flies from subgenus *Sophophora* (including invasive species such as *D. melanogaster* and *D. suzukii*, among others) were collected over baits inoculated with *H. uvarum* (68 in a total of 81 = 84%) than over *S. cerevisiae* (13 in 81 = 16%); while flies of the *tripunctata* group (subgenus

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Drosophila) were more attracted to baits inoculated with *S. cerevisiae* (93 in 121 = 77%) than to *H. uvarum* (23%).

Da Cunha, Shehata & De Oliveira (1957) sampled yeasts from crops of *Drosophila* collected in *Serra da Mantiqueira*, Brazil. They found 58.9% out of 17 *S. cerevisiae* isolates were obtained from *tripunctata* species crops, while only 9% out of 24 *H. uvarum* isolates were isolated from flies of the same group. However, the opposite pattern is observed for *willistoni* group (subgenus *Sophophora*), with 58% out of 24 *H. uvarum* isolates obtained and 11.8% of 17 *S. cerevisiae* isolates. Altogether, there are evidences in support of the natural association between *S. cerevisiae* and FIDS of the *D. tripunctata* group; while species of subgenus *Sophophora* such as *D. melanogaster*, may be naturally associated with apiculate yeasts. Thus, Hoang, Kopp & Chandler contention that “the results from *D. melanogaster*–*S. cerevisiae* laboratory experiments may not be fully representative of host–microbe interactions in nature” is probably right, but because *D. melanogaster* is an invasive species that is preferentially attracted in forests to apiculate yeasts, yet *S. cerevisiae* may be associated in natural environments with FIDS *Drosophila* such as *D. paraguayensis.*

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