

Yeast Succession in the Amazon Fruit *Parahancornia amapa* as Resource Partitioning among *Drosophila* spp.

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The succession of yeasts colonizing the fallen ripe amapa fruit, from *Parahancornia amapa*, was examined. The occupation of the substrate depended on both the competitive interactions of yeast species, such as the production of killer toxins, and the selective dispersion by the drosophilid guild of the amapa fruit. The yeast community associated with this Amazon fruit differed from those isolated from other fruits in the same forest. The physiological profile of these yeasts was mostly restricted to the assimilation of a few simple carbon sources, mainly L-sorbose, D-glycerol, DL-lactate, cellobiose, and salicin. Common fruit-associated yeasts of the genera *Kloeckera* and *Hanseniaspora*, *Candida guilliermondii*, and *Candida krusei* colonized fruits during the first three days after the fruit fell. These yeasts were dispersed and served as food for the invader *Drosophila malerkotliana*. The resident flies of the *Drosophila willistonii* group fed selectively on patches of yeasts colonizing fruits 3 to 10 days after the fruit fell. The killer toxin-producing yeasts *Pichia kluyveri* var. *kluyveri* and *Candida fructus* were probably involved in the exclusion of some species during the intermediate stages of fruit deterioration. An increase in pH, inhibiting toxin activity and the depletion of simple sugars, may have promoted an increase in yeast diversity in the later stages of decomposition. The yeast succession provided a patchy environment for the drosophilids sharing this ephemeral substrate.

Drosophila spp. are major vectors of yeasts, and yeasts provide a source of essential nutrients to these flies (34, 39). Some investigators consider *Drosophila* spp. to be ideal scouts for the presence of wild yeasts in the environment (33). Yeasts found in tropical *Drosophila* spp. correspond to those isolated from the presumed feeding substrates, and tropical flies discriminate yeasts to a greater degree than temperate flies (18). This is suggested to be a mechanism to diminish interspecific competition in regions where substrates and fly species are abundant and diverse (10, 28). The coexistence of species sharing ephemeral resources depends upon the nature of the resource and also upon the spatial-temporal fluctuations of both resource and competitors and does not necessarily imply resource partitioning (2, 38). The yeast flora used as food by the flies could be involved in the structuring of *Drosophila* communities. According to Lachaise et al. (23), food partitioning on patches of yeasts by drosophilids living sympatrically in the savanna of Lamto (Africa) reflects the substrate suitability and fly species that overlap in habitat eat different yeasts on the same resources.

Successional patterns of yeast colonization of fruits have been associated with *Drosophila* vectors (22, 26). In immature *Calymnia* figs, the pollinating fig wasp *Blastophaga psenes* introduces a specific microbiota which consists of *Candida guilliermondii* var. *carphophila* and *Serratia plymuthica*. These microorganisms persist and increase in number throughout the ripening period, attracting drosophilae that carry yeasts such as *Hanseniaspora valbyensis*, *Hanseniaspora uvarum*, and *Candida*

stellata, which cause active fermentative spoilage of mature fruits (26). The colonization of *Ficus* figs in the African savanna proceeds with a successive series of drosophilid species according to the microbial colonization of the fig receptacle. *Candida fructus* colonizes the early green receptacle, serving as food for the *Lissocephala* flies, and persists after inoculation of fermentative yeasts by *Zaprionus vittiger* and *Drosophila malerkotliana*. *Pichia membranaefaciens*, *Hanseniaspora valbyensis*, *Hanseniaspora uvarum* and its anamorph *Kloeckera apiculata*, and *Candida sorboxylosa* cause rapid fermentative breakdown (souring) of the fig and stimulate oviposition by *Drosophila fima*, *Drosophila greeni*, and *Zaprionus ornatus*. Thus, the fig may be regarded as a microhabitat for *Drosophila*-yeast interactions, and sequential fermentation of figs could be considered as a succession of microhabitat patches. Yeast diversity increases with ripening of the fruits, and thus, differential fig exploitation in time greatly decreases interspecific competitive pressure. Also, the habitat patches corresponding to yeast colonies allow different feeding sites to be available to drosophilids (22). Temporal variation in yeast species diversity, which increases as the resource ages, is found in cacao pods and *Opuntia* necrotic tissues (3, 12). The temporal variation should be mainly due to succession. A diagonal pattern of population colonization and extinction is also observed for the bacterial community of agria cactus rots when the presence of individual species versus temporal isolation is recorded. The average number of bacterial species increases with time until the community stabilizes, at the maximum diversity, and remains constant as long as the community remains stable (14). A succession of yeast species was demonstrated during wood degradation by the white rot fungus *Ganoderma applanatum* in Chilean rain forests (16). Different stages of decay in decomposing logs of *Pseudotsuga menziesii* also show yeast species succession over time, resulting in the dominance of ascomycetes over basidiomycetous species (9).

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TABLE 4. Yeast diversity and niche measures of the *Drosophila* spp. feeding and breeding on fruits of amapa

Host	Sex ^a	Yeast species diversity ^b		Feeding niche ^d	Fruit	Diet overlap ^e		
		WP ^c	Crop			<i>D. willistoni</i> group		<i>D. malerkotliana</i> (F)
						F	M	
<i>D. willistoni</i> sp. group ^f	F	8.5	3.2	0.18	0.23			
	M	6.5	2.9	0.21	0.23	0.39		
<i>D. malerkotliana</i>	F	2.2	1.1	0.43	0.28	0.48	0.48	
	M	3.0	0.9	0.43	0.27	0.49	0.50	0.69
<i>D. sturtevantii</i>	F	2.8	1.6	0.41	0.39			
	M	1.1	0.6	0.33	0.28			

^a F, female; M, male.^b Calculated by the method of Kvalseth (21).^c WP, flies that walked on plates; Crop, crops of flies.^d Reference 19.^e Reference 31. The number of samples of *D. sturtevantii* was not sufficient for analysis.^f Not identified to the species level.

range of 28 to 37°C, which includes the maximum growth temperatures of most yeasts (4, 5). This may have contributed to the variation in response to temperature among the yeast strains, since the environmental heterogeneity of a habitat is predicted to promote greater variation in characteristics of populations living there (37, 44).

The higher yeast species diversity in amapa fruits at Mocambo compared with the diversity at Bacurizal could have been due to the different levels of heterogeneity of the two areas. Bacurizal has unusually high frequencies of amapa and bacuri trees, whereas Mocambo is a protected area with a high plant species diversity (7). Yeast species composition and diversity could indicate habitat heterogeneity in these ecosystems, as was noted previously for yeasts associated with *Drosophila* spp. in forests of Rio de Janeiro (27). Environmental degradation and patch removal seem to extinguish populations and decrease diversity (32). The fruits of *Anacardium giganteum*, the *Helycostis* sp., and *Platonia insignis* were colonized predominantly by fermentative yeast species. The nonedible fruit of *Clusia grandiflora*, with medicinal properties (8), had a higher yeast species diversity than the fruits studied other than amapa. The yeasts, except for *K. apiculata*, isolated from this fruit were different from those of other communities. Some of these yeasts, such as the black yeasts and *Cryptococcus humicola*, are regarded as typical inhabitants of leaf surfaces and soils (34).

The fallen amapa fruits are among the most important sites of oviposition and sources of nutrition for *Drosophila* species and other insects in the sites studied (25). The volatile cues produced from the fermentative breakdown of fruits by the initial microbial colonizers attracted the *Drosophila* flies, which in turn inoculated new yeast species. The yeast growth and metabolism contributed to modification of the fruit tissues and probably to production of a hospitable substrate for both larvae and adult flies. Volatile cues resulting from the microorganism-substrate interaction may stimulate mating and oviposition of the flies (13). The most frequently isolated yeasts represented typical fruit-associated yeasts such as the ubiquitous species *K. apiculata*, which is also associated with spoiling fruits and *Drosophila* spp., in temperate ecosystems (34). *P. kluyveri* var. *kluyveri* is a killer toxin-producing yeast commonly isolated from rotting fruits such as tomatoes (11, 41) and oranges (42) and from cactus necrotic tissues and fruits (40). We have isolated this yeast only from amapa fruit and rarely from *Drosophila* spp. in the present study. *C. krusei* and the new species *C. amapae* (29) were isolated only from amapa fruit,

not from *Drosophila* vectors, and so they may have been inoculated by other vectors prior to deterioration of the fruits. *C. amapae* could also be an autochthonous resident of the amapa fruit, since it was not isolated from other sources.

The yeast species diversity increased with deterioration of the fallen amapa fruit (Table 3). The availability of simple sugars and favorable pH may have resulted in the high yeast species diversity and counts in the initial phases of deterioration. Yeasts colonizing the early phases of the fallen fruit had physiological profiles restricted mostly to assimilating a few simple sugars together with rapid fermentation of glucose and sucrose. The intense visiting of amapa fruits by *Drosophila* spp. and other insects on the second and third days after the fruit fell probably inoculated yeasts, including *C. guilliermondii*, the *C. sorboxylosa*-like complex, *K. apiculata*, the *P. kluyveri*-like complex, a *Geotrichum* sp., *P. kluyveri* var. *kluyveri*, *Pichia pijperi*, and the killer- and non-killer-producing *C. fructus* strains. Some species that colonized fruit in the early stages were replaced by other species. The increase in competition for resources and the lower availability of simple sugars probably caused the decrease in populations from the fourth day to the tenth day. Killer activity is a mechanism of competitive interference conferring an advantage during the early stages of population growth in ephemeral substrates such as fruits (15, 40, 41). The presence of killer yeasts may also have contributed to the decrease in diversity observed on the fourth day. For example, the killer activity of *P. kluyveri* var. *kluyveri* and *C. fructus* could have interfered with the sensitive strains of *Candida rugopelliculosa*, *C. sorbosa*-like complex, *C. sorboxylosa*-like complex, *Issatchenkia occidentalis*, *K. apis*, *Pichia acaciae*, and *P. kluyveri*-like complex (1), which disappeared from fruits on the fourth to sixth days. The yeast diversity increase on the sixth day could have been due to the cessation of the killer activity when the pH of the fruit increased from 4.0 to 6.0. This higher pH inactivated the killer factors of both the *P. kluyveri* var. *kluyveri* and *C. fructus* strains isolated from the amapa fruit (1). The lower diversity on the eighth day could have resulted from the exhaustion of soluble nutrients since yeasts with broader physiological abilities were isolated from fruits 10 to 14 days after the fruits fell. In the successional environment, the species with the highest reproductive rate under the current growth conditions is expected to dominate by outgrowing other species (6) and successional stages would correspond to different patches of habitat available to the yeasts.

The drosophilid community utilizing the fallen amapa fruits as resources transported the yeasts colonizing fruit in the early