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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 willistoni* 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 florae 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 *Calymirna* figs, the pollinating fig wasp *Blastophaga psenes* introduces a specific microbiota which consists of *Candida guilliermondii* var. *carpophila* 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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In this paper we describe the succession of yeasts in the ripe fallen amapa (*Parahancornia amapa*, Apocynaceae) fruit and try to demonstrate the interactions that determine the successional pattern of colonization. The *Drosophila* species feeding preferences for different yeasts may be at least partially responsible for the yeast distribution along temporal succession in the amapa fruit. We found that yeast species competitive interactions through the production of killer toxins may also play a role in shaping the yeast community of the amapa fruit.

MATERIALS AND METHODS

Sampling sites. Samples were taken at the Mocambo Forest Reserve site during January, February, and March of 1991 and 1992 and in the Salvaterra Ecological Reserve in February and March of 1992. The Mocambo Forest Reserve is situated near the city of Belém and belongs to the Rio Guama Research Station. This upland (terra-firme) forest area corresponds to 5.7 ha surrounded by a seasonally flooded (igapo) forest. The Bacurizal Forest is a disturbed area used extensively for gathering wood and fruit from the Salvaterra Ecological Reserve, on Marajó Island, Pará. The amapa (*Parahancornia amapa*) is an endemic fruit tree distributed in the upland and seasonally flooded forests of the Amazon Region. The tree is usually 40 to 50 m high and produces large fruits with thick skin and two indehiscent mericarps: a fibrous external layer and a soft fleshy inner layer involving the flat seeds. The fruits are edible and are known to serve as food to animals such as monkeys living in the forest canopy. They are visited by insects, including *Drosophila* species, that feed and breed on the deteriorating fruits on the ground (25).

Isolation of yeasts from Amazonian fruits. The yeasts were isolated in random collections during January, February, and March during the fruiting period of the amapa tree. The fallen fruits of amapa were numbered and labeled with plastic cards attached to wooden stakes placed in the soil near each fruit. The fruits were collected aseptically in sterile plastic bags and taken to the laboratory within a maximum of 5 h for processing. Fallen fruits of *Anacardium giganteum* (cajuí, Anacardiaceae), *Clusia grandiflora* (cebola-da-mata, Clusiaceae), a *Helycostis* sp. (Moraceae), and *Platonia insignis* (bacuri, Guttiferae) were collected and processed as noted for amapa fruits. The "fruit" of *A. giganteum* is a pear-shaped receptacle bearing an external kidney-shaped nut. The fleshy receptacle, which was sampled for yeast isolation, has a thin red skin and soft edible acid pulp. The fruits of *C. grandiflora* are similar in form to an onion but larger and presenting a thick skin and fibrous sticky layers intermixed with an oleaginous pulp. The *Helycostis* fruits have a hard coat and firm pulp. The fruit of *P. insignis* has a thick coat, a tough resinous external mesocarp, and a sugary soft flesh intermixed with the seeds. Unwashed whole fruits or parts of fruits broken during falling or partially eaten by animals were homogenized individually in a blender with 10% (wt/vol) sterile 0.85% NaCl, and 5 ml containing 0.5 g of each was further homogenized in a handheld glass Teflon tissue homogenizer. The pH was measured with indicator paper (Merck). A loopful of the homogenized tissue was streaked directly on YMA (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose, 2% agar supplemented with 100 mg of chloramphenicol per liter, adjusted to pH 3.7 to 4.0 with 0.7% 1 N HCl) plates. Plates were incubated at room temperature ($25 \pm 3°C$), and yeasts were isolated. These data are described as the frequency of occurrence of yeasts, representing the presence or absence of each yeast species in each sample of fruit. Successive decimal dilutions of the homogenized tissue were spread on the YMA plates for the amapa and other fruits sampled, and yeast counts are reported as CFU per gram of fruit; representative colonies were isolated.

Yeast succession during amapa fruit deterioration. Fruits (13 to 20 of each age) were collected randomly during 14 days after they fell to the ground from one amapa tree in the Mocambo Forest Reserve during February and March of 1992 and processed as described above. The area under the tree canopy and its limits were defined prior to the experiments, and fruits and fruit residues were removed from this area. Falling of the fruits was monitored, and each fruit was labeled with a numbered plastic card on the day it fell.

Isolation of yeasts from flies. *Drosophila* flies were aspirated (27, 28) from the surroundings of the ripe fallen amapa fruits and transferred immediately to petri dishes containing YMA, where they were allowed to walk for 5 to 8 h and then recovered for identification. The yeasts obtained from these flies are carried predominantly on the external surface and should include, as minor components, yeasts from regurgitation and fecal pellets and also yeasts carried on the ovipositors of females. These yeasts have the potential to be dispersed to new environments and represented the populations for which the flies served as vectors. Another set of flies were surface disinfected in 70% ethanol for 1 min and transported to the laboratory in tubes containing sterile saline (0.85% NaCl) solution. After being identified, the flies were reexposed to 70% ethanol for about 30 s and dissected. Crops obtained from them were streaked on YMA. The yeasts isolated from crops represented the species used as food by the flies.

Yeast identification. Individual yeast colonies of each morphological type were purified and characterized by standard methods (43). The Diazonium Blue B test was done on 3-day-old yeast-carbon base-urea agar slants (17). The production of and resistance to killer factors were tested on YMA with 0.03% methylene blue (45), and the pH was adjusted to between 3.2 and 7.0 with citrate buffer. *Candida glabrata* NCYC 388 was used as the sensitive strain (41). Yeasts were identified according to the keys of Kreger-van Rij (20) and Barnett et al. (4). The species-like epithet was used to designate strains differing in a few (usually three to five) tests from the standard description of the species. The designation of complexes was due to variations in growth temperature, fermentative ability, and assimilation of D-glucosamine, glycerol, DL-lactic acid, succinic acid, and citric acid among the strains.

Data analysis. Yeast species diversity was calculated for each fruit species and also for the transported yeasts and crops of the flies. Species diversity was measured by the odds measure of diversity, OD, where $OD = (\Sigma p^2)^{-1} - 1$, which describes the odds that two randomly selected individuals of the same sample will be of different species (21). p corresponds to the proportion of a given species i in a sample j, calculated as the frequency of the occurrence of i/total frequency of the n species in the sample j. The feeding niches of adults and larvae were calculated by the average niche width measure of Hulbert (19). Overlap between yeasts isolated from the flies and the amapa fruit yeast communities as well as diet overlap were measured with Morisita's index of similarity (31). The overlap between yeasts from the crops and from the amapa fruits is expected to show the utilization of this yeast community as food by the *Drosophila* spp.

RESULTS

Yeast community of the fruits. The yeast population in the amapa fruits was generally between 5×10^5 and 1×10^6 CFU/g during 14 days after falling, with a peak of 2×10^7 and 8×10^6 CFU/g on days 2 and 3, respectively. The fruits of *Anacardium giganteum*, *Clusia grandiflora*, the *Helycostis* sp., and *Platonia insignis* had yeast populations of 5×10^4 CFU/g. *K. apiculata* (anamorph of *Hanseniaspora uvarum*) and *Candida norvegensis* (anamorph of *Pichia norvegensis*) constituted the highest populations in amapa fruits. The most frequent species in amapa fruits were *K. apiculata*, representing 16% of the total isolated yeasts, *Kloeckera apis* (anamorph of *Hanseniaspora guilliermondii*), *Candida amapae* (possible anamorph of *Endomycopsella crataegensis*), *Candida krusei* (anamorph of *Issatchenkia orientalis*), *Candida sorbosa*-like complex, *C. fructus*, *C. sorboxylosa*-like complex, and *Pichia kluyveri* var. *kluyveri* (Table 1). These species constituted 50% of the total isolates. In the amapa fruits collected at the Mocambo site, *K. apiculata*, *C. amapae*, *C. krusei*, *C. sorbosa*-like complex, *P. kluyveri* var. *kluyveri*, and *C. sorboxylosa*-like complex were predominant. In the Bacurizal Forest site, *K. apiculata* and *K. apis* were the most frequently isolated species. Twenty-one strains of *P. kluyveri* var. *kluyveri* and four strains of *C. fructus* were isolated from amapa fruits and had killer activity against *C. glabrata* NCYC 388 in a pH range of 3.8 to 4.6. The most frequently isolated species in fruits of *Anacardium giganteum* were *C. guilliermondii* (anamorph of *Pichia guilliermondii*), *K. apiculata*, and the *P. membranaefaciens*-like complex. In *Helycostis* fruits, *K. apiculata*, *K. apis*, and *C. sorboxylosa*-like complex were the most frequently isolated yeasts. In the *Platonia insignis* fruits, *K. apiculata* and *C. sorboxylosa*-like complex were commonly isolated. In *Clusia grandiflora* fruits, black yeasts, *Cryptococcus humicolus*, and *K. apiculata* were frequently isolated. The yeast species diversity of the different fruit species is shown in Table 2.

Physiological abilities of the yeast community associated with the amapa fruit were mostly restricted to the use of glycerol, cellobiose, DL-lactate, salicin, and L-sorbose among the 36 carbon compounds tested (36). Fermentative species made up more than 82% of the isolates, as was expected for yeasts associated with deteriorating fruits. The frequency of yeasts capable of growing at 37°C was high compared with that of other tropical isolates (27, 30). Growth at 37°C was positive for strains of *Candida citrea*, *Candida deformans*-like, *C. fructus*, *Candida karawaiewii*, and *C. norvegensis*, contrary to their descriptions, and for *Candida holmii* and *Candida diversa*, described as variable for this characteristic. Among our isolates,

TABLE 1. Number of yeast isolates from fruits and *Drosophila* flies in two Amazon rain forest sites

Yeast	Fruits[a]						*Drosophila* surfaces						*Drosophila* crops					
	Amapa		Caj (10)	Hel (10)	Bac (10)	Clu (9)	Will[b]		Mal		Stu		Will[b]		Mal		Stu	
	Mo[c] (99[e])	Ba[c] (28)					F[d] (19)	M (26)	F (10)	M (16)	F (5)	M (6)	F[d] (26)	M (39)	F (11)	M (9)	F (3)	M (3)
Black yeasts						6												
C. amapae sp. nov.[f]	29																	
Candida berthetii	1						1	2			1	1						
C. blankii-like[g]	1																	
C. citrea	1	3					5	9										
C. deformans-like						1												
C. dendrica						1												
Candida diddensiae-like						1												
C. diversa	18						5	3										
C. fructus	15	8	1							3				2	1			
C. guilliermondii	2		9		5									1				
C. holmii						1												
C. insectamans	10						2	2					10	15	1			
C. karawaiewii	4	2																
C. krusei	27																	
C. lipolytica-like	2																	
C. norvegensis	5		1	2	1								3	6				
Candida parapsilosis	1	7																
C. quercitrusa-like	2																	
C. rugopelliculosa-like	1																	
C. sorbosa-like complex[h]	25		5				9	7					8	1				
C. sorboxylosa-like complex	51	8		8	8				2	6				5	3	1		
Candida tereba-like										1								
C. versatilis	1																	
Cryptococcus humicolus						5		1										
Debaryomyces vanriji var. *yarrowii*	10																	
Geotrichum sp.	5	9		2		1	2										1	
H. guilliermondii/K. apis	24	18		8	1		1		1				2		1			
H. occidentalis	1		1				3									1		
I. occidentalis	1																	
Kloeckera africana	1	3																
K. apiculata	60	35	9	10	9	5	12	20	10	14	4	5	26	34	11	9	2	3
Kloeckera japonica		6																
P. acaciae	1										2							
Pichia beckii									2	2	2							
Pichia etchelsii	7												3					
P. fermentans			3		2	2		1										
P. kluyveri var. *kluyveri*	21						1											
P. kluyveri-like	26	9					8	2	3	3				2				
P. membranaefaciens-like	19	13	10	5	6		20	24	3	8			1	15	1	1		
Pichia muscicola-like	1																	
P. pijperi	13												2	1				1
Pichia sargentensis-like	3																	
Prototheca sp.[i]	7			6	1							2						
Total no.	396	121	39	35	38	24	69	72	21	36	9	8	55	82	16	13	4	4

[a] Fruit species: Amapa, *Parahancornia amapa*; Caj, *Anacardium giganteum* (cajuí); Hel, *Helicostys* sp.; Bac, *Platonia insignis* (bacuri); Clu, *Clusia grandiflora*.
[b] Flies: Will, *D. willistoni* group; Mal, *D. malerkotliana*; Stu, *D. sturtevanti*.
[c] Site of collection: Mo, Mocambo Forest; Ba, Bacurizal area.
[d] Sex: F, females; M, males.
[e] Number of samples.
[f] New species (29).
[g] Probable new species similar to the species indicated.
[h] Heterogeneous group of strains that represent different varieties, presenting variable physiological reactions to fermentation and growth temperatures.
[i] Yeast-like colorless algae.

only *Candida dendrica* and *Hanseniaspora* and *Kloeckera* spp. other than *H. guilliermondii* and its anamorph *K. apis* failed to grow at 37°C. Ten strains of *C. fructus*, eight strains of *C. guilliermondii*, two strains of *Pichia fermentans*, and five strains of *Pichia pijperi* grew at 40°C. The *C. krusei*-like, *C. sorbosa*-like, and *C. sorboxylosa*-like complexes presented similar as-

similation profiles, with few positive results and variations in response to assimilation of D-glucosamine, DL-lactic acid, succinic acid, and citric acid and to maximum temperature of growth and fermentation of glucose. The *C. krusei*-like complex comprised those strains negative for L-sorbose assimilation and positive for growth at 40°C and 12 of 55 strains

TABLE 2. Yeast species diversity in the fruits

Fruit species	Locality	Diversity
Parahancornia amapa	Mocambo	11.70
Parahancornia amapa	Bacurizal	4.06
Anacardium giganteum	Mocambo	4.41
Helycostis sp.	Mocambo	3.69
Clusia grandiflora	Mocambo	6.29
Platonia insignis	Bacurizal	4.82

positive for growth at 42°C. The *C. sorbosa*-like complex corresponded to those strains positive for L-sorbose assimilation, negative for D-xylose assimilation, and variable for growth at 40°C. The *C. sorboxylosa*-like complex included the strains that assimilated both L-sorbose and D-xylose and included 59 strains positive for growth at 40°C. The strains of the *Pichia* complexes were considered to be *P. kluyveri*-like when they fermented glucose vigorously and *P. membranaefaciens*-like when they were weakly fermentative or nonfermentative. Both complexes presented variation in growth on L-sorbose, D-glycerol, DL-lactic acid, succinic acid, and citric acid and in growth at high temperatures (40 and 42°C) among the strains. Of 55 *P. kluyveri* complex strains, 28 grew at 40°C and 12 grew at 42°C; of 126 *P. membranaefaciens*-like complex strains, 74 grew at 40°C and 32 grew at 42°C.

Yeast succession on the ripe amapa fruits. The yeast populations and species frequencies fluctuated during deterioration of the amapa fruit. The yeast species diversity and population numbers in the fruits are presented in Table 3. The order of occurrence of the most frequent (>10% of the total isolates) yeast species of the amapa fruits during 14 days after falling from the trees is shown in Fig. 1. The first isolation of each species demonstrates a diagonal trend representing succession. Five species were frequently isolated on the day the fruits fell, and three of them were not isolated after the third day. Eight species were frequent on fruits from days 1 to 6. Four species were consistently isolated from days 3 to 8 and represented a third stage of succession. Likewise, four species isolated from days 8 to 14 had not been previously detected and represented a later successional stage. Two species, *C. krusei* and *C. amapae*, were isolated throughout the course of the succession.

Fluctuations in the populations of individual yeast species during the deterioration of the amapa fruit varied from 2×10^5 to 34×10^5 CFU/g on the day the fruits fell and on day 1. On day 2, *K. apis* and *K. apiculata* had the highest counts, 1.1×10^7 and 9.26×10^6 CFU/g, respectively. These species were no longer detected on days 3 and 4. On day 3, individual species

TABLE 3. Yeast species diversity during successional stages of deterioration of the ripe *Parahancornia amapa* fruit after its fall to the ground

Days after falling	Yeast population, CFU/g	Odds diversity measure
0	5.2×10^5	6.1
1	4.5×10^7	6.7
2	8.4×10^8	9.9
3	4.2×10^8	11.7
4	4.6×10^7	8.6
6	4.5×10^7	11.2
8	5.0×10^4	5.9
10	1.7×10^6	15.7
12	1.4×10^6	16.0
14	1.7×10^6	16.8
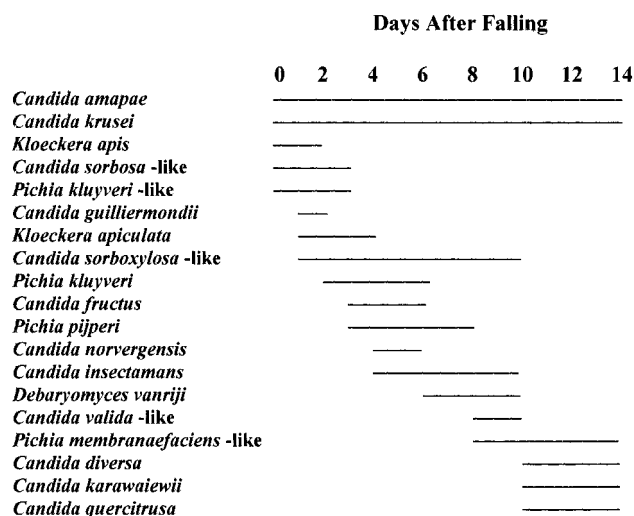
FIG. 1. Order of appearance of the most frequently isolated yeast species (<10% of the total) colonizing the successively deteriorated *Parahancornia amapa* fruit.

reached stable counts around 1.0×10^6 to 4.0×10^6 CFU/g. The killer yeasts *P. kluyveri* var. *kluyveri* and *C. fructus* were isolated from days 2 and 3 to day 6. After the day 8, the lower but stable counts included species not isolated in the early stages of deterioration.

Yeast-*Drosophila* interactions. The yeasts most frequently vectored by the flies included *K. apiculata*, *P. membranaefaciens*-like complex, and *C. citrea*. The most frequently isolated yeasts in the crops of flies were *K. apiculata*, which represented 45% of the isolates, *Candida insectamans*, and the *P. membranaefaciens*-like complex. Among the males of the *Drosophila willistoni* group, *C. insectamans* was isolated only from *D. willistoni* and was the most frequently isolated yeast in crops of these flies. The *P. membranaefaciens*-like complex was frequently isolated from crops of *Drosophila paulistorum* only, and it was isolated only rarely from crops of females of this and other groups. *Drosophila tropicalis* was frequently associated with *C. norvegensis*, *C. fructus*, and varieties of the *C. sorboxylosa*-like complex. The six isolates of *C. norvegensis* were obtained from crops of *D. tropicalis* males and, together with two isolates of *C. fructus* and three isolates of the *C. sorbosa*-like complex, constituted the total yeast isolates in crops of these flies. Males of the three *D. willistoni* group species had a low diet overlap, 0.26 between *D. willistoni* and *D. paulistorum* and 0.12 between *D. tropicalis* and each of the two other species of the group. The species diversity of yeasts transported by, and from the crops of, the flies and also the measures of the feeding niche and diet overlap are shown in Table 4.

DISCUSSION

Many of the isolates varied from the descriptions of known species, confirming the need for further taxonomic studies of yeasts from tropical forests. Five groups were assigned to species complexes owing to the inability to differentiate within each group on the basis of the physiological and morphological characteristics used in conventional taxonomy. These varieties differ in response to a few carbon sources as well as in fermentation of glucose or maximum temperature of growth. Rain forests present high temperatures, with variation of up to 6°C between night and day, and high temperatures typically in the

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 | | | *D. willistoni* group | | *D. malerkotliana* (F) |
						F	M	
D. willistoni sp. group[f]	F	8.5	3.2	0.18	0.23			
	M	6.5	2.9	0.21	0.23	0.39		
D. malerkotliana	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
D. sturtevanti	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 Kvålseth (21).
[c] WP, flies that walked on plates; Crop, crops of flies.
[d] Reference 19.
[e] Reference 31. The number of samples of *D. sturtevanti* 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 humicolus*, 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

stages but probably not fruit 10 to 14 days after its fall. The yeasts obtained during the last phase of deterioration, such as the *Candida blankii*-like complex, *C. diversa*, *C. karawaiewii*, the *Candida lipolytica*-like complex, *Candida quercitrusa*, the *C. rugopelliculosa*-like complex, and *Candida versatilis* were not isolated from the flies. This indicated that *Drosophila* spp. were not the only vectors of the yeasts associated with amapa fruits. Vectoring by the flies of some yeast species not found in fruits indicated that these *Drosophila* species visited other habitats in the forest. The species diversity of yeasts transported by females was greater than that transported by males, suggesting that females visited a greater variety of substrates than males, probably in search of oviposition sites (28). *Drosophila* species fed selectively on the yeasts of ripe amapa fruits, since yeasts transported by the flies were more diverse than those found in their crops. The low values of diet overlap with the yeast community of the fruit also indicate selective feeding behavior. The amapa fruit yeasts contributed extensively to the diet of these *Drosophila* spp. because only yeasts from the amapa fruit community were recovered from their crops.

Differential exploitation of fruits and segregation in different fruits depending on their stage of deterioration could explain the coexistence of different *Drosophila* species in the amapa fruit habitat. Different preferences and strong feeding niche separation among the flies were not noted, except among the *D. willistoni* group species, in which males of different species fed predominantly on different yeasts (data not shown). The *D. willistoni* group species are forest residents and may rely on diet partitioning to avoid competition among closely related species, as was noted for this group in forests of Rio de Janeiro (27). These flies were associated with *C. insectamans* and the *P. kluyveri*-like complex, which were found in higher frequencies in their crops than in the fruits and colonized the fruits only 6 to 10 days after the fruit fell. The opportunistic invader *D. malerkotliana* (24) apparently used the most abundant and frequent species in fruits, *K. apiculata*, indiscriminately in the early phases of fruit deterioration. The preferences for different yeast species among the *D. willistoni* flies may be a partitioning of the yeast dimension of their feeding niche to preserve a minimal resource overlap (22, 35). The niche separation between the *D. willistoni* group and *D. malerkotliana* occurs through the differential occupation of the habitat in time, leading to differential dispersion of yeasts among fruits of different ages. The different stages of deterioration of the amapa fruit should be regarded as a microhabitat mosaic in which fly species coexist in some patches but are segregated in others. The yeast species with higher populations and growth rates should attract and be dispersed by the early colonizers, mainly *D. malerkotliana*, but these yeasts were unable to maintain high populations in the intermediate to later stages of deterioration. Species associated with the *D. willistoni* group and other forest resident flies occupied the later stages of fruit deterioration. Studies of yeast diversity in fruits should be planned so as to cover the various stages of succession if they are to show the true extent of diversity in these microhabitats.

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