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Comparisons of Yeast Florae from Natural Substrates and Larval Guts of Southwestern *Drosophila*

James C. Fogleman[1], William T. Starmer[2], and William B. Heed[1]

[1] Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721, USA
[2] Department of Biology, Syracuse University, Syracuse, New York 13210, USA

Summary. The yeast florae in the natural substrates of four desert and three non-desert *Drosophila* species were compared both qualitatively and quantatively to the yeast present in the guts of *Drosophila* larvae living in those substrates. The desert species breed in rotting cacti and the other *Drosophila* were found breeding in necrotic oranges. Larvae of one cactophilic species, *D. mojavensis*, and larvae of all of the species utilizing oranges (*D. melanogaster*, *D. pseudoobscura*, and *D. arizonensis*) were found to contain non-random samples of the yeasts available in their respective substrates. Larval preference behavior is most likely responsible for these differences. The other cactophilic *Drosophila* (*D. nigrospiracula*, *D. mettleri*, and *D. pachea*) did not exhibit significant differences when the yeast florae of their larvae and substrates were compared. Selective feeding by larvae appears to be related to the degree of polyphagy in that only larvae of polyphagous species are selective. Trade-off between generalism and specialism at two biological levels is discussed.

Introduction

There are two geographic regions which have served as centers for research on *Drosophila* ecology: the Hawaiian Islands and the Sonoran Desert (Zouros, 1974). Each area has its interesting and unique aspects which are primarily responsible for its popularity. The characteristics of the Sonoran Desert which make it appropriate for the study of *Drosophila* ecology are its relative simplicity as an ecosystem and, because of this, the fact that the breeding and feeding sites of endemic *Drosophila* species are well known (Fellows and Heed 1972; Heed 1977, 1978). The four species of *Drosophila* that are endemic to the Sonoran Desert are: *D. pachea*, *D. nigrospiracula*, *D. mettleri*, and *D. mojavensis*. These cactophilic flies utilize necrotic sections of the giant columnar cacti (or soil which has been inundated with juices from necrotic tissue) for all stages of their life cycles. The major insect-host plant relationships are: *D. pachea*-senita (*Lophocereus shottii*), *D. nigrospiracula*-saguaro (*Carneigea gigantea*) and cardón (*Pachycereus pringlei*), *D. mettleri*-soaked soils from saguaro and cardón, and *D. mojavensis*-agria (*Stenocereus gummosus*) and organpipe (*Stenocereus thurberi*).

Food sources are important aspects of the ecology of animals, and yeasts are considered to be the major adult and larval food sources for most *Drosophila* species (Begon 1981). Although research concerned with the micro-organisms upon which *Drosophila* feed dates back about 35 years, the taxonomic knowledge and the techniques necessary for the accurate characterization of the yeast florae of both cactus rots and *Drosophila* have only been available for about 5 years. The initial stage of this research was concerned mainly with the formal description of the cactus yeasts species. These studies served as the basis for several qualitative surveys of the yeasts associated with necrotic cacti and adult *Drosophila* (Starmer et al. 1976 and Heed et al. 1976). Recently, Starmer et al. (1981) reported a cummulative and extensive, qualitative survey of yeasts in *Drosophila* and cacti of the Sonoran Desert.

Research on the yeasts associated with *Drosophila* and their substrates have essentially neglected the study of the yeasts ingested by larvae. Yet, larval feeding behavior is relevant to all aspects of insect population biology and may represent a more specialized and delicately adapted behavior than adult feeding (Begon 1981). The techniques necessary for quantifying yeast populations and communities are now possible since the qualitative nature of cactus yeast communities are known. Thus, comparisons of the types and frequencies of yeasts in a naturally occurring cactus rot with the types and frequencies found in the digestive tracts of larvae living in the rot are possible. Investigations of this type bear directly on the questions: How are larvae utilizing their resources? Do they feed randomly, selectively, or optimally with respect to the yeasts which are available? Fogleman, et al. (1981a) reported that larvae-substrate comparisons for *D. mojavensis* showed selective feeding behavior by the larvae. Their experiments demonstrated that larvae are capable of distinguishing between patches of different yeast species and spend more time feeding in patches of the preferred yeast.

This report presents the larvae-substrate comparison data for all four of the cactophilic *Drosophila* species endemic to the Sonoran Desert as well as several comparisons involving non-desert species which use necrotic citrus fruits as feeding and breeding sites.

Materials and Methods

Isolation and quantitative analysis of yeasts from larval guts and specific substrates were initiated by examining naturally occurring necroses for the presence of *Drosophila* larvae. If present, several 1 gram samples of the rotting tissue were removed from the area of the rot containing the larvae. Third instar or late second instar larvae were also collected. In substrates that typically contain larvae of more than one species (e.g. rotting oranges), species classification of larvae was made using the relative size of the anterior spiracles as a diagnostic character. The larvae were surface sterilized in 70% ethanol for one minute,

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rinsed twice in sterile water, and ground up in a small, glass homogenizer. Dilutions of both the tissue and larval samples were made in sterile water. Appropriate dilutions were plated on acidified sythetic media containing a single carbon source utilized by only one or two of the yeast species expected to be present. A complete medium (acidified yeast extract-malt extract agar, YM) was utilized to select unexpected rare species. These media were acidified with enough 1.0 N H_3PO_4 to make the pH 3.7 to 3.8 in order to reduce the growth of bacteria. A key to frequently recovered cactus yeasts based on carbon source utilization has been presented by Starmer et al. (1981). The predominant yeasts associated with necrotic oranges have been studied by Vacek et al. (1979). After a sufficient incubation period (usually 7–10 days at 25° C), the yeast species present on each selective plate were enumerated. A representative of each species was brought into pure culture by 2–3 successive platings on yeast extract-malt extract agar (Difco YM agar). Each representative was then identified to the species level by contemporary yeast taxonomy (Van der Walt 1970). Statistical significance of comparisons of the relative frequencies of yeasts in larval guts and substrates were determined by Students *t*-tests performed on arcsine transformed data.

Results

The data for the larvae-substrate comparisons are reported as relative percentages averaged over replications ± standard errors. Table 1 lists the yeast florae comparisons for *D. mojavensis* larvae and three of their major cactus substrates: agria, organpipe, and cochal. In all three comparisons, the yeast, *P. cactophila*, was more frequent in the larvae than in the substrate. The comparisons with agria and cochal had larvae-substrate differences on the order of 20 to 30%. Statistically, these differences are highly significant. The comparison involving organpipe showed that *P. cactophila* could hardly have been more frequent in the larval gut since it represented almost 100% of the gut contents. Two of the three comparisons involving *C. sonorensis* show significant reductions in frequency from substrate to larvae. Although the agria-larvae comparison of *C. sonorensis* was not statistically significant, an identical comparison from a different location did show *C. sonorensis* to be significantly lower in relative frequency in the larval gut (Fogleman et al. 1981a).

Table 2 compares the relative frequencies of yeasts in necrotic saguaro with those in the digestive tracts of *D. nigrospiracula* larvae. Differences in relative frequency between substrate and larvae are not statistically significant, and the actual relative frequency differences are around 3 to 5% – almost an order of magnitude less than those in the previous table.

The yeasts found in *D. mettleri* larvae and two of their natural substrates are presented in Table 3. Although soils which are inundated with organpipe rot juice are reportedly used by *D. mettleri* (Fogleman et al. 1981b), the relative rarity of such substrates compared to saguaro soaked soils suggests that the former are only secondary in the ecology of this species. Two of the larvae-substrate comparisons in this table are statistically significant ($P<0.05$). In saguaro soaked soil, *P. amethionina var. pachycereana* and *C. ingens* are relatively more frequent in the substrate than in larvae. However, three points can be made regarding these comparisons. First, neither of these yeast species represent primary yeasts in saguaro since they occur in relatively low frequencies (less than 5% of total yeasts). Second, like the previous larvae-substrate comparison involving *D. nigrospiracula*, the differences in relative frequency from substrate to larvae are on the order of 2 to 3%. Finally, the statistical significance of these comparisons is most likely due to the truncation of the data involving yeasts of very low frequency rather than any real biological differences. Although compensatory arcsine transformations were performed on the data prior to statistical testing, the truncation effect is still apparent for yeasts occurring at frequencies of around 5% or less. No significant differences were found in the comparisons of the yeasts in organpipe soaked soil and *D. mettleri* larvae.

The last of the set of comparisons of cactophilic *Drosophila* larvae and their substrates is shown in Table 4. None of the yeast comparisons concerning necrotic senita and *D. pachea* larvae were statistically significant. However, one yeast species,

Table 1. Comparisons of the yeasts in the larva guts of *D. mojavensis* and natural substrates: agria (*Stenocereus gummosus*), organpipe (*Stenocereus thurberi*), and cochal (*Myrtillocactus cochal*). Numbers are relative percentages averaged over replications ± standard errors (data from Fogleman et al., 1981a)

Yeast species	agria	Larval gut	*t*	Organpipe	Larval gut	*t*	Cochal	Larval gut	*t*
Replications	4	4		3	3		4	4	
Pichia cactophila	8.0 ± 0.6	36.7 ± 3.5	9.614***	95.4 ± 1.9	99.4 ± 0.3	2.569	76.0 ± 4.5	96.8 ± 1.3	5.286**
Candida sonorensis	8.4 ± 2.8	14.7 ± 5.5	1.041	4.5 ± 1.9	0.6 ± 0.3	2.664	15.1 ± 2.7	1.3 ± 0.5	6.252***
Pichia amethionina[a]	14.7 ± 2.3	29.8 ± 2.7	4.210**	–	–	–	8.6 ± 1.7	1.8 ± 0.7	4.270**
Kluyveromyces marxianus	19.6 ± 2.0	11.6 ± 2.0	2.889*	–	–	–	–	–	–
Clavispora opuntiae	47.4 ± 2.3	7.0 ± 1.2	15.003***	–	–	–	–	–	–
Cryptococus cereanus	1.9 ± 0.4	0.2 ± 0.2	3.991***	–	–	–	–	–	–
log (total yeast concentration)	6.300/g	2.658/larva		7.415/g	4.628/larva		6.509/g	4.216/larva	

[a] variety *amethionina*

* $P<0.05$ ** $P<0.01$ *** $P<0.001$

Table 2. Comparison of the yeasts in necrotic saguaro (*Carneigea gigantea*) and guts of *D. nigrospiracula*. Numbers are relative percents averaged over four replications ± standard errors

Yeast species	Cactus rot	Larval gut	*t*
Pichia cactophila	25.1 ± 1.1	29.5 ± 1.6	2.242
Pichia heedii	52.2 ± 2.7	57.0 ± 2.6	1.293
Pichia amethionina[a]	10.6 ± 0.3	13.4 ± 1.3	2.001
Candida ingens	12.0 ± 1.5	–	–
log (total yeast conc.)	6.186/g	3.577/larva	

[a] variety *pachycereana*

Table 4. Comparison of the yeasts in necrotic senita (*Lophocereus schottii*) and larval guts of *D. pachea*. Numbers are relative percents averaged over three replications ± standard errors

Yeast species	Cactus rot	Larval gut	*t*
Pichia heedii	66.1 ± 3.9	82.2 ± 9.3	1.453
Candida ingens	10.5 ± 1.8	2.9 ± 2.6	2.247
Cryptococcus cereanus	23.4 ± 2.2	14.9 ± 9.6	0.028
log (total yeast conc.)	7.056/g	5.077/larva	

Table 3. Comparisons of the yeasts in soaked soils and larval guts of *D. mettleri*. The soils had been moistened by juices from necrotic saguaro (*Carneigea gigantea*) or necrotic organpipe (*Stenocereus thurberi*). Numbers are relative percents averaged over four replications ± standard errors

Yeast species	Saguaro soaked soil	Larval gut	*t*	Organpipe Soaked soil	Larval gut	*t*
Pichia cactophila	53.6 ± 3.6	58.1 ± 3.8	0.875	79.1 ± 2.6	83.3 ± 3.3	0.993
Pichia heedii	18.1 ± 5.7	13.7 ± 6.5	0.738	–	–	–
Pichia amethionina[a]	4.4 ± 0.8	1.7 ± 0.3	3.510*	–	–	–
Candida sonorensis	14.2 ± 1.8	22.3 ± 5.2	1.578	20.4 ± 2.6	16.7 ± 3.3	0.880
Candida ingens	3.2 ± 0.6	0.7 ± 0.3	3.440*	–	–	–
Candida boidinii	3.4 ± 1.0	3.0 ± 0.8	0.331	–	–	–
Rhodotorula rubra	1.8 ± 0.7	0.5 ± 0.1	2.274	–	–	–
Cryptococcus laurentii[b]	1.2 ± 0.3	–	–	–	–	–
log (total yeast conc.)	7.201/g	4.182/larva		7.901/g	4.487/larva	

* $P < 0.05$
[a] variety *pachycereana*
[b] variety *magnus*

Table 5. Comparisons of the yeasts in necrotic oranges (*Citrus sinensis*) with the yeasts present in the digestive tract of the larvae using the orange as a substrate. Numbers are relative percents averaged over replications ± standard errors

Yeast species	Orange #1	*D. melanogaster*		Orange #2	*D. arizonensis*	
		Larval gut	*t*		Larval gut	*t*
Replications	3	3		3	3	
Torulopsis stellata	53.1 ± 7.6	70.9 ± 2.3	2.197	13.8 ± 7.3	97.2 ± 0.5	6.771**
Pichia fermentans	33.5 ± 2.2	26.5 ± 2.9	1.821	0.9 ± 0.2	0.0	–
Hanseniaspora uvarum	13.4 ± 5.4	2.6 ± 0.6	2.021	85.3 ± 7.3	2.8 ± 0.5	8.010**
log (total yeast conc.)	8.586/g	5.565/larva		6.050/g	2.883/larva	

** $P < 0.01$

P. heedii, went from 66.1% in the substrate to 82.2% in the larvae. Although not statistically significant due to the high variance among larval replications, the magnitude of this difference (16.1%) certainly suggests selective feeding and warrants further examination or repetition of the entire sample.

Tables 5 and 6 present the substrate-larvae comparisons for necrotic oranges and three resident *Drosophila* species: *D. melanogaster, D. arizonensis,* and *D. pseudoobscura*. Although the data vary from one comparison to another, some generalizations are possible. The most noticeable changes in frequency occur in comparisons involving the yeast, *T. stellata*. All six substrate-larvae comparisons of this yeast species show increases in relative frequency in the larval digestive system over the relative frequency in the substrate. These increases range from 13.7% to 83.4%. Three of the comparisons are statistically significant. In one set of comparisons, the relative frequency went from 86.2% in orange #4 to 100% in *D. arizonensis* larvae and 99.9% in *D. pseudoobscura* larvae. Unfortunately, the lack of variation between larval replications does not allow the use of the *t*-test, but it seems reasonable to suggest that any increase to fixation (100%) over replications is significant. Only one of the comparisons involving *T. stellata* (orange #3) was not statistically significant due to the high variance among larval replications. Still, the relative frequency of this yeast was 18.1% greater in the larvae than in the substrate.

Two other yeasts, *Pichia fermentans* and *Hanseniaspora uvarum*, were found in most samples. Of the three possible comparisons involving *P. fermentans* where the frequency in the substrate

Table 6. Comparisons of the yeasts in necrotic oranges (*Citrus sinensis*) with the yeasts in the digestive tracts of the larvae using the orange as a substrate. Numbers are relative percents averaged over replications ± standard errors

Yeast species	Orange #3	*D.a.*[a]		*D.p.*[b]		Orange #4	*D.a.*	*D.p.*
		Larval gut	*t*	Larval gut	*t*		Larval gut	Larval gut
Replications	3	2		2		3	2	2
Torulopsis stellata	25.1 ± 6.6	43.2 ± 19.6	1.076	86.1 ± 6.6	4.933**	86.2 ± 4.9	100.0 ± 0.0	99.9 ± 0.0
Pichia fermentans	63.3 ± 2.8	42.1 ± 5.1	3.960*	13.8 ± 6.7	4.453*	–	–	–
Candida vini	7.3 ± 3.9	11.8 ± 11.8	0.123	–	–	–	–	–
Saccharomycodes ludwigii	0.7 ± 0.3	2.2 ± 2.1	0.567	–	–	–	–	–
Hanseniaspora uvarum	3.6 ± 1.3	0.7 ± 0.6	1.693	–	–	–	–	–
Saccharomyces bailii[c]	–	–	–	–	–	13.6 ± 4.9	0.0	0.0
log (total yeast concentration)	8.942/g	5.196/larva		5.434/larva		7.354/g	5.609/larva	5.915/larva

[a] *D.a.* = *D. arizonensis*
[b] *D.p.* = *D. pseudoobscura*
[c] variety *bailii*
* $P < 0.05$ ** $P < 0.01$

was greater than 1%, all three were decreases from substrate to larvae. Two of the three were statistically significant at the 0.05 level. Similarly, all three of the comparisons involving *H. uvarum* were reductions in relative frequency from substrate to larvae. Only one (orange #2-*D. arizonensis*) was statistically significant.

The logarithms of the total concentrations of yeasts in the substrates and larvae averaged over replications are given at the bottom of each table. The units for substrates and larvae are per gram of tissue (or soil) and per larva respectively. In substrates, the numbers of cells per gram range from 1.1 million to 875 million. If the three substrate types examined in the study are listed in order of increasing average total yeast concentration, the first would be necrotic cactus tissue (8.8 million cells/gram) followed by soaked soils (47.8 million cells/gram) followed by necrotic oranges (321.1 million cells/gram). In larvae, the number of cells (per larva) range from about 450 to 822,000. The correlation coefficient between logarithm total yeast concentration in substrates and the logarithm total yeast concentration in the larvae extracted from those substrates is +0.734. This coefficient is statistically significant at the 0.01 level. It appears that the amount of yeasts in a larva reflects the amount of yeasts available in the substrate.

Discussion

Selective feeding behavior and differential digestion of yeast species are two possible explanations for the observed differences between frequencies of yeasts in substrates and in digestive tracts of resident larvae. Fogleman et al. (1981a) reported that there was no significant difference in the rates of yeast digestion by *D. mojavensis* larvae. The ability of *D. movavensis* larvae to non-randomly ingest yeasts by spending more time feeding in patches of preferred yeasts was also demonstrated in the laboratory. Thus, larval selectivity appears to be the more likely explanation. If natural substrates are structured such that the yeast species exist in patches, differences observed in larvae-substrate comparisons of yeast frequencies could easily be produced by selective larval feeding. Patchiness is consistent with the colonial type growth of yeasts.

All of the yeast comparisons presented here can be separated into two categories: 1) comparisons which demonstrate large differences in relative frequencies between the substrate and the resident larvae. These differences are, in general, highly statistically significant. 2) comparisons which show relatively small substrate-larvae differences that are not statistically significant. Examples of the first category are comparisons of *D. mojavensis* with agria, organpipe, or chochal and comparisons of necrotic oranges with all resident species. The latter category is exemplified by substrate-larvae comparisons involving the rest of the cactophilic *Drosophila: D. nigrospiracula, D. mettleri,* and *D. pachea.* The important point here is that identical categories would be produced if the flies were separated into polyphagous and essentially monophagous groups.

Drosophila mojavensis is certainly the most polyphagous of the desert species. Besides organpipe and agria, they have been reared from cladodes and fruits of *Opuntia* (prickly-pear cactus), fruits and stems of saguaro, cochal stems, barrel cacti, and cina (*Stenocereus alamosensis*) stems (Fellows and Heed 1972). *Drosophila pachea*, on the other hand, is monophagous utilizing only senita. The dependence of *D. pachea* on Δ^7-sterols for larval viability and female fertility explains this host plant restriction (Heed and Kircher 1965). Senita is the only cactus species to contain Δ^7-sterols due to an interrupted sterol bisosynthetic pathway (Campbell and Kircher 1980). *Drosophila nigrospiracula* have been labeled oligophagic because they utilize both cardón and saguaro necroses (Fellows and Heed 1972). However, since the geographic distributions of these two cactus species are mutually exclusive, *D. nigrospiracula* can be considered essentially monophagous in any particular locality. *Drosophila mettleri* is an unusual species in that the larval substrate is soil which has been saturated by juices from necrotic cactus tissue (Heed 1977). Among the substrates reportedly used by *D. mettleri* are soils soaked by saguaro, cardón, and hecho (*Pachycereus pecten-arboriginum*). They have also been reared from soil soaked by organpipe rot (Fogleman et al. 1981b) and are capable of using senita-soaked soils (Fogleman et al. 1982). Despite the apparent polyphagy of *D. mettleri,* they are essentially as monophagous as *D. nigrospiracula* because of the extreme rarity of soils soaked by cactus rots other than those produced by saguaro or cardón. Like *D. nigrospiracula* then, they really have only one substrate type in any given locality.

Among the other *Drosophila* species that were examined, *D. melanogaster* is certainly the most widespread and perhaps the most polyphagous. Practically any decaying vegetation can be used as a substrate although they prefer decaying fruit (Atkinson and Shorrocks 1977). They have even been aspirated from rotting agria fruits in southern Baja California (Heed, unpublished data).

Drosophila arizonensis, a sibling species of *D. mojavensis*, is a desert fly breeding primarily upon cina cactus. These flies also utilize *Opuntia* fruits and cladodes, saguaro fruits, hecho stems, squash, and citrus fruits (Fellows and Heed 1972). Thus, *D. arizonensis* is also polyphagous. *D. pseudoobscura* have been found breeding in slime fluxes (Carson 1951) and in the contents of garbage cans in California (Carson 1971). In the deserts of southern Arizona and Sonora, Mexico, *D. pseudoobscura* have been reared from *Cina, Opuntia,* saguaro, and the Arizona barrell cactus (*Ferocactus wislizenii*). Another succulent, but non-cactus substrate, which is utilized is *Agave palmeri*, a resident of grasslands. In the mountains, the species has been reared from *Quercus gambelii* (Heed et al. 1976). In Tucson, citrus fruits are utilized (Vacek et al. 1979). The appearance of *D. pseudoobscura* in such a wide variety of substrates, some of which are highly domesticated, supports its classification as a polyphagous species.

It appears, then, that selective feeding behavior in *Drosophila* may be related to the degree of polyphagy. Larvae of the polyphagous species feed selectively and those of the essentially monophagous species feed more-or-less randomly. The relationship between polyphagy and selective feeding can be considered a special case of the generalism versus specialism paradigm. Polyphagous *Drosophila* have had the evolutionary latitude to specialize on widely distributed yeasts. Intrinsic in this explanation is the fact that the selected yeast must be an advantageous food source, but it is possible that being widely distributed is a sufficient advantage. The yeasts that were selected by larvae of *D. mojavensis* and the orange breeders are *P. cactophila* and *T. stellata,* respectively. *Pichia cactophila* is the most widely distributed yeast in the Sonoran Desert. It occurs with high predicatability (and usually in high relative frequency) in all of the substrates used by *D. mojavensis*. No corresponding data exist on the distribution of *T. stellata*. This species, however, was certainly the dominant species in the oranges that were sampled in these experiments. Monophagous *Drosophila,* being restricted to a single substrate type, cannot ecologically afford to specialize on a particular yeast because yeast florae are variable on a per substrate basis. Under these circumstances, the best strategy is to be a yeast generalist – eating whatever is available in a random fashion.

The concept of generalism versus specialism is therefore applied at two different biological levels. On a physiological level, there may be generalism or specialism (randomness or selectivity) with respect to yeast species. An ecological level would include generalism or specialism (polyphagy or monophagy) with respect to substrates. This concept was first proposed by McNaughton and Wolf (1970) and includes the idea of trade-offs between levels. Applying the concept to the material presented in this paper suggests that ecological generalists such as *D. mojavensis, D. melanogaster, D. arizonensis* and *D. pseudoobscura* are physiological specialists (selective feeders). Conversely, species that are ecological specialists (*D. pachea, D. nigrospiracula,* and *D. mettleri*) are, of necessity, physiological generalists (random feeders). This generalism versus specialism dichotomy should provide further insight in the field of insect foraging behavior.

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