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Analysis of the Community Structure of Yeasts Associated with the Decaying Stems of Cactus. III. *Stenocereus thurberi*

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

[1]Department of Biological Sciences, University of Denver, Denver, Colorado 80208, USA and
[2]Department of Biology, Syracuse University, Syracuse, New York 13210, USA

Abstract. Yeast communities in necroses of organpipe cactus (*Stenocereus thurberi*) were surveyed at 3 localities in Arizona. Quantitative analysis of random samples allows comparisons of the types and numbers of yeasts at 3 levels: within plants, between plants within a locality, and between localities. The analysis shows that the major source of variability is between plants. This pattern is identical with the pattern shown by agria cactus (*Stenocereus gummosus*) and is thought to be due to sampling different successional stages. No significant differences in estimates of the effective number of yeast species (ENS) in agria and organpipe samples were found. Comparisons of agria, organpipe, and prickly pear (*Opuntia*) cacti support the hypothesis that cactus chemistry is an important determinant of the yeast community structure which, in turn, is an important determinant of the diversity of *Drosophila* species which utilize necrotic cacti as feeding and breeding substrates.

Introduction

The deserts of northern Mexico and southwestern United States provide an excellent opportunity to study the ecology of a biological system consisting of plants, microorganisms, and insects. The system involves species of yeasts and *Drosophila* which live in decaying stems, fruits, and cladodes of cacti, specifically giant columnar cacti and species of *Opuntia* (prickly pear). The cacti provide a source of nutrients for microorganismic growth and the *Drosophila* feed upon the microorganisms. There is a limited set of about 12 yeast species that are specifically associated with necrotic cacti and about 10 predominant species of cactophilic *Drosophila* [4, 12]. The system, then, not only furnishes an opportunity to study yeast community structure but also to consider the ecological significance of yeast community structure since the relationships and interactions between organizational levels in this micro-ecosystem are fairly well known. Another attribute of this system is that the yeast-cactus and yeast-*Drosophila* associations can be viewed from an evolutionary or coevolutionary standpoint, since the chemical divergence of the cacti has been accompanied by the development of specific insect-host plant relationships and divergent yeast communities.

This is the third paper in a series of articles that analyze the community structure of cactophilic yeasts. The first paper analyzed the yeast community of decaying agria cactus (*Stenocereus gummosus*) with respect to spatial, temporal, and physiological organization [11]. Three distinct localities in the vicinity of La Paz, Baja California Sur, Mexico, were sampled during January 1981. The second paper examined yeast communities in decaying pads of 3 species of prickly pear cactus in Arizona and Texas during the period October–December of 1981 [13]. Comparisons of *Opuntia* species with agria indicated that samples of the former contain relatively more yeast species with broader physiological abilities in their communities than columnar necroses. This paper investigates the spatial organization of the yeast community in decaying stems of organpipe cactus (*Stenocereus thurberi*), a close relative of agria. Organpipe and agria are chemically similar [5], and necroses of these plants are used as feeding and breeding substrates by the same *Drosophila* species. Comparisons of all 3 cacti should provide further insights regarding the determinants of yeast community structure.

Methods

The general procedure for collecting samples of necrotic cactus tissue for community structure analysis of the yeast flora has been described in detail by Starmer [11]. Samples of necrotic stems of organpipe cactus were collected in December of 1982 from 3 separate localities in southern Arizona: (1) west Organ Pipe National Monument, (2) east Organ Pipe National Monument, and (3) the southern end of the Santa Rosa Wash. Localities 1 and 2 are separated by approximately 15 miles, and locality 3 is east of the National Monument and about 36 miles from locality 2. Within these limited areas, efforts were made to sample all rotting stems that were seen. Each rot was aseptically opened and three noncontiguous samples were placed in separate sterile Vacutainers. These samples were placed in a cooler and either returned to the laboratory for plating or plated in the field. Samples representing 3 rotting stems from each locality were chosen for study. All samples were plated within 10 hours of collection.

Qualitative and quantitative analyses of the yeast communities in rotting organpipe cactus were performed by diluting 1 g of the necrotic tissue in sterile water. The 10^{-4} and 10^{-6} dilutions were plated on both selective and complete media. All media were acidified with 1 N phosphoric acid to a pH of 3.8 in order to inhibit the growth of bacteria. However, some bacterial colonies appeared on some of the plates. The selective media consisted of yeast nitrogen base (YNB, Difco), agar, and various carbon sources (0.5% w/v). The choice of carbon sources was based on previous surveys of the yeasts associated with organpipe cactus and their physiological abilities [reviewed in 1, 12]. The plates were incubated at room temperatures (25°C), and colonies were counted after 7 days. A representative of each colony type from each sample was brought into pure culture by 2 successive streaks on complete medium (YM, Difco). Identification or confirmation of presumptive selective isolation was made by standard methods currently used in yeast taxonomy [15]. Isolates representing new or undescribed species were given arbitrary designations and are shown in Tables 1 and 3 in quotation marks.

The quantitative data from each sample, plant, and locality were transformed by taking the arcsine square root of the proportional representation of the yeast species in the community. The transformed data were analyzed using the nested analysis of variance procedure outlined in Sokal and Rohlf [10]. The levels in the nested ANOVA were within individual plants, between plants within a locality, and between localities.

Estimates of the effective number of species (ENS) were calculated by the method outlined in Lachance and Starmer [7]. These estimates were obtained in 3 different ways: (1) on a per sample basis, then averaged over all samples; (2) on a per plant basis by pooling all samples per plant,

then averaging over all plants; and (3) on a locality basis by pooling all samples for all plants per locality, then averaging over all localities.

Results

The frequency of each yeast species and the total yeast density for each sample of organpipe cactus are given in Table 1. For comparative purposes, the same data for agria are presented in Table 2 (data from Starmer [11] presented as frequencies). A comparison of the average total yeast density for the 2 cacti indicates that, not only is the yeast flora in agria significantly more dense, but also significantly more variable. The average density (± the standard error) for agria is $547.4 \pm 123.7 \times 10^5$ cells/g and the same parameter for organpipe is $150.7 \pm 40.6 \times 10^5$ cells/g. The difference between the means is statistically significant ($t' = 3.047$, $P < 0.01$), and the difference between the variances is also significant ($F = 11.332$; df = 33,27, $P < 0.001$).

The F statistics derived from nested analyses of variance for each yeast species in both cacti are shown in Table 3. These analyses show that, for most yeasts, the plant-within-locality source of variation is statistically significant, but, at the next level in the nested ANOVA, there are no yeasts for which there is significant variation due to the locality source of variability. Also for yeast species that are found in both agria and organpipe, no significant differences can be attributed to the cactus species themselves. *Pichia amethionina* was omitted from this analysis due to its rarity in organpipe.

Coefficients of variation (CV) for within-plant and plant-within-locality variation sources are also presented in Table 3. The relationship between these 2 sources of variation can be indicated by calculating a rank order correlation coefficient (Spearman's Rho). The calculated statistics for agria and organpipe are 0.790 and 0.145, respectively. The coefficient for agria is significant at $P < 0.05$ (df = 5), and the coefficient for organpipe is not significant at this level (df = 8).

Comparisons of the effective number of yeast species in agria and organpipe rots at the 3 hierarchical levels indicated no significant difference between the cactus species with respect to this parameter at any level. The calculated estimates for agria and organpipe, respectively, are 1.656 and 1.996 on a per sample basis ($t' = 1.687$, $P > 0.05$), 2.804 and 2.505 on a per plant basis ($t' = 0.390$, $P \gg 0.05$), and 4.743 and 4.740 on a locality basis ($t = 1.472$, $P > 0.2$).

Discussion

Comparison of the community structure of yeasts in organpipe and agria cactus can be made from the data in Table 3. Agria and organpipe are the same in that none of the resident yeast species show significant increases in variation of their proportional representation between localities. However, in both cacti, most yeast species have significant variation attributed to different plants within a locality. This suggests that different areas within the same rot are relatively homogeneous with respect to yeasts, and that species proportions are relatively

Table 1. Frequency[a] of yeast cells in necrotic organpipe

Locality	Plant	Sample	Yeast species[b]											
			P.C.	C.S.	P.A.	C.C.	C.V.	C.I.	P.M.	C.M.	C.CAS.	P.TM.	P.H.	Total no.[c]
1	1	1	0.806	0.019	0.000	0.126	0.000	0.042	0.003	0.001	0.000	0.000	0.000	65.7
		2	0.000	0.000	0.000	0.000	0.942	0.002	0.000	0.000	0.014	0.039	0.000	148.5
		3	0.072	0.008	0.016	0.002	0.000	0.007	0.000	0.000	0.000	0.000	0.892	82.9
	2	1	0.056	0.000	0.000	0.349	0.042	0.028	0.000	0.000	0.522	0.000	0.000	7.0
		2	0.019	0.000	0.000	0.040	0.068	0.014	0.000	0.000	0.019	0.837	0.000	20.5
		3	0.245	0.000	0.000	0.518	0.000	0.050	0.000	0.000	0.185	0.000	0.000	11.8
	3	1	0.455	0.000	0.000	0.531	0.000	0.000	0.012	0.000	0.000	0.000	0.000	175.5
		2	0.153	0.000	0.000	0.770	0.000	0.000	0.075	0.000	0.000	0.000	0.000	142.7
		3	0.388	0.000	0.000	0.378	0.000	0.000	0.232	0.000	0.000	0.000	0.000	72.1
2	1	1	0.000	0.507	0.000	0.353	0.000	0.000	0.140	0.000	0.000	0.000	0.000	10.0
		2	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.5
		3	0.000	0.722	0.000	0.222	0.000	0.000	0.055	0.000	0.000	0.000	0.000	7.2
	2	1	0.027	0.176	0.000	0.035	0.000	0.000	0.000	0.664	0.095	0.000	0.000	35.3
		2	0.017	0.175	0.000	0.024	0.000	0.039	0.000	0.605	0.136	0.000	0.000	51.2
		3	0.025	0.069	0.000	0.022	0.000	0.000	0.000	0.760	0.121	0.000	0.000	49.3
	3	1	0.000	0.000	0.000	0.000	0.995	0.004	0.000	0.000	0.000	0.000	0.000	42.8
		2	0.000	0.000	0.000	0.000	0.917	0.016	0.000	0.000	0.000	0.065	0.000	20.7
		3	0.000	0.000	0.000	0.000	0.487	0.378	0.000	0.000	0.000	0.134	0.000	18.7
3	1	1	0.009	0.003	0.000	0.007	0.878	0.011	0.000	0.002	0.004	0.082	0.000	227.6
		2	0.027	0.009	0.000	0.014	0.827	0.001	0.000	0.006	0.009	0.104	0.000	157.2
		3	0.080	0.002	0.000	0.008	0.457	0.013	0.000	0.002	0.000	0.435	0.000	459.0
	2	1	0.506	0.208	0.000	0.268	0.000	0.016	0.000	0.000	0.000	0.000	0.000	670.8
		2	0.751	0.207	0.000	0.035	0.000	0.005	0.000	0.000	0.000	0.000	0.000	36.2
		3	0.428	0.280	0.000	0.006	0.254	0.000	0.000	0.000	0.029	0.000	0.000	235.6
	3	1	0.454	0.317	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.227	220.2
		2	0.254	0.466	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.279	214.5
		3	0.191	0.513	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.293	885.5

[a] The frequency values for cells of each yeast species are given as the fraction of total yeast cells in each sample

[b] P.C., *Pichia cactophila*; C.S., *Candida sonorensis*; P.A., *Pichia amethionina* var. *amethionina*; C.C., *Cryptococcus cereanus*; C.V., *Candida valida*; C.I., *Candida ingens*; P.M., *Pichia mexicana*; C.M., *Candida mucilagina*; C.CAS., *Candida* sp. "caseinolytica"; P.TM., *Pichia* sp. "TM"; P.H., *Pichia heedii*

[c] $\times 10^5$ yeast cells/g of sample

Table 2. Frequency[a] of yeast cells in necrotic agria (raw data from Starmer [11])

Locality	Plant	Sample	Yeast species[b]							Total no.[c]
			P.C.	C.S.	P.A.	C.C.	C.V.	C.I.	P.M.	
1	1	1	0.575	0.308	0.091	0.014	0.010	0.000	0.000	285.0
		2	0.267	0.626	0.058	0.025	0.021	0.000	0.000	1,092.0
		3	0.465	0.389	0.101	0.025	0.017	0.000	0.000	668.0
	2	1	0.783	0.095	0.117	0.003	0.000	0.000	0.000	282.0
		2	0.160	0.636	0.056	0.146	0.000	0.000	0.000	1,458.0
		3	0.235	0.334	0.143	0.016	0.000	0.270	0.000	718.0
	3	1	1.000	0.000	0.000	0.000	0.000	0.000	0.000	1,406.0
		2	1.000	0.000	0.000	0.000	0.000	0.000	0.000	68.0
		3	1.000	0.000	0.000	0.000	0.000	0.000	0.000	1,400.0
	4	1	0.767	0.008	0.056	0.163	0.000	0.000	0.004	1,970.0
		2	0.959	0.000	0.003	0.031	0.000	0.000	0.005	767.0
		3	0.939	0.000	0.001	0.058	0.000	0.000	0.001	187.4
2	1	1	0.590	0.079	0.099	0.219	0.000	0.000	0.009	1,523.0
		2	0.702	0.068	0.101	0.120	0.000	0.000	0.007	2,574.0
		3	0.655	0.008	0.065	0.256	0.000	0.000	0.014	1,948.0
	2	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0
		2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0
		3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0
	3	1	0.020	0.027	0.027	0.565	0.255	0.075	0.027	145.0
		2	0.019	0.035	0.067	0.269	0.484	0.083	0.039	252.0
		3	0.014	0.207	0.029	0.232	0.173	0.079	0.262	202.0
	4	1	0.036	0.013	0.069	0.026	0.785	0.069	0.000	303.0
		2	0.119	0.507	0.238	0.119	0.014	0.000	0.000	67.0
		3	0.049	0.004	0.049	0.000	0.895	0.000	0.000	20.1
3	1	1	0.123	0.098	0.086	0.049	0.641	0.000	0.000	81.0
		2	0.800	0.133	0.032	0.013	0.019	0.000	0.000	306.0
		3	0.669	0.186	0.039	0.032	0.071	0.000	0.000	306.0
	2	1	0.032	0.000	0.000	0.000	0.967	0.000	0.000	3.1
		2	0.781	0.187	0.000	0.031	0.000	0.000	0.000	32.0
		3	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.2
	3	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0
		2	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.1
		3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0

[a] The frequency values for cells of each yeast species are given as the fraction of total yeast cells in each sample

[b] P.C., *Pichia cactophila*; C.S., *Candida sonorensis*; P.A., *Pichia amethionina* var. *amethionina*; C.C., *Cryptococcus cereanus*; C.V., *Candida valida*; C.I., *Candida ingens*; P.M., *Pichia mexicana*

[c] $\times 10^5$ yeast cells/g of sample

constant between localities. As previously stated by Starmer [11], the variation between plants within localities is most likely a result of samples representing different successional stages of the rotting process. Unlike agria, no temporal sequence of yeasts can be inferred from organpipe data because no consistent groups of yeasts can be detected. It seems reasonable to assume, however, that yeasts undergo succession in organpipe as well as in agria, but because of the vagaries of sampling, this point remains unclear.

Table 3. Variability of yeast species within and between plants[a]

Species	Agria				Organpipe				Between cactus species F
	CV/100		F		CV/100		F		
	Within plant	Plant within locality	Plant within locality	Locality	Within plant	Plant within locality	Plant within locality	Locality	
Pichia cactophila	0.55	0.44	2.91[b]	3.54	0.66	0.50	2.75[b]	4.22	0.949
Candida sonorensis	0.77	0.99	5.93[b]	0.41	0.44	1.17	22.30[b]	1.35	0.094
Pichia amethionina (var. a)	0.42	0.87	13.64[b]	0.76	—	—	—	—	—
Cryptococcus cereanus	0.58	0.95	9.08[b]	1.42	0.73	0.81	4.70[b]	2.16	0.337
Candida valida	1.51	0.97	2.25	1.37	0.95	1.48	8.20[b]	0.14	0.808
Candida ingens	2.90	1.55	2.65[b]	0.77	1.42	0.85	2.07	0.33	1.193
Pichia mexicana	1.49	1.94	6.07[b]	1.42	1.60	1.82	4.88[b]	0.61	0.140
Candida mucilagena	—	—	—	—	0.26	2.81	357.29[b]	0.93	—
Candida "caseinolytica"	—	—	—	—	1.13	1.65	7.44[b]	0.36	—
Pichia sp. "TM"	—	—	—	—	2.01	1.17	2.02	0.15	—
Pichia heedii	—	—	—	—	2.25	1.70	2.72[b]	0.52	—

[a] Arcsine square root percent transformed
[b] $P < 0.05$

The relationships between the coefficients of variation presented in Table 3 point out another difference between the 2 species of cacti. In agria, the rank order correlation coefficient relating the variability within and between plants is statistically significant, whereas this parameter in organpipe is not significant. Starmer [11] proposed that the correlation in agria was an extension of the Kluge-Kerfoot phenomenon [6] at the level of the community. This phenomenon is indicated by a positive correlation between the interlocality differentiation for a variable and the amount of within-population variation. Sokal [9] has suggested that such a relationship implies stability in the relative levels of variability for substantial periods of time. With this in mind, the comparison of agria and organpipe shows that the yeast community in agria may be more structured than in organpipe.

On the other hand, a recent article by Rohlf et al. [8] reanalyzed the data sets that had been previously used to demonstrate the Kluge-Kerfoot phenomenon. In their opinion, these data sets suggest that the phenomenon may be a statistical artifact, and the observed correlations of measurements of within and among population variability are mainly due to the fact that both of these parameters are functions of a third variable—the sample mean. Their main conclusion, however, was not that there is never a real and biologically meaningful correlation between levels of within and among population variability, but rather that ". . . previous studies do not provide adequate data to allow an investigation of such a relationship." Certainly, statements regarding these correlations should be made with caution.

Agria and organpipe cacti are very similar with respect to effective numbers of yeast species present in their necroses. The estimates of ENS increase in value with hierarchical level because pooling the data tends to include more species. In addition, pooling tends to smooth out variation, and the more even the frequencies of the different yeast species are, the higher the estimate will be. Maximum ENS occurs when all yeasts are represented with equal frequency. It is evident from Tables 1 and 2 that organpipe contains a higher absolute number of yeast species than agria, but their proportional representation is more variable.

This comparison differs from a similar comparison presented by Starmer and Phaff [13] in the second paper of this series. Their comparison of agria and organpipe was based on the frequency of qualitative occurrence in a much larger number of samples. The estimates of ENS obtained in this manner were 4.71 and 6.51 for agria and organpipe, respectively. The difference in the 2 sets of estimates most likely represents the difference in the quantitative vs qualitative nature of the experimental approaches.

It is appropriate to reiterate the conclusions of Starmer and Phaff [13] with respect to ENS measurements. Their results indicated that *Opuntia* necroses contain slightly higher numbers of yeast species in their microbial communities (average = 7.15) than necroses of the columnar subtribe Stenocereinae (average = 5.02 including agria and organpipe). They suggested that the difference in cactus chemistry is an important determinant of the numbers and types of yeasts present. With respect to chemical composition, agria and organpipe are certainly closer to each other than either is to *Opuntia* species [5]. Based on

these ideas, the yeast community structure in agria and organpipe should be similar, and the data presented in this paper support this statement.

Finally, the role of *Drosophila* in shaping the yeast community structure should not be ignored. *Drosophila* are known to be yeast vectors [3], and there is evidence that *Drosophila* may be the principal source of yeast inocula for new cactus rots [11]. An additional determinant of yeast community structure may, therefore, reside in the insect-plant relationship and the factors that attract *Drosophila* species to specific cactus species.

Once the yeast community has been established, it may be further affected by the resident *Drosophila* life stages that are feeding upon yeasts. Selective foraging by *Drosophila mojavensis* larvae on specific yeasts in naturally occurring necroses of agria and organpipe has been demonstrated by Fogleman et al. [1, 2]. Vacek et al. [14] found nonrandom samples of yeasts in the guts of adult *Drosophila* feeding on necrotic oranges. In this case, the yeast species that was preferentially ingested was not randomly distributed within the necrotic tissue, but rather was more associated with the surface of the rot because of its growth characteristics. In addition, necrotic *Opuntia* pads are used as oviposition substrates by more *Drosophila* species than are the columnar cactus species [16], and this might be the reason why *Opuntia* rots contain more yeast species than agria or organpipe rots. All of these phenomena may be affecting the community structure of the yeasts associated with the decaying stems of cactus.

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References

1. Fogleman JC, Starmer WT, Heed WB (1981) Larval selectivity for yeast species by *Drosophila mojavensis* in natural substrates. Proc Natl Acad Sci USA 78:4435–4439
2. Fogleman JC, Starmer WT, Heed WB (1982) Comparisons of yeast florae from natural substrates and larval guts of southwestern *Drosophila.* Oecologia 52:187–191
3. Gilbert DG (1980) Dispersal of yeasts and bacteria by *Drosophila* in a temperate forest. Oecologia 46:135–137
4. Heed WB (1982) The orgin of *Drosophila* in the Sonoran Desert. In: Barker JSF, Starmer WT (eds) Ecological genetics and evolution: the cactus-yeast-*Drosophila* model system. Academic Press Australia, Sydney, pp 65–80
5. Kircher HW (1982) Chemical composition of cacti and its relationship to Sonoran Desert *Drosophila.* In: Barker JSF, Starmer WT (eds) Ecological genetics and evolution: the cactus-yeast-*Drosophila* model system. Academic Press Australia, Sydney, pp 143–158
6. Kluge AG, Kerfoot WC (1973) The predictability and regularity of character divergence. Am Natur 107:426–442
7. Lachance MA, Starmer WT (1982) Evolutionary significance of physiological relationships among yeast communities associated with trees. Can J Bot 60:285–293
8. Rohlf FJ, Gilmartin AJ, Hart G (1983) The Kluge-Kerfoot phenomenon—a statistical artifact. Evol 37:180–202
9. Sokal RR (1978) Population differentiation: something new or more of the same. In: Brussard

PF (ed) Ecological genetics: the interface. Springer-Verlag, Berlin, Heidelberg, New York, pp 215–239
10. Sokal RR, Rohlf FJ (1969) Biometry. Freedman, San Francisco
11. Starmer WT (1982) Analysis of the community structure of yeasts associated with the decaying stems of cactus. I. *Stenocereus gummosus.* Microb Ecol 8:71–81
12. Starmer WT, Phaff HJ, Miranda M, Miller MW, Heed WB (1982) The yeast flora associated with the decaying stems of columnar cactus and *Drosophila* in North America. Evol Biol 14: 269–295
13. Starmer WT, Phaff HJ (1983) Analysis of the community structure of yeasts associated with the decaying stems of cactus. II. *Opuntia* species. Microb Ecol 9:247–259
14. Vacek DC, Starmer WT, Heed WB (1979) Relevance of the ecology of *Citrus* yeasts to the diet of *Drosophila.* Microb Ecol 5:43–49
15. Van der Walt JP (1970) Criteria and methods used in classification. In: Lodder J (ed) The yeasts, a taxonomic study. North Holland, Amsterdam, pp 34–113
16. Wasserman M (1982) Evolution of the *repleta* group. In: Ashburner M, Carson HJ, Thompson JN (eds) The genetics and biology of *Drosophila,* Vol 3B. Academic Press, New York, pp 61–140