The Biogeographic Diversity of Cactophilic Yeasts

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19.1 Introduction

Two commonly used measures of biological diversity are the number of unique species (species richness) and the uncertainty of species identity (diversity measured by H'; Pielou 1975). These metrics are affected by the availability of suitable habitats and are ultimately a function of speciation, extinction, and immigration patterns (Ricklefs 1987; Zobel 1997; Hubbell 2001). For this last function, it is generally thought that the size of an organism is important in its capacity to disperse and that very small organisms (microorganisms) freely disperse over large areas, usually as passive propagules in currents of air or water. In this situation the species in a local area are expected to be the same as species over larger, even global, expanses (Godfray and Lawton 2001). This "bugs are everywhere" hypothesis is, however, without foundation for many small organisms that have formed obligate relationships with vectors that constrain their dispersal patterns. Among the fungi, ascomycetous yeasts are often constrained by their vectors (Phaff and Starmer 1987). Two examples are the yeasts associated with drosophilids that feed and breed in the necrotic tissues of cacti (Barker and Starmer 1982) and yeast communities in blossoms of Convolvulaceae (morning glory, bindweeds) and *Hibiscus* species. This latter group has been reviewed and studied in a biogeographic context by Lachance et al. (2001b). They concluded that geographic factors may not act directly to determine yeast distributions but rather act indirectly through their insect vectors (i.e., Coleoptera) that distribute the yeasts to their flower–host resources. These insects have a major influence on the biogeographic diversity of flower-specific yeasts. The extensive records and wide ranging sampling that characterize studies of the flower-inhabiting yeasts make them a useful system for studying yeast ecology and insect–yeast interaction. The system is comparable to the cactophilic yeasts (Starmer and Fogleman 1986; Starmer et al. 1991).

The cactophilic yeasts have been collected and studied for over 30 years and provide insights into the origin of new species (Starmer et al. 1980), host and vector ecology (Ganter et al. 1986), community stability (Latham 1998), yeast diversification

(Lachance et al. 2000, 2001b), origin of yeast communities (Starmer et al. 2003), and the relationship of phylogeny to yeast community organization (Anderson et al. 2004). Only four dominant yeasts are found world-wide in the cactophilic yeast community [*Pichia cactophila*, *Candida sonorensis*, *Sporopachyderma cereana* (and related sister species) and *Clavispora opuntiae*]. Several species are very common (*Myxozyma mucilagina*, *S. amethionina* clade species, *Dipodascus starmeri*, *Pichia deserticola* and *Pichia kluyveri* v. *eremophila*). All of these species are cactus-specific; they are rarely recovered from other habitats such as sympatric fruit rots (Starmer et al. 1987) or slime fluxes of trees (Ganter et al. 1986). These species have diverse origins. They do not belong to a single cactus-yeast clade but evolved from distinct ancestors found in separate clades that show affinity to fruit-rot and tree-flux habitats (Starmer et al. 2003). Overall 80 species have been detected in decaying columnar-cactus stems or *Opuntia* cladode rots. In this chapter, we shall analyze the pattern of diversity for these 80 species found in cacti and discuss possible reasons for those patterns.

The biogeography of cactophilic yeasts has been described, compared and discussed in terms of host and geographic determinants of species and community distributions (Starmer et al. 1990). That analysis compared four community types (host cactus categories) across five semiarid regions of the southern USA, Mexico, northern Venezuela, as well as Caribbean and Bahamian islands. The analysis showed that yeast communities from the same host cactus type were more similar to one another across the five regions as compared with yeast communities from different host cactus types within regions. It thus appeared that the host plant had a larger influence on the diversity and the composition of the yeast community than geographic separation. However, this conclusion for community similarity was not a generality that extended to all species, where some geographic factors may be important to the diversification and speciation of some cactus specific species complexes (i.e., *Sporopachyderma* spp., Lachance et al. 2001b; *Phaffomyces* spp. Starmer et al. 2001; and *Starmera* spp. Starmer et al. 1990). Since the last review of the biogeography of the yeasts associated with cacti (Starmer et al. 1990) new collections and new species have been added to the database, and records from other continents (primarily Australia, where *Opuntia* cacti were introduced) have been incorporated. The additional data for Australian localities add another level to the determinants of biodiversity. This situation is unique because not only were the cacti introduced but the cactus yeasts (from the Americas) were also introduced when biological control was attempted in efforts to remove *Opuntia* from large geographic areas of eastern Australia (Starmer et al. 1987). In addition, the local yeasts already in Australia are expected to be significantly different from those on other continents. This difference in indigenous microbiota is expected to add significantly to the species richness and diversity in the rotting cladodes of *Opuntia* in Austrialia.

19.2 Methods

19.2.1 Measuring Diversity

Even though diversity can be quantified by many different metrics (for example, 24 measures of β diversity, Koleff et al. 2003), a useful method is to partition the global

diversity, γ, into diversity between and within levels of a hierarchy. The simplest partition is $\gamma = \alpha + \beta$, where α is the average diversity for all of the samples and β is the between-sample diversity or the difference between γ and α (Lande 1996; Loreau 2000). When several levels exist in a hierarchy, e.g., host plants, local areas, and regions such that n=3, then $\gamma = \alpha + \beta_1 + \beta_2 + \beta_3$. In this example α is the average diversity of the yeasts in the host plants, β_1 is the diversity between host plants, β_2 is the diversity between localities, β_3 is the diversity between regions, and γ is the total diversity.

The additive partition can be used for species richness S (defined as the total number of unique species in the entire collection), the Simpson index $d=1-\Sigma p_i^2$ or the Shannon–Weiner index $H' = \Sigma p_i \ln(p_i)$, where p_i is the proportional representation of each species in the level under consideration. This approach has been reviewed by Veech et al. (2002) and is becoming widely used by ecologists to understand the relationship between scale and biological diversity (Godfray and Lawton 2001). For an extensive discussion of β diversity see Vellend (2001). Veech et al. (2002) suggest converting the diversity components in the hierarchy into percentages or proportions of the total (γ) for comparison of the relative contribution to the total species richness (S), Simpson's index (d), or the Shannon–Weiner index (H'). We have followed their suggestion and present statistics for S and H'.

19.2.2 Hosts

Cactus hosts were categorized according to the systematic groupings outlined by Gibson and Nobel (1986) and conform to the listing given in Starmer et al. (1990). The major divisions we use in our analysis are given in bold in Table 19.1. They were chosen for study because they represent a nested taxonomic hierarchy and have adequate sample sizes.

19.2.3 Geography

The database used in this study includes records for 188 distinct collection localities in eight regions (listed in the following with the number of localities for each, see Fig. 19.1 for a map of the New World regions):

1. AU (44): Australia (Queensland and New South Wales)
2. CA (24): Caribbean Islands [Greater Antilles: Cuba, Cayman Islands, Jamaica, Navassa, Hispainola (Haiti and Dominican Republic); Lesser Antilles: Montserrat, US & British Virgin Islands, and Islas Los Roques]
3. FB (7): USA(Florida), Bahama Islands (Great Inagua and Conception Island)
4. HA (2): USA (Hawaii)
5. SM (25): Mexico (Chiapas, Guerrero, Hidalgo, Jalisco, Michoacan, Oaxaca, and Puebla), Honduras
6. SD (71): USA (Arizona and California), Mexico (Sinaloa and Sonora)
7. TX (5): USA (Texas)
8. VZ (10): Venezuela (northern)

Table 19.1 Cactus hosts sampled according to systematic groups. Those groups given in *bold* were used as categories for analysis. The number of yeast species and the number of plants sampled are in *parentheses*. Only the number of plants sampled is given for each genus

Family	Subfamily	Tribe	Subtribe	Genus
Cactaceae (80, 2649)	**Opuntioideae** (67, 1651), North America: (42, 950), introduced: (46, 701)			*Opuntia* (1602), *Nopalea* (49)
	Cactoideae (49, 998)	Cacteae		*Ferocactus* (13)
		Cereeae		*Cereus* (36), *Melocactus* (18)
		Hylocereeae		*Acanthocereus* (7)
		Leptocereeae		*Neoabbottia* (12)
		Pachycereeae	**Pachycereinae** (36, 335)	*Lophocereus* (80), *Carnegiea* (54), *Backebergia* (1), *Cephalocereus* (28), *Neobuxbaumia* (1), *Pachycereus* (37), *Pilosocereus* (134)
			Stenocereinae (38, 572)	*Stenocereus* (533), *Myrtillocactus* (28), *Escontria* (16)

The hierarchy available for cactus yeasts is easily viewed as a nested geographic set, starting from the global or continental level, which is divided into distinct regions, that are further divided into subregions composed of a group of localities in which individual rot pockets of plants are sampled to yield the incidence of yeast species. Thus, each yeast species has a plant, locality, subregion, region, and continental designation such that diversity can be viewed at all levels. Specific definitions of the levels for this study are as follows.

2.3.1 Plants

Individual plants are discrete. Even though a single plant can have multiple rot pockets, our collections have generally been limited to one sample per plant. The number of yeast species present in a plant-rot pocket is the species richness (S). The overall database does not allow calculations of p_i within a plant because the cell numbers for each species were not accurately estimated in all collections. As a consequence the diversity index H' could be calculated only for localities, subregions, regions, and continents. However, we do include the categories "within-plant" and "between

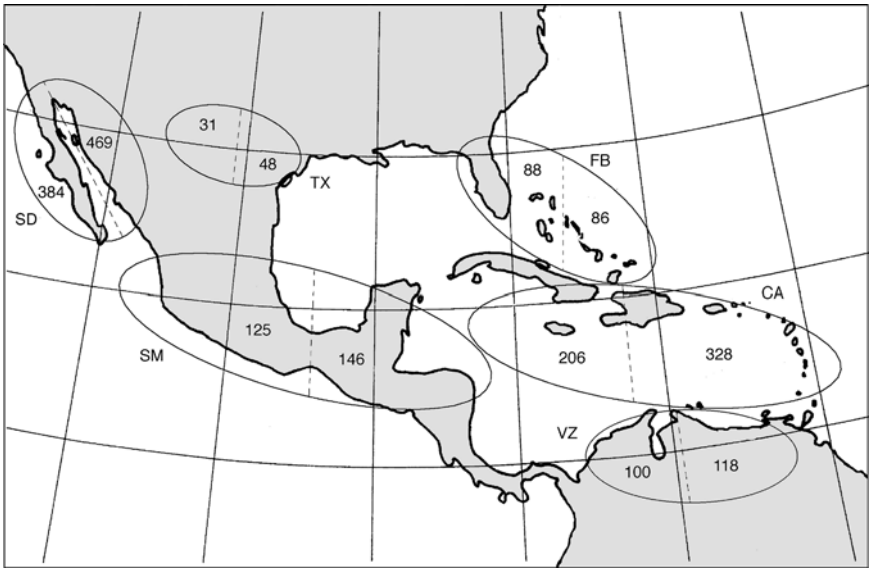

Fig. 19.1. Geographic areas where cactus necroses were collected. *Shaded oval areas* show the six distinct regions that were sampled (see Sect. 2.3 for the regional definitions). Each region is divided by a *dashed line* into subregions. The *numbers* within in each subregion are the number of plants sampled

plants" for studies of two *Stenocereus* species, *S. gummosus* (Pitaya agria) and *S. thurberi* (organpipe), for which detailed within-plant yeast-cell counts were made by selective-isolation methods (Starmer 1982; Fogleman and Starmer 1985). In order to eliminate noise from estimates of H', we excluded locations with small numbers of yeast isolates ($n<8$; e.g., a location with only one or a few plant samples).

2.3.2 Localities

Localities are somewhat subjective but were discrete. They range in size from a few square meters to several hectares. Separate localities were usually isolated in space by 10 km or more. Localities can be viewed as having more dispersal potential within than between.

2.3.3 Subregions

Subregional divisions of larger regions were mainly arbitrary but represented two more or less equal east–west contiguous partitions, except for Australia, where the division was north–south, and Hawaii, where no subregional division was warranted.

2.3.4 Regions

Regions were geographically distinct in the sense that dispersal between regions was severely limited by large expanses such as cactus-free terrain, bodies of water, or mountains.

2.3.5 Continents

Continental division was only possible for *Opuntia* hosts and is a "natural" versus "introduced" categorization of the *Opuntia* cacti. Those cacti in continental New World regions (North America, the Caribbean, the Bahamas, and northern Venezuela) were native, whereas those in Australia and Hawaii were introduced.

19.2.4 Yeasts

The localities were partitioned into eight regions and 15 subregions. Figure 19.1 shows the regional and subregional extent for North America. The number of plants sampled in each is shown for each subregion. The yeast taxa recovered from the entire collection and their frequency of isolation on a per plant basis are listed in Table 19.2 along with their isolation frequency in each of the eight regions.

19.3 Species Richness

Comparison of the proportion of diversity (S) explained as the scale of sampling increases shows a distinct trend for yeasts both from native columnar cacti and from *Opuntia* (Table 19.3, Fig. 19.2). The β diversity for species richness (S) between regions is over 50%. This differentiation at the highest level of division could have several causes that function to render the regions different from one another in their species composition: (1) the discontinuous geographic regions may severely limit the likelihood of dispersal from region to region, (2) allopatric speciation events result in differential community composition, (3) opportunistic local yeasts (non-cactus-specific) differ in the regions such that rare entry into the community causes the species richness to increase on pooling species from all regions, (4) cactus-specific species that were once widespread have become extinct in some regions but not all (i.e., relics), and (5) species from different regions are different but ecologically equivalent (Shmida and Wilson 1985).

19.3.1 Yeasts of Columnar Cacti

In order to evaluate the factors just listed we compared the species composition for the nature of species that were not shared among regions. Of the 49 species in the columnar community, 24 were unique (or essentially unique) to an individual region. In two instances the unique species were commonly recovered from cactus rots and also were closely related, i.e., *Phaffomyces antillensis* was found in the Caribbean region and the sister species *P. thermotolerans* in the Sonoran Desert; *Starmera caribaea* was exclusive to the Caribbean and *S. amethionina* in the Sonoran Desert.

Table 19.2 Number of each yeast species isolated from the entire collection and the eight geographic regions (see Sect. 2.3 for a description of each region). Yeast species listed as sp. "1", etc. are unknown or unidentified taxa

	Global	AU	CA	FB	HA	SM	SD	TX	VZ
Pichia cactophila	1,245	251	271	48	22	100	394	29	130
Candida sonorensis	862	314	106	54	19	35	220	54	60
Sporopachydermia cereana	592	45	108	29	2	54	282	26	46
Clavispora opuntiae	377	228	46	8	21	17	27	9	21
Myxozyma mucilagina	174	39	–	13	1	1	93	23	4
Starmera amethionina	166	27	–	–	1	–	125	13	–
Dipodascus starmeri	161	–	19	1	–	23	96	–	22
Pichia deserticola	140	–	47	–	–	15	70	8	–
Pichia kluyveri v. *eremophila*	119	–	–	–	–	10	76	33	–
Pichia mexicana	86	–	11	5	–	4	34	–	32
Pichia heedii	72	–	–	–	–	–	72	–	–
Pichia angusta	69	1	1	1	–	–	59	7	–
Cryptococcus albidus	67	30	3	–	1	5	21	2	5
Starmera caribaea	64	–	33	18	-	2	2	4	5
Candida boidinii	62	33	–	4	6	2	9	8	-
Pichia opuntiae	61	53	–	–	–	1	5	–	2
Pichia barkeri	57	41	8	4	–	4	–	–	–
Pichia kluyveri	48	15	12	8	3	3	6	–	1
Rhodotorula minuta	46	34	1	–	–	–	9	1	1
Pichia norvegensis	45	–	7	18	9	–	11	–	–
Cryptococcus laurentii	40	21	6	–	–	2	5	4	2
Pichia membranifaciens	39	4	–	1	–	2	1	–	31
Clavispora lusitaniae	32	–	–	–	–	29	3	–	–
Phaffomyces thermotolerans	26	–	–	–	–	–	26	–	–
Candida caseinolytica	26	–	–	–	–	1	24	1	–
Pichia pseudocactophila	25	–	–	–	–	5	20	–	-
Rhodotorula mucilaginosa	24	16	1	–	–	1	–	–	6
Debaryomyces hansenii	23	19	2	–	–	–	1	–	1
Kloeckera apiculata	22	11	3	–	3	–	–	5	–
Phaffomyces antillensis	20	–	20	–	–	–	–	–	–
Cryptococcus sp. "1"	15	–	–	–	–	–	–	–	15
Pichia guilliermondii	13	1	–	1	–	1	3	3	4
Kluyveromyces marxianus	13	–	–	–	–	–	12	1	–
Rhodotorula graminis	12	–	6	1	–	1	2	1	1
Williopsis californica	9	9	–	–	–	–	–	–	–
Rhodotorula glutinis	8	7	–	–	–	–	–	–	1
Cryptococcus macerans	8	8	–	–	–	–	–	–	–
Candida zeylanoides	8	7	1	–	–	–	–	–	–
Metschnikowia pulcherrima	7	7	–	–	–	–	–	–	–
Kloeckera apis	6	–	3	2	–	1	–	–	–
Cryptococcus luteolus	5	2	1	2	–	–	–	–	–
Candida sp. "2"	4	–	1	–	–	1	1	–	1
Candida vini	4	4	–	–	–	–	–	–	–
Candida orba	4	4	–	–	–	–	–	–	–
Trichosporon cutaneum	3	–	–	–	–	–	–	–	3
Rhodotorula fujisanensis	3	3	–	–	–	–	–	–	–
Pichia sp. "TM"	3	–	–	–	–	–	3	–	–
Candida viswanathii	3	3	–	–	–	–	–	–	–
Candida krissii	3	3	–	–	–	–	–	–	–
Yarrowia lipolytica	2	2	–	–	–	–	–	–	–
Rhodotorula aurantiaca	2	–	2	–	–	–	–	–	–

Continues

Table 19.2 Number of each yeast species isolated from the entire collection and the eight geographic regions (see Sect. 2.3 for a description of each region). Yeast species listed as sp. "1", etc. are unknown or unidentified taxa—*cont'd*

	Global	AU	CA	FB	HA	SM	SD	TX	VZ
Pichia onychis	2	–	1	–	–	–	1	–	–
Issatchenkia orientalis	2	–	–	1	–	–	1	–	–
Issatchenkia occidentalis	2	2	–	–	–	–	–	–	–
Cryptococcus skinneri	2	–	–	–	–	1	1	–	–
Pichia burtonii	1	1	–	–	–	–	–	–	–
Trichosporon sp. "2"	1	–	–	–	–	–	1	–	–
Trichosporon sp. "1"	1	1	–	–	–	–	–	–	–
Debaryomyces sp."1"	1	–	1	–	–	–	–	–	–
Rhodotorula sp. "2"	1	–	1	–	–	–	–	–	–
Rhodotorula sp. "1"	1	1	–	–	–	–	–	–	–
Rhodotorula marina	1	–	1	–	–	–	–	–	–
Rhodotorula auriculariae	1	1	–	–	–	–	–	–	–
Pichia strasburgensis	1	–	–	–	–	–	1	–	–
Pichia nakasei	1	1	–	–	–	–	–	–	–
Pichia glucozyma	1	1	–	–	–	–	–	–	–
Pichia farinosa	1	–	–	–	–	–	1	–	–
Kloeckera japonica	1	1	–	–	–	–	–	–	–
Dipodascus capitatus	1	1	–	–	–	–	–	–	–
Cryptococcus magnus	1	–	–	–	–	1	–	–	–
Cryptococcus infirmo-miniatus	1	1	–	–	–	–	–	–	–
Cryptococcus flavus	1	–	–	–	–	–	1	–	–
Candida sp. "3"	1	1	–	–	–	–	–	–	–
Candida sp. "1"	1	–	–	–	–	–	1	–	–
Candida vanderwaltii	1	1	–	–	–	–	–	–	–
Candida tropicalis	1	–	–	–	–	–	1	–	–
Candida tenuis "like"	1	1	–	–	–	–	–	–	–
Candida inconspicua	1	–	–	–	–	–	1	–	–
Candida diddensiae	1	1	–	–	–	–	–	–	–
Candida catenulata	1	–	1	–	–	–	–	–	–
Total	4,958	1,257	724	219	88	322	1,722	232	394

Both cases represent speciation events as sources of the overall species richness. Two other common, but unrelated cactus-specific yeasts, *Pichia heedii* and *Candida caseinolytica*, were region-specific (Sonoran Desert) and could represent old lineages that have only survived in the Sonoran Desert, i.e., relics. Alternately, they might be autochthonous members of other sympatric communities living in other plants but we have little evidence for this possibility (Ganter et al. 1986). *Pichia membranifaciens* was found frequently in cacti collected in Venezuela, occurring multiple times in different localities. This species is not cactus-specific and is found in a number of habitats, including fruit rots and tree fluxes. This may be a case where the species diversity is increased as a consequence of ecological equivalence. However, because the *P. membranifaciens* phenotype is convergent with a number of cactus-specific yeasts and because these strains are no longer available, it is not possible to verify their identity. They may in fact represent cryptic species similar to *P. membranifaciens*, as is known to be the case in recent studies of sap-flux yeasts in Costa Rica

Table 19.3 Species richness (S) and diversity (H') for host categories in the geographic hierarchy. *Numbers* in *bold* are the percentage contribution of the total species richness or diversity. β diversity represents contributions of between-level components. α diversity is the within-sample diversity (see Sect. 2.1 for details on calculations)

	Global	Continental	Regional	Sub-Regional	Local	Plant	Within plant
Opuntioideae:S	67	44	22.13	16.60	5.78	1.94	–
β_c:β_r:β_{sr}:β_l:β_p:α	**34.3**	**32.6**	**8.2**	**16.2**	**5.7**	**2.9**	–
H'	2.68	2.505	2.189	2.08	1.669	–	–
β_c:β_r:β_{sr}:β_l:α	**6.5**	**11.8**	**4.1**	**15.3**	**62.3**	–	–
Cactaceae:S	–	56	26.17	19.83	7.93	1.95	–
β_r:β_{sr}:β_l: β_p:α	–	**53.3**	**11.3**	**21.3**	**10.7**	**3.5**	–
H'	–	2.642	2.299	2.193	1.705	–	–
β_r:β_{sr}:β_l: α	–	**13.0**	**4.0**	**18.5**	**64.5**	–	–
Opuntioideae:S	–	42	20.17	14.67	5.67	1.94	–
β_r:β_{sr}:β_l:β_p:α	–	**52.0**	**13.1**	**21.4**	**8.9**	**4.6**	–
H'	–	2.552	2.197	2.044	1.671	–	–
β_r: β_{sr}:β_l: α	–	**13.9**	**6.0**	**14.6**	**65.5**	–	–
Cactoideae:S	–	49	20.2	14.3	5.57	1.59	–
β_r:β_{sr}:β_l:β_p:α	–	**58.8**	**12.0**	**17.8**	**8.1**	**3.2**	–
H'	–	2.525	2.091	1.959	1.619	–	–
β_r:β_{sr}:β_l: α	–	**17.2**	**5.2**	**13.5**	**64.1**	–	–
Pachycereinae:S	–	36	13.6	9.89	3.81	1.34	–
β_r:β_{sr}:β_l:β_p:α	–	**62.2**	**10.3**	**16.9**	**6.9**	**3.7**	–
H'	–	2.567	2.005	1.820	1.501	–	–
β_r:β_{sr}:β_l: α	–	**21.9**	**7.2**	**12.4**	**58.5**	–	–
Stenocereinae:S	–	38	17.5	12.38	4.85	1.44	–
β_r:β_{sr}:β_l:β_p:α	–	**53.9**	**13.5**	**19.8**	**9.0**	**3.8**	–
H'	–	2.299	1.986	1.871	1.579	-	–
β_r:β_{sr}:β_l: α	–	**13.6**	**5.0**	**12.7**	**68.7**	-	–
Stenocereinae[a]							
H'	–	2.299	1.986	1.871	1.420	0.9145	0.7075
β_r:β_{sr}:β_l: β_p:β_w: α	–	**13.6**	**5.0**	**19.6**	**22.0**	**9.0**	**30.8**

[a]Local, plant, and within-plant categories are estimated from data of *Stenocereus gumosus* and *S. thurberi* collections in the Sonoran Desert that selectively isolated and recorded cell counts of yeast species within plants, while the other categories include *Stenocereus* species from all regions.

and *Clermontia* flowers in Hawaii (Lachance et al., unpublished). All other species (17) that were unique to one region were either single isolates (11) or only occurred in low numbers (6). These include basidiomycetes such as *Cryptococcus* and *Rhodotorula* species and ascomycetous species found commonly in fruit rots or tree fluxes (*Kloeckera* spp. and *Pichia* spp.). The overall assessment of the 24 unique yeasts is four are due to speciation, two are relics, one is uncertain and 17 are rare opportunistic non-cactus species.

19.3.2 Yeasts of *Opuntia* Cacti

A similar comparison of the 42 yeast species in the *Opuntia* yeast community showed that 11 were found in only one region. Eight were single isolates, of which two were normally specific to columnar cacti (i.e., *Pichia pseudocactophila* and

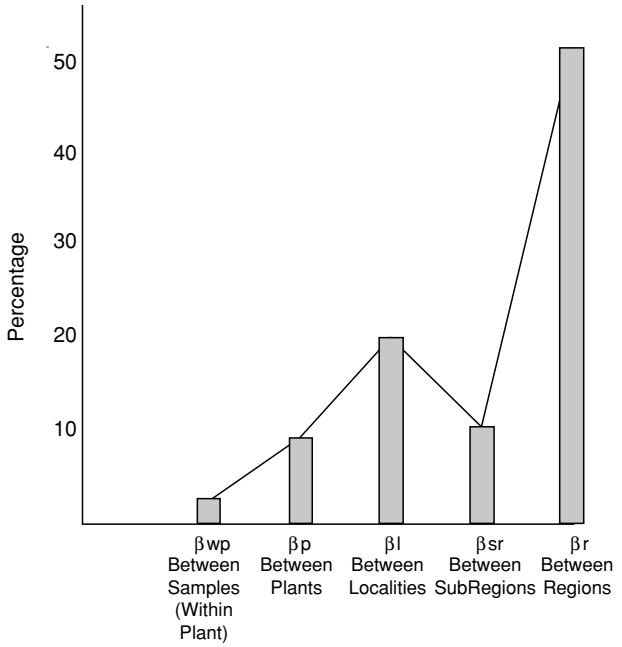

Fig. 19.2. Partition of species richness for yeasts in the Cactaceae (S=56) as a function of geographic level (Table 19.3)

P. thermotolerans) while the others were non-cactus yeasts usually associated with fruit or tree fluxes (*Kloeckera* spp., *P. membranifaciens*) or potentially airborne basidiomycetous species. These unique regional yeasts in *Opuntia* are mostly opportunistic and not products of speciation or extinction processes.

The global comparison of S for yeasts from *Opuntia* cladode rots shows a substantial increase in species richness when the Australian and Hawaiian yeast collections are included. Inspection of Table 19.2 shows that this increase is not due to replacement of one set of dominant cactus yeasts by another but is more likely a consequence of different rare indigenous yeasts in the local environments. Thirteen unique yeast species were recovered only once and only in Australia. A comparable number of single isolates were unique to the New World. Furthermore ten species found only in Australia had a low frequency of isolation (less than 10), whereas a number of common species in the New World have been found neither in Australia nor in Hawaii. These include seven species that were isolated between 161 and 45 times in the New World. Notable (Table 19.2) is that the top six cactophilic yeasts are found in Australia and other regions of the world (i.e., introduced and natural). It may be possible to trace the origin of the cactus-specific yeasts that found their way to Australia from the Americas during the campaign to eradicate the undesirable "prickly pear." However, because the introduction of yeasts and their vectors occurred a number of times (67 shipments of rotting plant material infected with

microorganisms and larval stages of insects) and from a number of different localities (59 from North America and eight from South America) in the New World (Dodd 1940; Starmer et al. 1988) multiple sources are likely.

19.3.3 Other Studies

Cactus rots have been investigated in Brazil by Rosa and colleagues (Morais et al. 1994; Rosa et al. 1995). Their species lists from columnar and *Opuntia* cacti contain the same dominant species we found in our surveys, with three to four additional unidentified or unknown *Candida* species collected in low numbers. It is likely that more extensive sampling of other localities in South America will increase the species richness, although the cactophilic yeast community clearly is very similar for both continents.

19.3.4 Biases Affecting Estimates of Species Richness

Among the factors that could bias our estimates of species richness are the methods that we use to detect yeasts in the individual rot pockets. Using general isolation media such as acidified yeast extract–malt extract agar or medium supplemented with chloramphenicol will necessarily cause one to overlook species present in low numbers, especially under conditions where the dominant yeasts are very numerous. We have shown this to be the case when we used selective isolation media to screen for rare yeasts and to obtain accurate counts of common yeasts (Starmer 1982; Fogleman and Starmer 1985; Latham 1998). However in those studies the yeasts that were revealed were still a subset of those found in the larger survey that only exposed those species with the greatest number of cells. Another factor that may prove important is the undetected diversification of taxa that we have called single species. For example, we expect the taxon *Pichia mexicana* to consist of a number of cryptic species. This was our initial finding when we identified the common dominant cactus yeast as *Pichia membranifaciens* in the mid 1970s (Heed et al. 1976; Starmer et al. 1976). Detailed study of host plant distribution, physiology, sexuality, GC content of the DNA, and DNA reassociations revealed several distinct species and complexes that were originally identified as *P. membranifaciens*. Among these were *Pichia cactophila*, *Pichia pseudocactophila*, *Pichia deserticola*, *Phaffomyces opuntiae, P. thermotolerans, Pichia eremophila,* and the *S. amethionina* complex, all of which have restricted physiologies that are convergent on a similar phenotype. In a like manner it is now recognized that *Sporopachydermia cereana* is a highly heterogeneous complex of species that show considerable geographic diversification (Lachance et al. 2001a).

19.4 Yeast Diversity (Shannon–Weiner)

The diversity index (H') has a very different pattern of change in the geographic hierarchy as compared with species richness (S). The most detailed comparison is available for yeasts found in the columnar cacti in the subtribe Stenocereinae (Table 19.3). In this case most of the variability occurs at three levels: within samples of

individual plants, between plants in a locality, and between localities in a region (Fig. 19.3). These three levels account for 72% of the diversity. Only a small amount of diversity remains for the other levels (i.e., within a plant or between samples, between subregions or between regions, Fig. 19.3). This result is explained by the following arguments.

Each sample of cactus tissue has about two to three species and if samples are taken repeatedly from the same rot they yield about the same number of cells of the same species, i.e. samples are homogeneous in the rot pocket and pooling them does not increase the diversity by much. However, each plant may represent a different inoculation history or may be at a different stage of the decay process. As a consequence there would be a large increase in diversity when plants from a single locality are pooled. Combining localities of a subregion also increases diversity but in this case the increase is likely due to changes in habitats and local species availability. This is reflected in the sharp increase in species richness (Fig. 19.2) seen for β diversity (between localities).

Comparison of the diversity index for categories where yeast cell numbers for each plant were not available gives no information for within-plant diversity. In these cases α diversity is for yeast species within localities. In all host categories the α diversity comprises most of the total diversity. The α partition accounts for

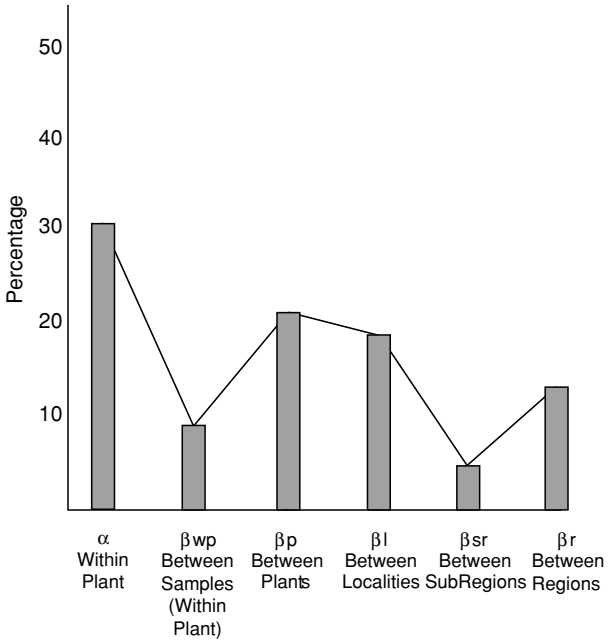

Fig. 19.3. Partition of species diversity for yeasts in the Stenocereinae (H'=2.299) as a function of geographic level (Table 19.3)

58.5–68.7% of the total diversity. This magnitude of increase in the metric likely reflects (as mentioned before) the inoculation history and the stage of the rotting process sampled in each locality. Likewise the increase (12.4–18.5%) for between-locality diversity is interpreted to be a function of the local species availability as a consequence of living in different habitats. There was little increase in diversity (4.0–7.2%) between subregions, whereas diversity between regions increase by 3 times as much (13.0–21.9%). The distribution of yeasts in the Stenocereinae would seem to argue in favor of the ubiquitous model of microbial diversity (Fenchel and Finlay 2004). However, it should be noted that these cacti are limited in distribution, such that the distinction between β and γ diversity would be expected to be less pronounced.

19.5 Conclusions

There is a striking difference when the geographic hierarchy is used to partition species richness (S) as contrasted to species diversity (H'). Most of the species richness is due to finding different relatively rare non-cactus-specific yeast species in different regions or continents. In this case the increase is most likely due to accidents, contaminants, or otherwise unusual circumstances and as such provides a "random" reason for an increase in biodiversity. Diversity as reflected in relative proportions of species and their uncertainty is influenced primarily by the number of cells (or abundance) of a small number of species (two or three) within a rotting sample (usually about 1 cm^3) of cactus tissue. Thus the α diversity, which almost always includes the core cactus-specific species, accounts for most of the information. This diversity metric has a biological meaning that reflects the salient factors mentioned in the "Introduction," i.e., habitat suitability, speciation, extinction, and immigration. Our previous work on habitat suitability and the insect (vector) yeast relationships has emphasized the likely mutualistic interactions among the core cactus yeasts and has shown experimentally that the cactus-yeast community is mutualistic with their vectors (Starmer et al. 1991). These relationships are not obligate but apparently strong enough to (1) maintain yeast communities that are stable over time and space (Latham 1998) and (2) restrict them to decaying cactus stem and cladode tissues (Ganter et al. 1986). These factors argue strongly that not all microorganisms are everywhere and that not all microorganisms freely and passively disperse to achieve world-wide distributions.

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