Chapter 1

Yeast Biodiversity: How Many and How Much?

Marc-André Lachance

Department of Biology, University of Western Ontario, London, ON, Canada N6A 5B7 (e-mail: lachance@uwo.ca)

1.1 Introduction

Biodiversity is now a common word. Harper and Hawksworth (1995) tabulated the frequency of use of the term in *Biosis* and reported its first occurrence in 1988 followed by an increase to approximately 900 by 1994. A similar search of the PubMed database yielded a cumulative total of 1,361 hits by the end of 2003. By comparison, the number of articles using the word "yeast" is approaching 100,000. If the present trend continues, by the year 2016 searches for either word will produce in excess of 36,000 hits for that year only. The task at hand is to make similar predictions about yeast biodiversity.

Biodiversity means different things to different individuals. Gaston (1996) reviewed several definitions and concluded that the concept is an abstract expression of all aspects of the variety of life. Recent publications dealing with yeast *diversity*, had they appeared only 15 years earlier, might have used instead such terms as *taxonomy*, *ecology*, or *survey* (Nout et al. 1997; Buzzini and Martini 2000; Fell et al. 2000; Poliakova et al. 2001; Gadanho et al. 2003; Granchi et al. 2003; Lachance et al. 2003a; Ganga and Martinez 2004; Renker et al. 2004) or even *enzymology* (Lamb et al. 1999). The Convention on Biological Diversity (Anonymous 1992) defines biological diversity as "the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems." As with most things in our society, biodiversity became a tangible reality when it could be assigned a significant economic value. And as with most things in science, the recognition of biodiversity as a worthy research topic is predicated on measurability and the generation of testable hypotheses. The current urgency of the scientific study of biodiversity stems from the realization that only a small fraction (approximately 8%) of the total diversity of life is known (Stork 1999) and that species extinction is occurring at a measurable and increasing rate (Purvis and Hector 2000).

1.2 Measurement and Significance of Biodiversity

1.2.1 Levels of Diversity

The inclusion of any level of biological variation in the definition of biodiversity could lead to a trivialization of the concept, as variation is the very essence of biology. A more restrictive circumscription should limit the term to ecological and evolutionary variation. Theoretical ecologists, following the model of Whittaker (1960), often subdivide species diversity into three hierarchical components, namely within-sample (α), between-samples (β), and global (γ) diversity. These components may be considered additive ($\gamma = \alpha + \beta$, Crist et al. 2003). The main units of measurement are richness (simple species count) and heterogeneity (relative abundance of each species in a community). The two measures can be examined simultaneously in relative abundance plots, which contrast the number of species in a sample as a function of the number of individuals representing each species. Considerable interest in the underlying causes of such distributions was stimulated by the pivotal publication of MacArthur and Wilson's (1967) treatise on island biogeography. A recent model (Hubbell 2001) attributes a large portion of the species composition of a community to chance. Implicit to this view (but perhaps not sufficiently explicit) is that membership of a species in a community depends initially on its fundamental niche, in other words, the sum of its intrinsic properties. For example, the community of floral nectar rarely contains basidiomycetous yeasts. This is not due to chance, but to the fact that such a habitat favours fermentative, osmotolerant, copiotrophic species, which are found most often in the Saccharomycetales. In the neutral model, a community is seen as an assemblage of ecologically equivalent species, where the abundance of each species within a local community is not so dependent on the fundamental niche. Instead, species composition is affected by speciation in the metacommunity, the rate of influx of species, the size of the local community, and the local rate of extinction. This is almost entirely analogous to Kimura's (1983) neutral model of evolution. Natural selection remains the preliminary screen that causes rapid elimination of deleterious mutations and rapid fixation of adaptive alleles, just as the environment determines whether or not a species can enter a community. The majority of species in the community have already "passed the test" of selection, and are equally adapted. As is the case for selectively neutral mutations, the relative abundance of a species will be due not so much to some intrinsic property, but to chance.

Application of such theories to yeast communities is not yet completely practical. Most models of community ecology were developed for communities where members can be identified and enumerated rapidly, e.g., forest trees and insect biota. The recent development of identification methods based on DNA sequencing (Kurtzman and Robnett 1998; Fell et al. 2000) has not yet resulted in practical means of identifying yeasts instantly, in the field, but such technologies are no doubt forthcoming. An attempt to explore the factors that underlie community structure was made recently (Lachance et al. 2003a). The yeast biota of morning glory flowers and associated nitidulid beetles was characterized in a "forest island" (*kīpuka*) on the slope of the Mauna Loa volcano in Hawaii. The yeast community is highly

specialized, consisting almost entirely of members of two clades with affinities to *Metschnikowia* and *Wickerhamiella*: the former clade is vectored primarily by the beetles, and the latter by drosophilid flies. Although the resulting community is a mixture, each clade can be studied separately with selective media. The *Metschnikowia* clade members in the community consists of six species and whose frequencies follow the expected log series distribution, from abundant to rare. Two of the species (*Metschnikowia hawaiiensis* and *Candida kipukae*) are probably Hawaiian endemics. The others have also been found in Central America and are thought to have reached Hawaii in recent history. The six species are similar physiologically, suggesting that they might be mutually neutral with respect to niche. However, their distribution within the *kīpuka* is not random and follows closely the distribution of the host beetles. The latter consist of two major species, one Hawaiian endemic and one that was introduced in the early twentieth century. Maximum growth temperature and insect choice may be important factors in the local distribution, such that a completely neutral model would have to be ruled out. The study is in progress, and increased sampling is hoped to provide a test of the neutral hypothesis.

1.2.2 Diversity Within Species

Even if one agrees that species abundance is central to the characterization of biodiversity, genetic diversity is an essential feature of the species itself. Even orthodox proponents of the phylogenetic/autapomorphic species concept would have to agree that a "species" that is completely devoid of variation can hardly be regarded as a species (Wheeler and Meier 2000). Variation among members of a species has long plagued pragmatic systematists in their search for stable diagnostic (autapomorphic) characters (Lodder and Kreger-van Rij 1952). As DNA sequence analysis took the study of yeast diversity by storm, the recurring dream of an invariant species trait was temporarily rekindled (Kurtzman and Robnett 1998). However, the sequencing approach has in some instances brought to light considerable variability among individuals that share a common gene pool and thus are members of the same biological species. One response might be to denounce the biological species concept as antiquated and inoperable (Wheeler and Meier 2000). Another would be to accept that the genes that are most amenable to phylogenetic construction are not necessarily involved in conferring a common evolutionary destiny to members of a species, and that species cannot be defined on the basis of invariance in gene sequences.

In a study of the distribution of yeasts in seawater, Gadanho et al. (2003) subjected 234 isolates to microsatellite-primed PCR fingerprinting and demonstrated that in most cases multiple isolates of various basidiomycetous yeast species contain a substantial amount of genomic variation. Ascomycetous species recovered in that habitat exhibited less variation.

Intraspecific variability has been examined in the two species in the genus *Clavispora*, both of which occur in nature as heterothallic, haploid mating types. This offers the advantage that species boundaries can be assessed by mixing of compatible strains and observation of ascospores. *Clavispora opuntiae* has so far

been isolated exclusively from necrotic tissue or tunnels of moth larvae found in cacti. Hundreds of specimens have been recovered globally and preserved for study. Although the growth responses of most isolates are generally constant, polymorphisms have been detected at the level of the ribosomal DNA (rDNA) gene cluster (Lachance et al. 2000b). In some 500 isolates examined by restriction mapping, over 40 variants were recognized. These correlated to a large degree with geography, host plant species, and insect vectors. Most of the variation was shown by sequencing to be located in the intergenic spacer region, although a small amount of polymorphism was also detected in the large subunit rRNA gene. Strains representing the extremes of that variation had been shown previously to exhibit a lower degree of interfertility (Lachance et al. 1994) and perhaps represent the beginning of a speciation event.

C. lusitaniae is similar morphologically and physiologically to *C. opuntiae*, but exhibits much less habitat specificity, having been recovered in cactus fruit, agave rots, industrial wastes, clinical specimens, and several other sources. Mating compatibility and large subunit rDNA sequences were determined in 37 strains (Lachance et al. 2003c). The sequences could be assigned to ten types belonging to two families that differed by as much as 32 substitutions in the D2 domain. The variation was not correlated with mating intensity or abundance of mature asci.

Although these studies do not allow generalizations about the evolutionary or ecological significance of genetic diversity within yeast species, they would seem to support the view that variability is an intrinsic property of species.

1.2.3 Species Diversity

From the first to the current edition of the *The yeasts, a taxonomic study*, the number of species described has grown from 164 in 1952, to 349 in 1970, to 500 in 1984, and to 700 in 1998 (Lodder 1970; Kurtzman and Fell 1998). Extrapolation of these numbers leads to the prediction that an eventual 2016 edition would contain approximately 1,000 species. However, this number may very well be exceeded in the forthcoming fifth edition, planned for 2005. The increase is due to several factors, including methodology and species concepts. In the first edition (Lodder and Kreger-van Rij 1952), species were circumscribed on the basis of morphology and a small number of growth tests. The doubling in the number of species found in the second edition was due in part to the use of a much larger battery of nutritional properties. Early application of molecular approaches had a considerable impact on the third edition, but was not entirely accountable for species proliferation, as the shift to a genomic basis for species delineation also caused the merger of physiological or morphological variants into larger and more diverse species. The publication of the fourth edition coincided with early application of DNA sequencing in yeast identification and phylogenetic reconstruction, although the full impact of this approach came later. Again, the result is a mixture of species fusions and subdivisions.

The definition of species is fundamental in the generation of meaningful estimations of biodiversity, which accounts in part for the heartiness of the debate on that subject (Wheeler and Meier 2000). The species problem as it applies to bacterial and

fungal diversity has been discussed by O'Donnell et al. (1995), who pointed out the lack of a common standard. Although species concept controversies are not alien to yeast systematics, many practitioners agree that species should, whenever possible, represent cohesive evolutionary units. Individual researchers may disagree on how best to document the boundaries of such units, but the result is nonetheless a relatively stable consensus. As the issue is far too complex to be examined here in detail, it will be expedient to assume, rightly or wrongly, that taxa which are recognized at any given time constitute genuine and meaningful species.

Sequence analysis resulted in an enormous increase in the ease and speed of identification, making intense biodiversity surveys almost manageable. Many species descriptions currently being published come from material collected in the past and stored in collections in the hope that new technologies would eventually facilitate meaningful species assignments. The sequencing approach has fulfilled this need. Unfortunately, the clarifications brought forward by sequencing have done little to improve our understanding of the natural history or ecology of the species being described. Unless the ecological context of species is also documented, Linnean binomials will remain no more than mere labels of little relevance to biodiversity. By their very nature as unicellular heterotrophs, yeasts are inexorably dependent on other fungi, bacteria, animals, and plants for their existence, and ideally species descriptions should include data on these interactions. The old precept, "everything is everywhere", although no longer tenable, sadly continues to influence yeast taxonomy. An inordinate amount of energy is devoted to transforming sequence data into "correct" trees, at the expense of the yeasts themselves and their biology. Another important consequence of the dependence of yeasts on other life forms is the urgency of documenting their natural history before their very habitats disappear. Unless conservation efforts are intensified, it will become easier to determine the rate of extinction of yeasts than to estimate the number of extant species. The fact that the construction of a comprehensive inventory of life on Earth is seen as a priority by an increasing number of researchers, governments, and granting agencies (Mulongoy et al. 1999) should be viewed with optimism. Equally encouraging is the emergence of more frequent studies aimed at characterising whole yeast communities in relation to their insect vectors. A case in point is a recent description of 16 closely related species originating from fungivorous beetles and their habitats (Suh et al. 2004). Members of the Coleoptera associated with tree decay have long been known to harbour numerous yeast species, as evidenced by the work of pioneers such as L.J. Wickerham, J.P. van der Walt, and H.J. Phaff. These yeasts are suspected to engage in intimate symbiotic relationships with insects, although the nature of the interaction remains elusive in most cases. Recent studies of yeasts found in tropical bees led to the discovery of the genus *Starmerella* (Rosa and Lachance 1998), the nucleus being a growing clade whose membership has increased from 12 described species in 1998 to 29 putative species at the last published count (Rosa et al. 2003). Studies of nitidulid beetles associated with ephemeral flowers have resulted in the near doubling of described species of *Metschnikowia* (Lachance et al. 2003b) and a significant expansion of the formerly monotypic genera *Kodamaea* (Lachance et al. 1999) and *Wickerhamiella* (Lachance et al. 2000a).

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