Chapter 3
Yeast Community Composition and Structure

Andrey Yurkov and María I. Pozo

Abstract Yeasts are globally distributed, but different species occur in different climates and environments. With a few exceptions, yeasts do not occur in their natural environments as a pure culture but co-occur with other microscopic eukaryotes and prokaryotes and comprise microbial communities. The observed yeast diversity in natural environments is a combined result of the response of each species to habitat conditions, including arrival, growth, and further dispersal, and the biotic interactions among species. In this chapter, we review some recent concepts and tools developed in community ecology and discuss how they may help understand yeast diversity in nature. We address species recognition approaches and the effects of the intraspecific variation and application of molecular operational taxonomic units on the yeast community parameters. Community ecology tools discussed in this chapter include diversity (taxonomic and functional), quantity, priority effects, species richness estimators, and species-abundance distribution. Additionally, we compare the use of community composition and community structure parameters in the literature. Concepts such as frequent (vs. rare), autochthonous (vs. transient or allochthonous) and specialist (vs. generalist) yeast species are also discussed through this chapter.

Keywords Species • Diversity • Quantity • Community parameters • Ecology tools

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P. Buzzini et al. (eds.), *Yeasts in Natural Ecosystems: Ecology*,
DOI 10.1007/978-3-319-61575-2_3

3.1 Introduction

Yeasts are globally distributed, but different species occur in different climates and environments, and they also vary in the morphological (e.g. pigmentation, forcibly ejected propagules, hyphal growth, chlamydospores), physiological (e.g. fermentation, utilisation of low-weight aromatics, vitamin-free growth) and physiochemical (e.g. psychrophily, production of siderophores and acids) traits. Linking these features to the spatial distributions of species is fundamental to the understanding of yeast diversity. With a few exceptions, yeasts do not occur in their natural environments as a pure culture but co-occur with other microscopic eukaryotes and prokaryotes. Thus, yeast diversity in natural environments is a combined result of the response of each species to habitat conditions, including arrival, growth, and further dispersal, and the biotic interactions among species.

For example, flowers, rotting cacti, and tree fluxes are rich with simple sugars or alcohols and offer certain groups of yeasts a suitable habitat. These substrates are frequently visited by insects that vector yeasts, thereby, largely determining the membership of the yeast community. In the absence of vectors, these habitats might not bear any yeasts (reviewed by Starmer and Lachance 2011). To ensure transportation between mosaic habitats, yeasts steer the dispersal by targeting attraction of the vector with volatiles (e.g. Becher et al. 2012; Davis 2015; Holighaus and Rohlfs 2016) or, conversely, by masking their presence in the habitat (Mittelbach et al. 2016a). Passive propagation can also be facilitated by several morphological properties, including formation of ballistospores by phylloplane yeasts (also some mycoparasites), “aeroplane” (cross-like configuration) cells of the nectar yeast *Metschnikowia gruessii* and hydrophobic cells (rotting cacti), among them (Brysch-Herzberg 2004; Fonseca and Inácio 2006; Starmer and Lachance 2011; Pozo et al. 2012). Research on yeast co-growth in the habitat received some attention in the past, and studies report a broad range of naturally occurring interactions between species (reviewed by Starmer and Lachance 2011). Recently, environmental alteration (also called priming) by early-arriving yeasts was also

recognised as an important factor influencing flower nectar communities (see below).

In this chapter, we review some recent concepts and tools developed in community ecology and discuss how they may help understand yeast diversity in nature. Although we mainly focus on synecology (community ecology), we recommend reading book chapters summarising the knowledge on yeast autecology (species ecology) and diversity (Lachance and Starmer 1998; Lachance 2006; Starmer and Lachance 2011; Buzzini et al. 2017). Through the chapter, we shall frequently refer to the niche concept described previously by Lachance and Starmer (1998) and Starmer and Lachance (2011). The chapter is composed of two sections: we will first review the ways in which the structure (such as species composition and diversity) of communities is described and then discuss the ecological processes that are thought to determine community structure.

3.2 Community Composition and Diversity

Biodiversity has been a hot topic in community ecology for a long time, and studies addressing diversity in microbial ecosystems abound. The majority of studies listed yeast species isolated from different habitats (Fig. 3.1). As the approaches to species identification and circumscription evolved, the definition of the species as an ecological unit was changing gradually with more sophisticated techniques involved in the characterisation of yeast isolates (Barnett 2004; Lachance 2006).

3.2.1 Species Recognition

Ecological interactions among different taxa determine diversity of a community and ecosystem functioning. By "taxa", we normally refer to genus and species, and those are characterised by cell morphology (e.g. spore shape, cell division), physiology (e.g. carbon and nitrogen source assimilation tests) and phylogenetic relatedness, which is mostly estimated, in the case of yeast, by comparing sequences of the D1/D2 domains of the 26S (or LSU) rDNA gene (e.g. Kurtzman and Robnett 1998; Fell et al. 2000; Scorzetti et al. 2002). In the *Origin of the Species*, Darwin stated that "I look at the term species, as one arbitrarily given for the sake of convenience to a set of individuals closely resembling each other, and that it does not essentially differ from the term variety, which is given to less distinct and more fluctuating forms. The term variety, again, in comparison with more individual differences, is also applied arbitrarily, and for mere convenience sake" (Darwin 1872). Like other organisms, yeast species should represent cohesive evolutionary units. Currently, there is considerable controversy on the best way of documenting the boundaries of such units (e.g. Lachance 2006, 2016; Lachance et al. 2010). Kurtzman and Robnett (1998), by conducting a large-scale study of D1/D2

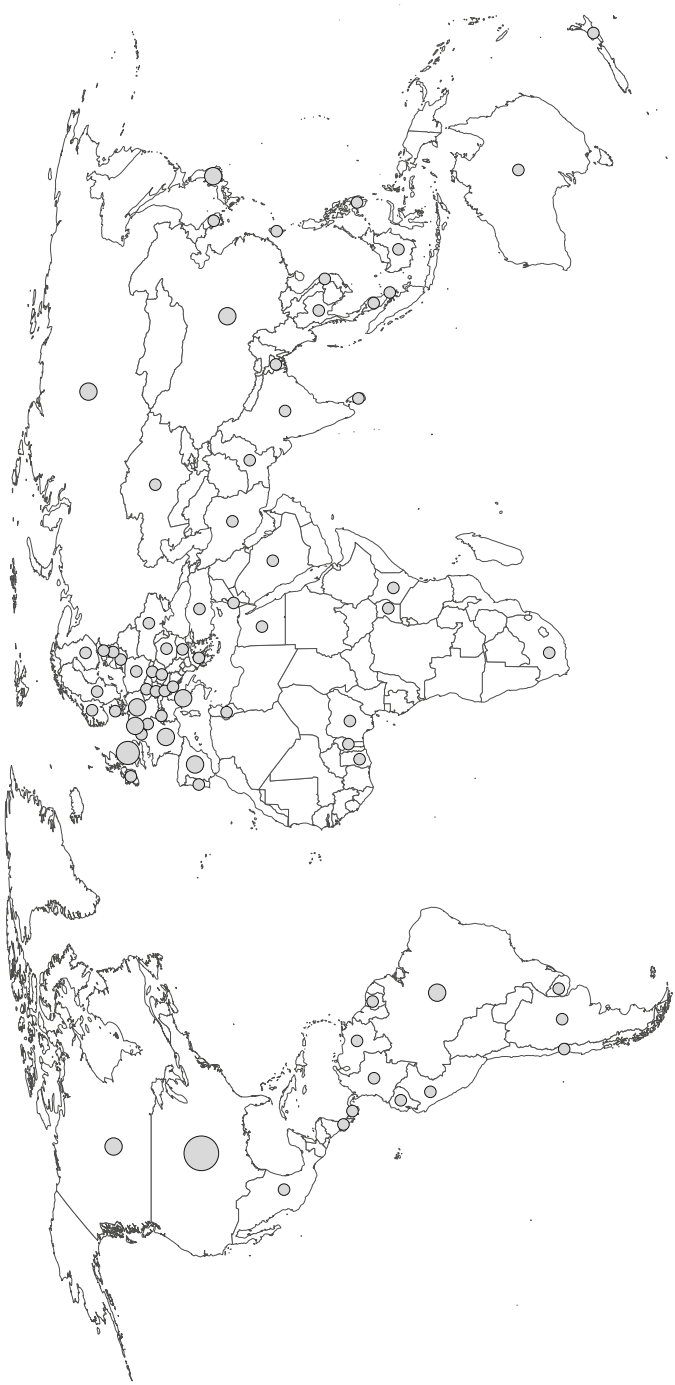

Fig. 3.1 Geographic distribution of publications in which natural yeast communities have been surveyed. A total of 1528 references have been collected using Web of Science and Google Scholar databases (citations from the English-speaking literature published from 1980 onwards have only been considered, stand December 2016). The size of *circles* is proportional to the number of publications

ribosomal LSU sequences of 500 species of ascomycetous yeasts, showed that strains that differ by less than 1% nucleotide substitutions are likely to be members of the same species. As a result, this threshold has been widely accepted as a rule to delineate species of yeast, instead of the 3% prevailing rule for many other organisms. Despite distant evolutionary relationships between ascomyceteous and basidiomycetous yeasts, the "1% rule" was used as an argument to delimit species in the latter group. However, another large-scale study of LSU sequence heterogeneity in Basidiomycete yeasts performed by Fell et al. (2000) did not show any suitable cut-off value. Similarly, the follow-up study did not reveal a common threshold, neither in LSU nor in ITS, in this group of fungi (Scorzetti et al. 2002). As new molecular identification methods develop, Lachance and Starmer (1998) foresaw that "one can easily conceive of the possibility that a primary isolation plate, a microscope slide, or even a fixed section of a yeast habitat could be treated with a mixture of DNA probes, each tagged with a different chromophore, in such a way that direct identification of individual colonies or even single cells in situ would be achievable". Although quick and affordable identifications of yeasts based on the DNA sequences have become feasible, the determination of a yeast's physiological characteristics will never cease to be of importance in understanding its ecology.

The species concept has a great influence on diversity studies because different approaches to yeast species recognition often result in different entities. A review that aimed at resolving the impact of the species concept on biodiversity studies showed remarkable differences, with surveys based on a phylogenetic species concept detecting 48% more species (300% more for fungi) and an associated decrease in population size and range (Agapow et al. 2004). The same holds true for widespread basidiomycetous yeasts identified with physiological and phylogenetic approaches (Yurkov et al. 2015a). Specifically, several phylogenetic species of the genera *Filobasidium* and *Vishniacozyma* were nested in "phenotypic" *Cryptococcus albidus* and *Cryptococcus laurentii* (Yurkov et al. 2015a). This study also showed that species recognition approach might affect our understanding of community structure and the distribution range of yeast species and require a larger sampling effort.

3.2.2 Strain Variation

Dissimilarities between isolates of the same species may represent an annoyance in practical taxonomy, is also a component of biodiversity, and communities must comprise this level of sampling. The conventional approach of taking one colony as a representative of a species (Lachance and Starmer 1998) does not capture functional diversity within species. Researchers are becoming increasingly conscious about intraspecific variance. For example, the general occurrence of within-species variation was detected by a PCR fingerprinting in sea water basidiomycete communities (Gadanho et al. 2003). Similarly, strains of the same species isolated

from forests in Germany, Portugal and Russia showed variation in ribosomal gene sequences (Yurkov et al. 2012a, 2016) and PCR fingerprints (Yurkov et al. 2015a). Yeasts have quick life cycles, and a larger number of offspring can be produced asexually instead of following a sexual cycle. The resulting yeast populations may constitute distinct biotypes subject to unusual forms of selection. In this scenario, mutation can be easily fixed as a result of diversifying selection, as shown in ascomycetous yeasts (Herrera et al. 2011). Although the sexual cycle of ascomycetous yeasts is commonly observed in the laboratory, teleomorphic stages of many basidiomycetous yeasts are known from the field studies only (e.g. Tremellomycetes, Ustilaginomycetes) and are rarely observed under laboratory conditions. Thus, in many cases, the sexual cycle remains little understood in basidiomycetes, although the sexual recombination can be revealed with a genomic approach (Coelho et al. 2011; Yurkov et al. 2015b). A diverse origin of the isolates, which also reflects different environmental constraints, typically favours high levels of intraspecific variation in wild yeast populations (Pozo et al. 2015; Yurkov et al. 2015a, b).

3.2.3 Units Other than Species and Operational Taxonomic Units (OTUs)

Although community analyses are commonly made using species, other units can be also used. Yeasts were the first group of fungi, where a DNA barcoding was adopted for species identification (Kurtzman and Robnett 1998; Fell et al. 2000; Schoch et al. 2012). Fast development of sequencing techniques and availability of sequence databases resulted in an enormous increase in the ease and speed of identification, making intense biodiversity surveys almost manageable.

Once taxa are delineated, the characterisation of yeast communities encounters additional difficulties. Biodiversity estimates are often biased on taxa that are easily cultivable in the lab (see the discussion on rare species below). Only less than 1% of the estimated microbial diversity is thought to be cultivable in laboratory conditions due to the low growth rate of many environmental microorganisms (Amann et al. 1995). To what extent this constraint can be applied to yeasts is yet unknown, although the majority of the lineages comprised by yeasts are cultivable. The description of a microbial community and the quantification of diversity associated with several habitats such as soil, phyllosphere, insect-flower system and aquatic environments have increasingly applied culture-independent methods (Steven et al. 2007; Jumpponen and Jones 2010; Redford et al. 2010; Mašínová et al. 2017). Culture-independent methods include denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), terminal restriction fragment length polymorphism (T-RFLP) and clone libraries, which are based on a DNA barcoding fragment of a conserved gene and can be used to quickly and cheaply determine the main components of fungal communities. High-throughput

sequencing with meta-barcoding of DNA has improved species detection by providing a large amount of sequence data, although all from short sequencing reads.

Computational issues nowadays compromise the efficiency of culture-independent methods and thus make it difficult to arrange large numbers of sequence reads into biologically meaningful information (e.g. classification). An automated identification of sequence data relies on a reference database, variability of the selected DNA marker and a clustering algorithm. Each of these steps has its own limitation (e.g. Amend et al. 2010) that results in an incomplete identification (assignment to the known reference sequences) of the pool of nucleotide sequences. Therefore, a pragmatic approach based on operational taxonomic units (OTUs) is commonly used. Nucleotide sequences are grouped according to their similarity (95–99% threshold) and analysed using common ecological tools implemented in various software packages. However, a reliable use of molecular OTUs as yeast species proxies is presently unlikely. Cut-off values are selected artificially and may not reflect the actual variability of the genetic marker across different phylogenetic lineages (e.g. Ascomycota and Basidiomycota). The size of the most commonly analysed DNA marker, the ribosomal ITS region, is rather stable in basidiomycetous yeasts but varies in Saccharomycetes ranging from about 300 to 1000 nucleotides. Also, the interspecific polymorphism of ribosomal gene regions (e.g. *Clavispora lusitaniae*, *Barnettozyma californica*) complicates the application of molecular OTUs. Although yeasts were detected among fungi in almost every sampled substrate, most of them were not identified to a certain species or even to the genus level. Apart from species inventories, culture-independent studies often detect thousands of units exceeding by far the number of all described fungi or yeasts (e.g. Blackwell 2011; Mašínová et al. 2017). How these molecular OTUs correspond to the species and to what extent the yeasts detected with these techniques are cultivable are a matter of debate. A recent study that analysed soil yeasts with an amplicon sequencing technique showed that several OTUs defined with a conservative 97% threshold were matched to the same yeast species (Mašínová et al. 2017). Thereby, common identification pipelines can potentially overestimate yeast diversity.

3.3 Community Composition and Diversity

By revisiting the classic literature on yeasts, we can see that communities that are studied nowadays have also been studied in the past (Figs. 3.1 and 3.2). The main criticism against past studies is that yeast isolation has not gone much beyond the mere nomenclatural description of new species, together with the assessment of species richness. Below, we summarise some of the ecological concepts that can be assessed by studying yeast communities in their natural habitats.

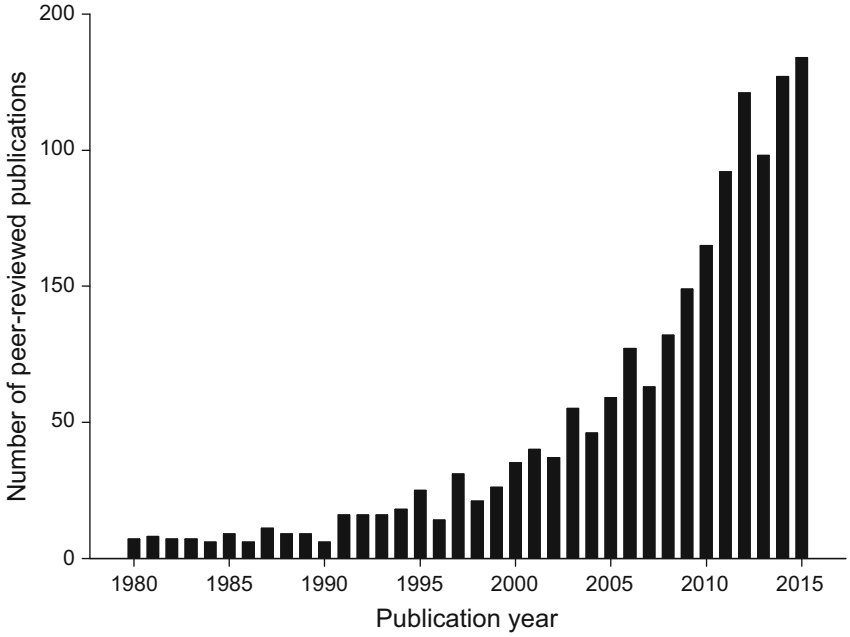

Fig. 3.2 Number of peer-reviewed studies dealing with natural yeast communities published since 1980. Other details as for Fig. 3.1

3.3.1 General Parameters

Community assembly rules aim to predict spatial species distributions and the mechanisms underlying the distribution patterns. One of the early proposed rules is that competition is responsible for determining the patterns of assemblage composition (Diamond 1975). However, their generality has been debated for over 20 years, and the debate continues today (Gotelli and McCabe 2002). Their strong spatial reference makes them highly amenable to phyllosphere studies and less amenable for aquatic environments, for instance. Below, we address a few empirical community distribution patterns in the section dedicated to the structure of yeast communities. Spatial aspects of the distribution of yeasts are reviewed in Chap. 4 of this book.

Diversity encompasses species richness (simple species count) and heterogeneity (relative abundance of each species in a community). Both types of information can be summarised in a rank abundance curve, which displays species richness and species evenness (see below). Diversity can be statistically partitioned into three hierarchical components: alpha, beta and gamma diversity (Whittaker 1960). Following the original idea, the hierarchically organised diversity partitioning is commonly used on a large spatial scale and includes local, regional and global diversity. The question is, however, whether or not the same approach can be

applied to the microbial systems, which are smaller in size than those of plants and animals. If one considers flower nectar community, which is discrete in space and is spatially limited due to the range of animal vectors, the three diversity components can be reasonably translated into the following categories: (1) nectar in a given flower (alpha diversity), (2) nectar from different plant species growing together (beta diversity) and (3) nectar of flowers pooled from different parts of an agricultural field or forest (gamma diversity). One example of this study for the given microsite could be the work by Pozo et al. (2011), who combined the study of yeast alpha and beta diversities with the assessment of their sampling effort. While the alpha diversity assessment showed that most of the individual flowers were dominated by a few *Metschnikowia* species, differences at the plant species level (beta diversity) revealed the occurrence of rare species that also suggested a need for more intense sampling at this scale.

Average number of species is a potentially useful measure to characterise species richness of a yeast community. Because the number of observed species naturally depends on the sampling intensity, species recovery depends on the community structure and the proportion or frequency of detection of each species. Therefore, Chernov (2005, 2013) introduced an index that reflects species diversity in relation to the intensity of sampling. Instead of using the total species richness values, a proportion of the observed number of species to analysed (purified) colonies or plates was calculated. Absolute and relative numbers of species differed substantially in samples with a few dozens of colonies per plate, whereas in samples with more than 100 colonies, there was no statistically significant difference detected between the estimates of these two parameters.

3.3.2 Functional Diversity

In ecology, the group of species that use the same resources in a similar manner is called a guild. Although yeasts share a common adaptation, the ability to grow in a unicellular manner, they differ in their ability to use different nutrients. The habitat has the largest effect on the composition of species, all of which should possess a pool of adaptations to colonise it. Thus, the yeast community can be viewed from the position of these capabilities or potential functions in the habitat. Species descriptions provide a set of characters, which can be used to assess the physiological profiles of the communities. A study of yeast communities associated with *Drosophila* flies across the USA showed that the habitat was the major factor influencing the physiological ability of the community (Lachance et al. 1995). Observation of species, almost indistinguishable physiologically (identical fundamental niche), in similar habitats was a driving force for understanding the ecology and distribution of yeasts (e.g. di Menna 1965; Babjeva and Chernov 1995; Starmer et al. 2003; Buzzini et al. 2012). A few approaches have been made to classify yeasts into functional groups, which would reflect the common physiological (or morphological) adaptations for the habitat or lifestyle. Babjeva, Chernov and

co-workers (Babjeva and Chernov 1995) studied distributions of yeasts across most typical biomes in the USSR and categorised them according to their phenotype and occurrence in the substrates, i.e. species assemblages (originally: complexes) in phyllosphere (e.g. *Vishniacozyma* spp. as "phenotypic *Cr. laurentii*"), litter (e.g. *Tausonia pullulans*) and soil (e.g. *Lipomyces* spp., *Solicoccozyma* spp.). This approach was based on the previous classification of the life strategies of yeasts, i.e. phytobionts (living plant material), pedobionts (soils), saprobionts (decomposing material) and humidobionts (humid sugar-rich substrates). The ability to assimilate complex substances was more pronounced in species inhabiting litter and soils. This adaptation is presumably linked to the dependency on the derivatives of plant decomposition (reviewed by Botha 2006) and was found to be common for several soil yeasts (Di Menna 1965; Sláviková and Vadkertiová 2000; Botha 2006; Mestre et al. 2011). Physiological profiles of the community can be also analysed qualitatively. Chernov (2005) studied the number of assimilated compounds (index of polytrophy) by yeast assemblages in the biogeographic context in the most common types of substrates. As a result, most polytropic communities were observed in tundra, and the number of assimilated substances decreased towards the lower latitudes. Among the surveyed substrates, availability of simple sugars strongly affected polytrophy of the communities as the number of assimilated compounds (also complex substances) was increasing in litter and soils.

Recently, a tool FUNGuild was developed to parse fungal molecular OTUs into ecologically meaningful categories such as functional guilds (Nguyen et al. 2016). The original selection of the ecological categories highlights the ecological diversity of yeasts as they can be assigned to the 7 out of 12 guilds, namely, animal pathogens, lichenicolous fungi, mycoparasites, plant pathogens, undefined root endophytes, undefined saprotrophs and wood saprotrophs. But even as saprotrophs, yeasts should not be synonymised with the saccharolytic lifestyle only. Because of their taxonomic complexity and heterogeneity, yeasts display a multitude of metabolic properties, which are routinely recorded for every described species. This makes yeasts an attractive object to study functional aspects of the community ecology.

3.3.3 Diversity and Quantity

The composition of an ecological community depends on the compatibility of species to the local environment. Moreover, their abundances in the community can be the result of complex interactions and processes (Fukami 2015). Concepts in ecology that might explain yeast coexistence and therefore the conformation of microbial communities are neutralism, commensalism, amensalism, predation and parasitism (Atlas and Bartha 1993; Starmer and Lachance 2011). Neutralism describes the occurrence of sparse-independent populations sharing the same habitat. This can be exemplified by lack of direct contact in the particular case of simple, low populated communities such as soils, in which samples with higher cell

numbers (CFU) are more species rich than those with lower cell counts (e.g. Yurkov et al. 2011; Chernov 2013). The number of isolated species positively correlated with cell density in species-poor communities (e.g. soils) following a logarithmic curve (Chernov 2005, 2013). Other yeast habitats with simple communities, such as floral nectar, are also amenable for neutralism, despite harbouring denser yeast populations (Pozo et al. 2016). Irrespective of host plant species, yeast communities of floral nectar in Europe are largely composed by two species of the genus *Metschnikowia* (Brysch-Herzberg 2004; Pozo et al. 2011). On one hand, nectar habitats are sugar rich and might be not limited with nutrients as soils. On the other hand, the co-growth of the ecologically similar species might be due to the fine niche partitioning, either in the consumption of resources or physical growth preferences. For example, physiological profiles indicated that *Metschnikowia reukaufii* and *M. gruessii* did not compete for most carbon and nitrogen sources but even support co-growth in a more restrictive culture medium (Pozo et al. 2016). Likewise, several *Saccharomyces* species co-occur on Mediterranean oaks, and their niches are strongly determined by growth temperature preferences (Sampaio and Gonçalves 2008; see also Chap. 5 of this book). The cross-feeding (syntrophy) and substrate priming further promote efficient nutrient utilisation by yeast communities (see below).

We should admit, however, that the neutral model in yeast community ecology has been met with some scepticism (Lachance 2006). As in the aforementioned examples, niche occupancy by yeasts is not random but is steered by interspecific interactions and, more importantly, by interactions with their vectors or hosts (reviewed by Starmer and Lachance 2011). Besides neutral interactions, competition, amensalism, predation, and parasitism are considered in the literature (Atlas and Bartha 1993; Starmer and Lachance 2011). The first one, competition, refers to a wide array of antagonistic interactions, including growth inhibition, contact inhibition and competition for nutrients, among others. The diversity of mechanisms used by yeasts to outcompete other species is broad and involves killer activity (killer proteins and mycocines), substrate depletion (nitrogen or vitamins), acidification, ethanol production and mineral sequestration (iron acquisition with pulcherrimin). These abilities have been reviewed previously (e.g. Golubev 2006; Starmer and Lachance 2011) and are addressed in more details in the following chapters of this book.

3.3.4 Priority Effects

The history of the substrate also plays an important role in community composition and can affect the growth of arriving yeasts. For example, the two yeasts *Hanseniaspora vineae* and *Metschnikowia pulcherrima*, which naturally coexist during wine fermentation, affected the fermentation kinetics by having a superior nutrient intake rate compared to *Saccharomyces cerevisiae* (Medina-Rland et al. 2012). The effect was even more pronounced when *S. cerevisiae* was inoculated

24 h after the initial stage of fermentation with a non-*Saccharomyces* yeast, compared to co-inoculation. This temporal succession affects the strength of interference, or facilitation, and is, therefore, named as a priority effect (Chase 2003). The aforementioned timing of the species inoculation and the subsequent modification of the media that, in turn, favours or inhibits later-arriving species change, thereby, the yeast community composition. This approach overlies the specific history of community assembly into the known rules of community ecology (Fukami 2015). The relevance of this ecological theory in the particular case of microbial ecology has been recently acknowledged, and the number of known examples is steadily growing. Specifically, nectar yeast communities have been repeatedly used as a model to test priority effects. Although secreted flower nectar does not carry yeasts, floral visitors such as bees, birds, bats or ants vector yeasts propagules to this specific habitat. Those yeasts may or may not establish and produce substantial changes (e.g. amino acid and sugar composition and concentration) in nectar as a substrate for future incoming taxa (Herrera et al. 2008; Peay et al. 2012; Vannette and Fukami 2016; Mittelbach et al. 2016b). By using experimental manipulation in microcosms, Peay et al. (2012) demonstrated the strength of priority effects for most of the yeast species found in the floral nectar of a hummingbird-pollinated shrub in California. This study concluded that late-arriving species experienced strong negative effects from early-arriving species, but also warned about the relevance of the species phylogenetic relatedness. Variation on the strength of priority effects was stronger between closer relatives. Such an outcome can be explained by changes in functional diversity. Both carbon and amino acid consumption profiles indicated that competition between closer relatives was more intense owing to higher ecological similarity (Peay et al. 2012). In another experiment that investigated the growth of nectar yeasts, *Candida rancensis* was not affected, whereas cell densities of *M. reukaufii* increased when the microcosm was inoculated with a second species (Mittelbach et al. 2016b). The basidiomycetous yeasts *Vishniacozyma victoriae* and *Itersonilia pannonica* (originally *Udeniomyces pannonicus*) showed a negative effect of a priming species. Killer yeasts are often preponderant in the very sugar rich and favourable for yeast development substrate, i.e. decaying fruits. Because the antagonistic activity of the killer toxins has a specific taxonomic range (Golubev 2006; see also Chap. 9 of this book), the first-arriving yeast would largely determine species composition in the community. This would usually result in a typical fruit yeast community, but depending on the local conditions (e.g. prevalence of another community type), the community type could change it towards allochthonous, untypical for fruits, species (Starmer, personal communication).

The cross-feeding (syntrophy) phenomenon encompasses the associations where the growth of one partner is improved or depends on the nutrients, growth factors or substrate provided by the other partner. The nutritional interdependence is known in microbiology, especially between symbiotic prokaryotes and some microbial consortia (e.g. Seth and Taga 2015). Although the topic is less studied for yeasts, cross-feeding may play an important role in the composition of yeast communities. In the aforementioned example, frequently vectored to flowers, basidiomycete yeast

It. pannonica did not grow in artificial nectar without an accompanying species (Mittelbach et al. 2016b). A similar situation may be present in the yeasts found in soapberries (*Sapindus* sp.) in Hawaii where some yeast species co-occur more often than expected, and corresponding physiological tests suggest cross-feeding relying on starch use may be involved (Starmer, personal communication). Likewise, yeasts that excrete riboflavin into the medium imply that the riboflavin may be a useful vitamin source for other organisms as well as co-occurring yeasts (Starmer, personal communication). The phenomenon of cross-feeding is probably involved in some other cases of yeast co-growth in the environment.

3.3.5 Community Stability

Community stability can be measured as resilience or resistance of a community to perturbations, either mechanic or with the introduction of an exotic species (Grimm and Wissel 1997), which does not originate from the same environment. The relationship between community stability and ecosystem functioning is a matter of lively discussions in ecology (Bezemer and van der Putten 2007).

Some of the most prominent efforts to understand diversity in higher organisms, such as mammals, are derived from the original Lotka-Volterra model (1925–1926). These assumptions converged into the concepts of "bottom-up" and "top-down" regulations of community diversity during the twentieth century. While "top-down" effects refer to the control that consumers exert on the remaining community members, "bottom-up" effects focus on how access to resources may affect community composition. Most researchers emphasised initially the association between the species richness and the availability of resources ("bottom-up" regulation); however, the recognition of the role of consumers and predators is gaining strength (reviewed by Leroux and Loreau 2015). Although the dichotomy between the two forces has motivated ecological research over the last century, microorganisms have received little attention to date (Meyer and Leveau 2012). The study of both regulatory forces in microbial communities can be seen as the global ("top-down") versus temporal ("bottom-up") resource limitation (Crowther and Grossart 2015). For example, strong nutrient limitation in soils could exemplify the "top-down" processes, whereas phylloplane communities constitute a good model to study temporal ("bottom-up") limitation.

3.3.6 Species Richness Estimators and Sampling Effort

In most of their natural habitats, yeasts hardly occur as a pure culture. The ultimate importance of sampling and isolation approaches has been highlighted repeatedly (Lachance and Starmer 1998; Boundy-Mills 2006). Cultivation techniques still provide us with the majority of data on yeast biodiversity and distribution. During

the twentieth century, these methods have been substantially improved to facilitate the discovery of yeasts (Boundy-Mills 2006). Because ecological surveys need to find an optimal sample size to reveal most of the real diversity with a bearable sampling intensity, sampling strategies have been studied for yeasts (reviewed by Boundy-Mills 2006). Sample size will depend on the richness and heterogeneity of those yeast communities. Lachance and Starmer (1998) determined empirically that a minimum of 15 independent samples is required to obtain an accurate reflection of community composition for cactus necroses, insects, tree exudates or flowers. Those are all species-poor communities, heterogeneous in terms of the abundance of single species. The number of samples also varies according to the frequency of empty samples. For example, the authors suggested the sampling size should be corrected to obtain a minimum of eight non-empty samples, when empty samples are present (Lachance and Starmer 1998). Rotting cacti, tree exudates and flowers are fairly homogeneous substrates compared with phylloplane and soils. The number of analysed samples should be further increased for heterogeneous and species-rich substrates. Insufficient sampling intensity and geographical sampling bias (Fig. 3.1) both compromise our knowledge of natural yeast communities. A few available estimations suggest that only a small fraction (approximately 5–10% depending on the habitat) of the total diversity of fungi is known (e.g. Hawksworth 2001; Blackwell 2011). The same is true for yeasts even though the number of described species has increased tenfold during the last 60 years (Lachance 2006). In our opinion, these numbers reflect the fact that the majority of the total yeast diversity is not known. This also justifies the need for further sampling in different habitats and regions.

Species richness is an intuitive measure of diversity and community composition, but despite its simplicity, this index can differ substantially from the real diversity. The problem arises when the community inventory is growing steadily with the number of samples analysed—the more samples are processed, the more species that will be found. As a result, species richness values presented in different publications (all of which used a different sampling intensity) cannot be directly compared. Another methodological problem is that both the expected species richness and the number of samples sufficiently describing the community are unknown. Thus, rarefaction curves provide a more reliable estimate of the "real" species richness, by plotting the number of species as a function of the number of analysed samples or purified (also identified) colonies. Those curves are created by randomly resampling (with replacement) the pool of N samples and plotting the average number of species (calculated after several rounds of resampling) found in each sample, i.e. in one sample, two samples, etc. (Sanders 1968). Rarefaction curves generally grow rapidly at first, as the most common species are found, but approach a plateau as only rare community members remain to be sampled (see Magurran 2004). Statistically, the use of rarefaction controls for differences in species richness values (Gotelli and Colwell 2011). For example, the analysis of rarefaction curves demonstrated that the yeast community is more species rich on

plant material than in soils irrespective of the sampling depth (Babjeva et al. 1999). Also, the use of this approach showed that yeast communities are more species rich in forest biotopes than in tundra, steppe or deserts (Maksimova and Chernov 2004). Likewise, Takashima et al. (2012) demonstrated that subtropical forests were more species rich than temperate forests above ground, whereas below ground, the opposite trend was observed.

Many attempts have been made to correct the sampling bias in species estimations. Statistical approaches to describe the species richness (or other units) have been successfully adapted to bacterial or fungal communities (Hughes et al. 2001; Bohannan and Hughes 2003; Unterseher et al. 2005, 2011; Schnittler et al. 2006). However, less than 10% from about 1500 papers (Fig. 3.2) studying yeast communities reported their sampling efficacy in soils (Yurkov et al. 2011, 2016; Orgiazzi et al. 2012; Takashima et al. 2012; Bellemain et al. 2013; Taylor et al. 2014), plant-related substrates (Pereira et al. 2002; Glushakova and Chernov 2010; Pozo et al. 2011; Takashima et al. 2012; Alvarez-Perez and Herrera 2013; Jacquemyn et al. 2013a, b; Morais et al. 2013) and insects (Ort et al. 2012; Niu et al. 2015). A few studies did not focus on a specific habitat but analysed the total yeast richness in a biotope (Maksimova and Chernov 2004; Yurkov et al. 2004) or compared species richness in different substrates (Babjeva et al. 1999). In aquatic environments, best sampling strategies still led to the assessment of just 60% of the species present (Kutty and Philip 2008; Fell 2012), whereas soils can be fairly well sampled (e.g. Babjeva et al. 1999; Yurkov et al. 2011). The utility of the rarefaction has been also shown for nectar yeast communities in Europe, which were studied with a different sampling intensity by the four aforementioned studies, whereas three of four nectar surveys observed less than 10 yeast species in a maximum of 120 samples, whereas sampling of a total of 600 flowers doubled the species richness values (Alvarez-Perez and Herrera 2013).

Next to the rarefaction, species richness estimators represent a useful tool in community ecology. The main difference between the two approaches is that estimators use the ratio of species discovery to predict the number of species by using different computational algorithms. Thus, the application of a species richness estimator can show the number of species retrieved with increased sampling effort. Alternatively, it can answer the question on whether the present sampling effort is sufficient to discover the majority of expected species in a community. In other words, it tests for undersampling. Insufficient sampling depth is an important concern in heterogeneous habitats, like soils, which require higher sampling intensities due to rare species (e.g. Yurkov et al. 2011, 2016). These infrequent yeasts may constitute the majority of the population in some environments, such as nectar (Alvarez-Perez and Herrera 2013), rotting cacti (Starmer et al. 2005) and soils (e.g. Yurkov et al. 2011, 2016). The estimators ICE and ACE improve rarefaction curves by taking into account the minimum number of samples that allow us to identify rare (in ICE) and minor (ACE) species (reviewed in Gotelli and Colwell 2011). The bootstrap estimator does not differentiate the species frequency and the first-order jackknife, and Chao 1 richness estimators additionally rely on the number of species only found once. Chao 2 estimator is distinct from the other

species richness estimators as it is an incidence-based estimator of species richness, which relies on the number of singletons and doubletons, i.e. species found in only one and two sample units.

3.4 Community Structure

The simplest description of diversity is a species inventory such as a list of yeasts isolated in a study. In community ecology, this is also called community composition. These data can be further analysed as presence or absence of a species in a sample. It does not include any quantitative information such as dominant or frequent species. Additional information about species abundances describes the structure of communities. Depending on the study design, it can include either incidence or abundance data, or both. Abundance-based community structure is usually based on colony counts, namely, number of colonies of each yeast species observed in cultivation experiments (see also Boundy-Mills 2006). Incidence-based community structure corresponds to the frequency of isolation of a species. It does not take into consideration the number of colonies but counts the detection of a yeast in the sample only. Both these approaches have their own limitations. Isolation of yeasts is usually made on solid media, where a sample is inoculated. Inoculated plates are incubated, and growing yeast colonies can be differentiated into morphological groups based on their appearance on the medium by recording, for instance, colour, shape, texture, formation of filaments and media colourisation. Representative colonies are then picked, transferred to pure culture and identified. Even though morphological characters are often not unique, their combination can often provide a suitable differentiation of yeasts in a sample. It is important to document, however, that closely related yeast species may show similar or even indistinguishable colony morphology and, thus, can be mistaken when counted on plates. Compared to counts based on colony-forming units, incidence-based community structure does not extrapolate identification results to abundance but records a species detection only. Representative cultures or even randomly purified colonies can give an impression on how frequent is the certain yeast species in a sample. This approach is likely to provide fair estimates in communities composed of species, which can be mistaken based on the morphological characters. When yeast incidence is recorded, the community structure can be expressed as frequency of occurrence. It doesn't provide information about the dominance but aims to detect most common species within sampled colonies.

When the composition of a yeast community is well characterised and rather stable and can be assessed with a reasonable sampling effort, selective isolation can be further employed to quantify morphologically indistinguishable species. Selective isolation involves any unique physiological condition (either of a yeast or habitat) and tolerance to inhibitors. This cultivation method can also reveal rare and minor species (see below). But it is important to remember that an additional replicate with a complete medium should be always used to ensure the complete

assessment of the community. This approach was successfully used to study yeast communities in rotting cactus tissues (Starmer, personal communication).

3.4.1 Diversity Indices

A quantitative measure that reflects how many different species there are in a habitat and simultaneously takes into account the proportion of each entity in the sampled community is known as a diversity index. Diversity indices, the Shannon diversity index (also known as Shannon entropy, the Shannon-Wiener or sometimes erroneously the Shannon-Weaver index) and the Simpson diversity index (also known as the inverted Simpson index) are among the most frequently used in the literature. They both take into account species richness and proportion (number of individuals or relative abundance) of species. Sometimes, values given by diversity indices are used as a synonym of alpha diversity. This is not entirely correct, as the diversity indices do not consider rarity of species in a community. Diversity values are commonly used to make comparison between habitats to reflect the degree of the diversity alteration (e.g. succession) or to provide a quantitative measure of differences in the structure of communities between samples. Importantly, the indices reflect changes in both the species richness and the evenness of the community. The latter is used to measure how close in numbers (or as a proportion) each species in a community is. Among communities with the same number of species, the one with more similar proportions of members would be characterised by a higher diversity value. Thus, the decrease or decline of the diversity can be caused by either of these parameters, i.e. community composition (species richness) and structure (evenness).

For example, diversity of yeasts in oligotrophic lakes was higher off the coast than in the coastal zone and resulted from a more even species distribution (Brandão et al. 2011). Likewise, the Shannon diversity values calculated for phyllosphere yeasts on *Sphagnum* moss were higher in swamp than in forest biotopes, although both yielded the same number of species (Kachalkin and Yurkov 2012). On the contrary, higher species richness values in the rhizosphere and bulk soil underneath *Nothofagus pumilio* resulted in a higher diversity in these soil fractions than in the ectomycorrhizosphere (Mestre et al. 2011). An interesting observation was made by Starmer and co-workers in the review of the biogeographic diversity of cactophilic yeasts (Starmer et al. 2005). Communities of yeasts in rotting cactus tissues are highly specific and composed of a small number of dominant species, all of which have shown a restricted geographic range that is determined by insect vectors and their hosts. These communities occasionally include rare non-cactus-specific yeasts, which are regionally diverse (dissimilar between locations) and are randomly transported from the surrounding environments. Because the core cactus-rot communities are very similar among locations, diversity on a regional scale is largely determined by an almost random subset of

rare non-cactus-specific yeasts. Consequently, communities in single location show a lower diversity than those on a regional scale.

Not only the number of individuals affects the observed species diversity but also the size and heterogeneity of the sample. Homogenisation of large and heterogeneous samples can potentially equalise the proportions of yeast species in cultivation experiments. This approach has been applied repeatedly for the investigation of phylloplane and soil yeasts. For example, several individual leaves pooled in a composite sample can be further subsampled (e.g. cut into pieces), washed and used to inoculate plates instead of plating leaf washings of a single leaf (e.g. Inácio et al. 2010). Likewise, soils can be homogenised (mixed) and sieved to reduce the heterogeneity and dissimilarities between subsamples and replicates (e.g. Yurkov et al. 2011).

3.4.2 Species-Abundance Distribution

Species-abundance (SAD) and rank-abundance distributions (RAD) are helpful to understand the structure of yeast communities and can be used as an additional tool in community analyses. As such, SAD and RAD are the most basic way to describe the abundance of each species in a community (reviewed by McGill et al. 2007). Abundance values, either absolute (colony counts) or relative values (proportion of each species), are arranged in descending order and plotted as a hollow-curve histogram. A visual comparison of community structure across samples implies a substantial overlap in species lists among the samples and can be done either with SAD or RAD. When a few or even no species are found in common, RAD is employed and the order or rank of a species in a community is recorded but not its identity. For example, soil yeast communities in grasslands and forests in Germany had only one species, *Apiotrichum dulcitum* (originally *Trichosporon dulcitum*), in common (Yurkov et al. 2012a). Nevertheless, the application of the RAD for the analysis showed the influence of the vegetation type on yeast communities.

Species lists and colony counts are commonly collected data during yeast surveys. At the same time, SAD is different from an abundance table as it enables easy research of community properties such as evenness and proportion of rare species. When plotted as a histogram, SAD typically produce the typical hollow curve, which is one of ecology's oldest and most universal laws (McGill et al. 2007). Several models have been proposed to describe distribution of plant and animal species in forms of SAD and RAD, including logseries, lognormal and geometric and broken stick, among others (reviewed by McGill et al. 2007). These models tried not to just describe empirical curves but explain the composition of the communities using a set of ecological tools such as species niche concepts, competition for resources, dispersal and reproductive strategy. Babjeva, Chernov and co-workers analysed yeast population on living plant material (vascular plants, mosses and lichens), senescent plants, litter and soils collected on the territory of the former USSR (reviewed by Babjeva and Chernov 1995). Later, Chernov (2005,

2013) studied the distribution of species ranks (RAD) obtained from ca. 7000 samples and employed Pearson's chi-squared tests to predict distribution models' characteristic for each substrate. Communities in decomposing plant material (forest litter, peat) were well described with the geometric distribution, whereas yeast assemblages on fresh and senescent leaves showed more flat RAD curves corresponding to the broken stick (random niche appointment) model (Chernov 2005, 2013). The authors suggested the shape of a SAD reflects the availability of resources in the community and the competition between single species.

The incline of the hollow curve reflects the evenness of the community with more even communities resulting in more gentle slopes of the SAD. McGill et al. (2007) reviewed several empirical patterns of SADs such as incline, skew and modality. In a series of experiments, more flat curves corresponded to communities with a higher productivity, for example, plant communities in late successional stages. To what extent these observations can be applied to yeast communities is yet unclear. As in the case of the observations by Chernov (2005, 2013), the steep incline of the curve could suggest a strong limitation of the resources, which is a very likely scenario for decomposed organic substrates such as litter and soils. On plant surfaces, sugars and other low-weight carbon sources are not as limited as in soils, so the phylloplane generally supported a more even yeast community, i.e. broken stick vs. a geometric model. Recent analysis of soil yeasts along a land-use gradient confirmed the previous rule regarding the shape of SADs below ground but also showed that more even communities were found under intensively managed grasslands, which were subjected to fertilisation in the past (Yurkov et al. 2012a). In another study, yeast communities underneath decomposing wood logs were more even than those sampled just 1 m apart under forest litter (Yurkov et al. 2012b). The observed difference also correlated with the higher amount of the dissolved organic carbon in soils underneath dead wood logs. Although SADs of yeast fungi in their natural habitats did not receive much attention, we believe that this tool has potential usefulness for community analyses.

3.4.3 Frequent and Rare Species

It is intuitive and understandable that some yeasts are observed in the environment more often than the others (Magurran and Henderson 2003). However, it is extremely difficult, if at all possible, to suggest any artificial cut-off values for frequent and rare species. Obviously, different ecosystems and substrates provide diverse conditions for yeast communities. Therefore, it is important to sample the habitat sufficiently to reveal the structure of the yeast community. It has been repeatedly documented that frequent (core community) and rare species also show different distribution patterns (Magurran and Henderson 2003; Unterseher et al. 2011). Although the importance of rare (or minor) species is not well understood, a few studies suggest that their diversity is important for ecosystem

functioning and stability, e.g. resistance against colonisation by exotic plants in a grassland system (Lyons and Schwartz 2001; Lyons et al. 2005).

To our knowledge, little effort has been made to distinguish ecological roles of frequent and rare species of yeasts in their habitats. While frequent species are usually well studied, rare community members, though often outnumbering the core group, are recovered as single isolates. The situation is further complicated by the extraordinary dispersal abilities of yeast cells, so rare resident species are difficult to distinguish from the transient species from outer sources (see the below discussion). A good understanding of yeast autecology could help to answer the question whether a yeast might or might not be able to live in the sampled environment. This might be, however, more difficult for yeasts that show broad chemical, physical and physiological capabilities (broad fundamental niche), which also allow them to colonise diverse habitats. Although our knowledge of distribution patterns of rare and frequent species is scarce, this community parameter can be potentially useful. For example, forest stand properties such as projective cover or a management history affect soil yeast communities (Yurkov et al. 2012a, 2016). Compared with managed areas, low-managed and near-natural forests harbour a higher number of rare yeast species, which also results in higher estimations of species richness values (Yurkov et al. 2011). Likewise, the fragmentation of the forest tree cover also increases the number of rare yeasts in soils (Yurkov et al. 2016). Whether or not the diversity and proportion of rare species show a meaningful trend in other yeast habitats should be addressed in future studies.

3.4.4 Resident (Autochthonous and Indigenous) and Transient (Allochthonous and Alien) Species

Although the terminology may differ between studies, the main intention to distinguish true inhabitants from alien or "accidentally observed" species is intuitively clear. As in the case of frequent and rare species, this question can be addressed from two different perspectives, namely, autecology and synecology (community ecology). Laboratory observations of autecological properties can help predict the range of suitable habitats for a yeast. For example, fast-growing fermenting ascomycetes are better adapted to sugary substrates, while soilborne basidiomycetes often possess the ability to assimilate low-weight aromatic compounds (Botha 2006; Starmer and Lachance 2011). Carotenoid pigmentation together with the dispersal by the forcibly ejected buds (ballistoconidia) is believed to be an adaptation to exposed environments such as plant surfaces (Fonseca and Inácio 2006; Starmer and Lachance 2011). The adaptations can be partitioned further to consider conditions of a single microhabitat or a yeast's potential interactions with vectors. In this regard, the development of more sensible isolation techniques allowed to discern tongue, crop and gut yeast communities—all of which were previously considered as an insect community (Malloch and Blackwell 1992; Hu et al. 2015).

Similarly, floricolous yeast can be separated onto nectar, corolla and anther communities (Pozo et al. 2012). A further downsizing of a yeast habitat is also feasible for nectar habitats where the oxygen gradient from bottom to top of the nectar content would allow the establishment of microaerophilic and aerobic communities, respectively (Lachance 2006).

A combination of abundance- and incidence-based approaches to describe the structure of yeast communities can be used to uncover ecological preferences of isolated yeasts. Indigenous (autochthonous) community members are species that are expected to be both abundant and frequent in a habitat (see also Lachance and Starmer 1998; Starmer and Lachance 2011). These yeasts usually comprise a core community and are easy to isolate even with a limited effort. Yeasts frequently detected in low numbers could represent minor but indigenous community members. For example, basidiomycetous yeasts were commonly considered as resident (allochthonous) in the nectar environment (e.g. Brysch-Herzberg 2004). However, the species *Cystofilobasidium alribaticum* (originally cited as *Cystofilobasidium capitatum*) and the members of the genus *Vishniacozyma* (originally cited as *Cryptococcus carnescens*, *Cryptococcus heimaeyensis* and *Cryptococcus victoriae*) were frequently observed in association with bird-visited flowers and were able to grow in artificial nectar (Mittelbach et al. 2015). Similarly, orchids' nectar contained large number of basidiomycete species (Jacquemyn et al. 2013b). In phylloplane and soils, Basidiomycetes commonly prevail over the ascomycete yeasts (e.g. Botha 2006; Fonseca and Inácio 2006; Starmer and Lachance 2011). However, infrequent ascomycetous species were successfully linked to plant surfaces (e.g. *Candida oleophila* and *M. pulcherrima*) and forest soils (*Candida vartiovaarae* and *Kazachstania piceae*) based on their occurrences in the environment (e.g. Glushakova et al. 2007; Glushakova and Chernov 2010; Yurkov et al. 2012a). In contrast to minor community members, infrequent but numerous yeasts may originate from sources other than the studied substrate (transient or allochthonous) or be restricted to a certain microhabitat in a heterogeneous environment. Isolation of rare species in low numbers is difficult to interpret due to the risk of undersampling. Often these species are minor transient community members from neighbouring substrates, and their origin can be revealed in a biotope-wide analysis including various substrates. Because the place of yeast isolation is not necessarily where they live, a careful examination of substrates that serve naturally as reservoirs (e.g. soil and water) is important to distinguish autochthonous from allochthonous species (see also Lachance and Starmer 1998; Starmer and Lachance 2011). We believe that a repeated sampling with a sufficient sampling effort can help to reveal true habitats of many species in the future.

Variation of frequency and abundance of yeasts between single samples can further complicate community analyses when occurrence and colony counts of a species differ substantially. A useful tool to account for spatial and temporal variation is the probability of dominance, which is calculated as the number of samples, where a species showed the highest abundance, relative to the total number of samples, where this species was observed (e.g. Maksimova and Chernov 2004; Glushakova and Chernov 2010; Yurkov et al. 2012a). This measure records

the cases when a yeast becomes dominant in a community, thereby accounting for heterogeneity between habitats, samples or replicates. For instance, despite little overlap between yeast communities on plant material, litter and soils, Maksimova and Chernov (2004) revealed the most typical yeasts for each type of substrate using the aforementioned parameter. When the probability of dominance is additionally considered, it is possible to distinguish rare minor community members from rare yeasts, which colonise the substrate only in a certain period of time.

3.4.5 Specialists and Generalists

A combination of physiological abilities, metabolic capacities and physical-chemical limitations determined in laboratory experiments defines a potential habitat where a yeast species might live—its fundamental niche. Narrow fundamental niches define physiological specialists. For example, *Hanseniaspora* yeasts commonly found on fruits and berries have limited physiological abilities. They ferment and respire glucose vigorously, utilise cellobiose as a source of carbon and require an external supply of vitamins (Starmer and Lachance 2011). Likewise, widespread nectar-borne yeasts *M. gruessii*, *M. reukaufii* and *C. rancensis* show a narrow spectrum of assimilated compounds. Although these species have a narrow fundamental niche, they still have a wide distribution in nature, due to the common occurrence of the corresponding habitat. Fruits and berries of different decay stages are common in nature and are regularly visited by insects that transmit yeasts between them. Even though flowers represent a short-lived and very fragmented habitat, the aforementioned nectar yeasts show no preference to a particular group of plants and are commonly found in an association with different insect vectors. The narrow fundamental niche can be further constrained by the limited distribution of its vector that would result in highly endemic yeast species (e.g. Lachance et al. 2003).

Alternatively to a narrow fundamental niche, some yeasts were found in different environments in considerable numbers and are believed to be adapted to a broader range of environmental conditions. For example, *Naganishia albida* (originally *Cr. albidus*) was isolated from soils, plants, water and cold habitats and displayed the capability to sustain cold, desiccation and oligotrophic conditions. Other examples include *Aureobasidium pullulans*, *Filobasidium magnum* (*Cryptococcus magnus*), *Vishn. victoriae* (*Cr. victoriae*), *Debaryomyces hansenii* and *Meyerozyma* (*Pichia*) *guilliermondii*, among others. These species may show opportunistic distribution, which is not restricted to a particular region or habitat. They usually possess broader physiological abilities and utilise diverse carbon sources (polytrophic). Due to the ability to live in various habitats and conditions, such yeasts are referred to in the literature as eurybionts, opportunists or generalists. They are opposed to yeasts with a narrow, geographical or fundamental niche, which are called stenobionts or specialists. To what extent the distribution of the polytrophic species is constrained by autecology and dispersal abilities is a matter of debate.

3.5 Concluding Remarks

Community ecology tools allow researchers to move from simple yeast inventory reports towards a better understanding of mechanisms structuring yeast communities. Yeast communities in their natural environments represent dynamic and open systems. Thus, it is important to distinguish typical inhabitants of the habitat from transient colonists, which may not able to grow properly in the given environment. The available knowledge suggests that properties of transient and resident community members in the habitat are different and are reflected in their distribution patterns. Application of community ecology tools also allows to study yeast observations in a larger context and compare studies across climatic and spatial transects. Because yeast habitats are often too different in respect to the number of species and their proportions in the community, it is important to ensure adequate sampling effort, which catches the majority of residing species. Physiological data traditionally collected for yeast species opens the possibility to describe additionally functional diversity of the community and analyse quantitatively relevant ecological traits and functional redundancy of the ecosystem.

Acknowledgements Tadashi Fukami and William T. Starmer are acknowledged for their valuable suggestions and for correcting the English text.

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