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Nectar yeasts: a natural microcosm for ecology

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Abstract

The species of yeasts that colonize floral nectar can modify the mutualistic relationships between plants and pollinators by changing the chemical properties of nectar. Recent evidence supporting this possibility has led to increased interest among ecologists in studying these fungi as well as the bacteria that interact with them in nectar. Although not fully explored, nectar yeasts also constitute a promising natural microcosm that can be used to facilitate development of general ecological theory. We discuss the methodological and conceptual advantages of using nectar yeasts from this perspective, including simplicity of communities, tractability of dispersal, replicability of community assembly, and the ease with which the mechanisms of species interactions can be studied in complementary experiments conducted in the field and the laboratory. To illustrate the power of nectar yeasts as a study system, we discuss several topics in community ecology, including environmental filtering, priority effects, and metacommunity dynamics. An exciting new direction is to integrate metagenomics and comparative genomics into nectar yeast research to address these fundamental ecological topics.

Keywords

Alternative stable states, metacommunity, nectar bacteria, nectar yeasts, pollination, priority effects

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Introduction

Naturalists have known for over a century that the nectar of flowers often contains yeasts (Baker and Baker, 1983; Boutroux, 1884; Capriotti, 1953; Ehlers and Olesen, 1997; Grüss, 1917; Jimbo, 1926; Nadson and Krassilnikov, 1927; Sandhu and Waraich, 1985; Schoelhorn, 1919; Schuster and Úlehla, 1913; Vörös-Felkai, 1957). It is only recently, however, that the ecology of nectar yeasts has begun to be studied (e.g., Herrera, de Vega, Canto, and Pozo, 2009; Peay, Belisle, and Fukami, 2011). Much of this ecological research is motivated by the realization that nectar yeasts have the potential to modify the way plants and pollinators affect each other (e.g. Herrera, Pozo, and Medrano, 2013; Schaeffer and Irwin, 2014; Vannette, Gauthier, and Fukami, 2013). This modification happens in part because nectar yeasts change the chemical properties of floral nectar and, consequently, the foraging behavior of pollinators. Although not as well appreciated, another motivation that has driven some recent studies on nectar yeasts is their utility as an illustrative example to uncover general principles of ecology. In this article, we will focus on this second motivation and propose that multi-species assemblages of nectar yeasts serve as powerful natural microcosms (*sensu* Srivastava et al., 2004) with which to bridge theoretical and empirical ecology. To this end, we will first discuss the characteristics of nectar yeasts that make them useful as a natural microcosm. We will then explore several outstanding ecological questions that can be addressed using nectar yeasts. Our main focus in this article will be yeasts, but they frequently co-occur with bacteria in floral nectar. In addition to yeasts, we will also consider these bacteria and their interactions with yeasts where appropriate to make our argument.

Aspects in nectar yeasts as a tool for advancing ecological theory

Nectar yeast research (Fig. 1) has addressed several topics in ecology, including environmental filtering, priority effects, and metacommunity dynamics (Fig. 2). The physical simplicity of the nectar environment and the low species richness of nectar yeast communities allow researchers to experimentally examine the contributions of abiotic and biotic factors to community assembly with relative ease. In addition, the spatially nested structure of flowers as microbial habitats, combined with animal-assisted dispersal across flowers, presents a rare opportunity for studying metacommunities through both field and laboratory experiments. One outstanding question that can be effectively addressed using nectar yeasts is the genetic basis of community assembly. What are the genes that govern the ability of species to disperse and proliferate and ultimately determine how multi-species communities assemble in local habitats? Applications of new techniques such as metagenomics and comparative genomics should help in pursuing this question. Nectar yeasts are also well suited for investigating another outstanding question: how do dynamic processes such as phenotypic plasticity, local adaptation, and epigenetics influence community dynamics?

Nectar yeast communities as a natural microcosm

We believe that four characteristics of nectar yeasts make them one of the most powerful natural microcosms, or “small contained habitats that are naturally populated by minute organisms” (Srivastava et al., 2004), available to ecologists. First, in most cases, floral nectar appears initially sterile before being inoculated with yeasts and bacteria by pollinators and other flower-visiting animals (Belisle, Peay, and Fukami, 2012; Herrera, Canto, Pozo, and Bazaga, 2010). Each flower, functioning as a well-delineated habitat island (Belisle et al., 2012), can therefore be considered a replicated instance of a microbial community undergoing primary succession. Because of the short generation times of microbes, studies can easily evaluate multi-generational succession (Toju et al., 2018). As such, nectar microbial communities serve as highly reproducible natural systems to study how communities develop across multiple spatial and temporal scales (Belisle et al., 2012).

Second, nectar yeast communities are simple enough to make it possible to study species interactions in detail. Nectar yeast communities are characterized by low species richness (but see Canto et al., 2017 for preliminary evidence suggesting that nectar yeast diversity might be higher in the tropics than in temperate regions), dominated by a small number of species in the class Saccharomycetes, especially those in the genus *Metschnikowia* (Brysch-Herzberg, 2004; Canto et al., 2015; Lachance, 2016; Pozo, Lachance, and Herrera, 2012), and potentially also by a few species in the basidiomycete class Tremellomycetes (Aleklett, Hart, and Shade, 2014; Brysch-Herzberg, 2004; Peay et al., 2011; Pozo, Herrera, and Bazaga, 2011; Pozo et al., 2012). These species have presumably evolved a set of traits that facilitate survival and growth in the high osmotic pressure of floral nectar (Herrera et al., 2010; Peay et al., 2011). Many nectar yeast species can be cultured (Peay et al., 2011), which allow for complementary experiments in the field (e.g., Vannette and Fukami, 2017) and in the laboratory (e.g., Vannette and Fukami, 2014) (Fig. 1).

Third, compared to most microbial systems, dispersal is relatively tractable in nectar yeasts. Yeasts are predominately inoculated by bees (Brysch-Herzberg, 2004; Good, Gauthier, Vannette, and Fukami, 2014; Herrera et al., 2013; Rering et al., 2018), birds (Belisle, Mendenhall, Oviedo Brenes, and Fukami, 2014; Mittelbach et al., 2015; Vannette et al., 2013), ants (de Vega and Herrera, 2012, 2013), and other flower-visiting animals (Herrera et al., 2010; Lachance et al., 2001), although they seem to be sometimes dispersed by wind. Microbial immigration can be quantified by observing pollinator visitation (e.g., using motion-activated camera traps) and can be experimentally manipulated by enclosing flowers in wire cages or mesh bags (Vannette and Fukami, 2017) or inoculating wild flowers with yeasts or bacteria (Toju et al., 2018). In the laboratory, researchers can mimic dispersal using pipettes (Vannette and Fukami, 2014) or pollinators' mouthpieces (Hausmann, Tietjen, and Rillig, 2017) (Fig. 1).

Fourth, the ways in which nectar yeasts modify the environmental conditions of their habitats can be easily characterized, allowing detailed investigations into species interactions driven by niche preemption and modification. In addition to altering the sugar composition and concentration in floral nectar (Canto and Herrera, 2012; Canto et al., 2015; Herrera, García, and Pérez, 2008; Misra et al., 2012; Pozo, de Vega, Canto, and Herrera, 2009; Schaeffer, Vannette, and Irwin, 2015), nectar yeasts can modify nectar secondary (specialized) metabolites (Vannette and Fukami, 2016), produce volatile organic compounds to attract pollinators (Golonka, Johnson, Freeman, and Hinson, 2014; Pozo et al., 2009; Raguso, 2004; Rering et al., 2018), draw down nitrogen in nectar (Dhami, Hartwig, and Fukami, 2016; Peay et al., 2011; Vannette and Fukami, 2014), and even increase nectar temperature (Herrera and Medrano, 2017; Herrera and Pozo, 2010). Researchers can use synthetic nectar to test how changing abiotic factors mediate biotic interactions between nectar microbes and other actors, such as pollinators.

No natural microcosm is perfectly suited for all ecological questions (Srivastava et al., 2004). To ensure an effective use of natural microcosms, it helps to be aware of their limitations, not just strengths. Here we list two primary limitations of nectar yeasts. First, nectar yeasts' primary means of dispersal, phoresy via flower-visiting animals, may distinguish them from many other organisms, with the notable exception of nectar-inhabiting mites that are also dispersed by hummingbirds and other flower-visiting animals including insects and mammals

(Colwell, 1973; Seeman and Walter, 1995; Tschapka and Cunningham, 2004). Besides being obviously distinct from active dispersal in animals, nectar yeast dispersal may also be fundamentally different from wind- or water-aided passive dispersal as observed for many other microbes or plants (Nemergut et al., 2013). For example, one thing that may be peculiar in nectar yeasts are their effects on pollinator behavior (Golonka et al., 2014; Pozo et al., 2009; Raguso, 2004; Rering et al., 2018; Vannette and Fukami, 2016), which can influence the direction of yeast dispersal.

Second, the simplicity of nectar microbial communities may make them fundamentally different in the way species interactions affect community assembly. Nectar yeasts have been hypothesized to engage in facilitative interactions (Álvarez-Pérez and Herrera, 2013; Herrera, 2017) and have been shown to compete among themselves and against nectar bacteria (Song, Vannette, Dhami, and Fukami, in revision; Toju et al., 2018; Tucker and Fukami, 2014). However, nectar microbial communities may lack the complex trophic interactions between predators and prey that characterize most other communities, even though this impression might reflect the current lack of information rather than actual rarity in nature. For example, viruses, if they exist, may affect yeast or bacterial populations as exploiters, similar to how predators affect prey populations in other systems (Aleklett et al., 2014). To our knowledge, no research has investigated viruses of nectar yeasts or bacteria in floral nectar, although other research has studied the ecological role of other mycoviruses (Ghabrial et al., 2015) and bacteriophage (Bohannan and Lenski, 2000). Additionally, *Crithidia bombi*, a parasite of bumble bees, have been observed in nectar, and they have been hypothesized to compete with nectar yeasts and bacteria (Cisarovsky and Schmid-Hempel, 2014).

Using nectar microbes to advance ecological theory

Many ecological principles have traditionally been developed through observations of the natural history of plants and animals (Fig. 1). With improved molecular identification techniques, microbes are now becoming increasingly popular as study systems with which to test and refine general concepts in ecology (Koskella, Hall, and Metcalf, 2017). Here, we will illustrate how nectar yeasts have advanced ecological theory, using environmental filtering, priority effects, and metacommunity dynamics as case studies (Fig. 2).

Originating in the study of plant communities (Bazzaz, 1991; van der Valk, 1981), the concept of environmental filtering posits that the abiotic environment functions as a sieve through which species with unsuited traits will be filtered out from local communities (Kraft et al., 2015) (Fig. 2). Recent studies have revisited the assumptions behind the environmental filtering hypothesis, noting that environmental filtering is more difficult to quantify than generally recognized because biotic interactions can similarly filter communities and interact with environmental (or abiotic) filtering to dictate community membership (Cadotte and Tucker, 2017; Kraft et al., 2015; Thakur and Wright, 2017).

Nectar yeasts provide a system that can be used to experimentally disentangle contributions of abiotic and biotic factors in determining species occurrence. Abiotic factors such as high osmotic pressure (Lievens et al., 2015), low nutrient availability (Dhami et al., 2016), and chemical deterrents (Carter and Thornburg, 2004; González-Teuber and Heil, 2009) may explain the low species richness and phylogenetic clustering of yeast communities found in floral nectar (realized community), as compared to the more diverse microbial communities found on pollinators (i.e., species in the potential species pool; see Fig. 2) (Herrera et al., 2010). However, research into the mechanisms that enable coexistence between nectar microbes, both yeasts and bacteria, (Pozo et al., 2016; Tucker and Fukami, 2014; Vannette and Fukami, 2014), show that biotic interactions also contribute to community composition and modify the environmental filter. All of these factors can be easily manipulated experimentally. Results from these experiments could greatly contribute to improving the environmental filtering concept.

For example, what is largely lacking in the concept is consideration of priority effects, in which the order and timing of species arrival influence the way species affect one another in local communities (Fukami, 2015). Environmental filtering can be highly dynamic in the presence of strong feedbacks between biotic and abiotic factors, which can cause priority effects through niche preemption and niche modification (Fukami, 2015). Generally, studying priority effects is challenging because researchers often lack historical data on community assembly and immigration. Nectar microbes are an appealing system to study historical contingency because of the unique characteristics of nectar yeasts that we discussed in the previous section and similar characteristics of nectar bacteria.

In fact, priority effects have already been studied with nectar yeasts (Mittelbach, Yurkov, Stoll, and Begerow, 2016; Peay et al., 2011; Vannette and Fukami, 2014) and some bacterial species that also colonize nectar (Tucker and Fukami, 2014). For example, strong priority effects are found between bacteria and yeasts, causing bacterium- or yeast-dominated nectar communities as two distinct alternative stable states (Tucker and Fukami, 2014). In a laboratory experiment, early-arriving yeasts or bacteria modified the chemical environment of nectar and prevented colonization by the other (Tucker and Fukami, 2014). This laboratory result is consistent with the field observation that wild flowers are either dominated by yeasts or bacteria, and rarely by both (Song et al., 2018; Toju et al., 2018), which may have fitness implications for the plants. Pollinators may be deterred by nectar colonized by bacteria, reducing plant pollination success and seed set (Vannette et al., 2013). Nectar yeasts may also mediate plant-pollinator interactions by suppressing bacterial growth and modifying secondary metabolites in nectar (Vannette and Fukami, 2016). These recent findings suggest that priority effects drive dynamic environmental filtering, with the filter being modified through niche preemption and modification as local microbial communities are assembled in flowers, affecting not just community structure (microbial species composition), but also community function (pollination and seed production). Furthermore, priority effects in flowers may also modify the pool of subsequent colonizers by affecting the foraging behavior of pollinators. All of these processes can be studied through field experiments that are designed to establish causal relationships (Herrera et al., 2013; Schaeffer and Irwin, 2014; Toju et al., 2018; Tsuji et al., 2016; Vannette and Fukami, in revision).

Metacommunity theory—the idea that dispersal of organisms across local habitats interact with local species interactions to affect communities at both local and regional scales—has emerged in an attempt to find general principles in community assembly (Leibold and Chase, 2017). Local dynamics had long been the focus of community ecology, and metacommunity ecology was developed to understand these local dynamics in the context of larger, regional biota (Holyoak, Leibold, and Holt, 2005; Leibold and Chase, 2017; Leibold et al., 2004). To reduce ecological complexity to an experimentally manageable scale, researchers studying metacommunity dynamics have often turned to laboratory microcosm experiments. However, these simplified systems may not approximate natural communities well, limiting the potential applicability of findings from these experiments. Natural microcosms like nectar yeast communities are a promising but largely under-exploited tool that can be used in conjunction with laboratory microcosms. Nectar yeasts allow researchers to disentangle the role that dispersal (Hausmann et al., 2017; Vannette and Fukami, 2017), species interactions, environmental variability (Canto, Herrera, and Rodriguez, 2017; Herrera, Pozo, and Bazaga, 2014; Mittelbach et al., 2015; Pozo, Herrera, and Alonso, 2014), and even eco-evolutionary dynamics (Wittmann and Fukami, 2018) play in structuring metacommunities, even over multiple generations of local habitats (Toju et al., 2018). Communities of nectar bacteria may also be structured by similar factors (Aizenberg-Gershtein, Izhaki, and Halpern, 2017) and understanding the interactions between nectar bacteria and yeasts will be key to understanding how nectar microbial communities assemble.

One particularly exciting new direction is to integrate metagenomics and genome editing into the toolbox used to study nectar yeasts as a natural microcosm. By pairing comparative genetics with laboratory experiments, transcriptomics, and genome editing, researchers can elucidate the genes and physiological pathways that underpin interspecific differences in colonization, coexistence, and priority effects. Already, whole-genome sequencing of the cosmopolitan nectar yeast *Metschnikowia reukaufii* has resulted in the identification of potential genes responsible for their strong priority effects (Dhami et al., 2016). Environmental heterogeneity has been indicated to affect genotypic diversity of nectar yeast populations (Herrera, 2014; Herrera, Pozo, and Bazaga, 2011), but the specific genetic drivers of community assembly and genetic population structuring remains largely unknown (Dhami et al., 2018). Genetic and genomic approaches can clarify how genetic diversity across landscapes underpins alternative stable states in hierarchically structured communities. One plausible hypothesis is that the wide genotypic diversity of nectar microbes (Herrera et al., 2011) allows them to be competitive in highly variable nectar environments. Preliminary evidence suggests that phenotypic plasticity (Pozo et al., 2015) and epigenetics (Herrera, Pozo, and Bazaga, 2012) may also contribute to their competitiveness. Studying the role of phenotypic plasticity, epigenetics, and local adaptation is still nascent in environmental microbiology (Bury-Moné and Sclavi, 2017; Kraemer and Boynton, 2017; Veening, Smits, and Kuipers, 2008) because of methodological constraints, including difficulty with culturing and single-cell sequencing (Bury-Moné and Sclavi, 2017). Fortunately, however, these constraints are less severe with nectar yeasts than with many other groups of microbes.

Conclusion

In a field like ecology, particularly community ecology, which is characterized by a high degree of contingency (Fukami, 2015; Lawton, 1999), developing overarching principles may seem impossible. We believe that nectar yeasts as a study system can help ecologists overcome this challenge and advance ecological theory. In cell and molecular biology, development of a few model systems has resulted in advancements far beyond their initial scope. We suggest that ecologists can similarly make use of natural microcosms like nectar yeasts to achieve more rapid progress in uncovering fundamental principles than otherwise possible.

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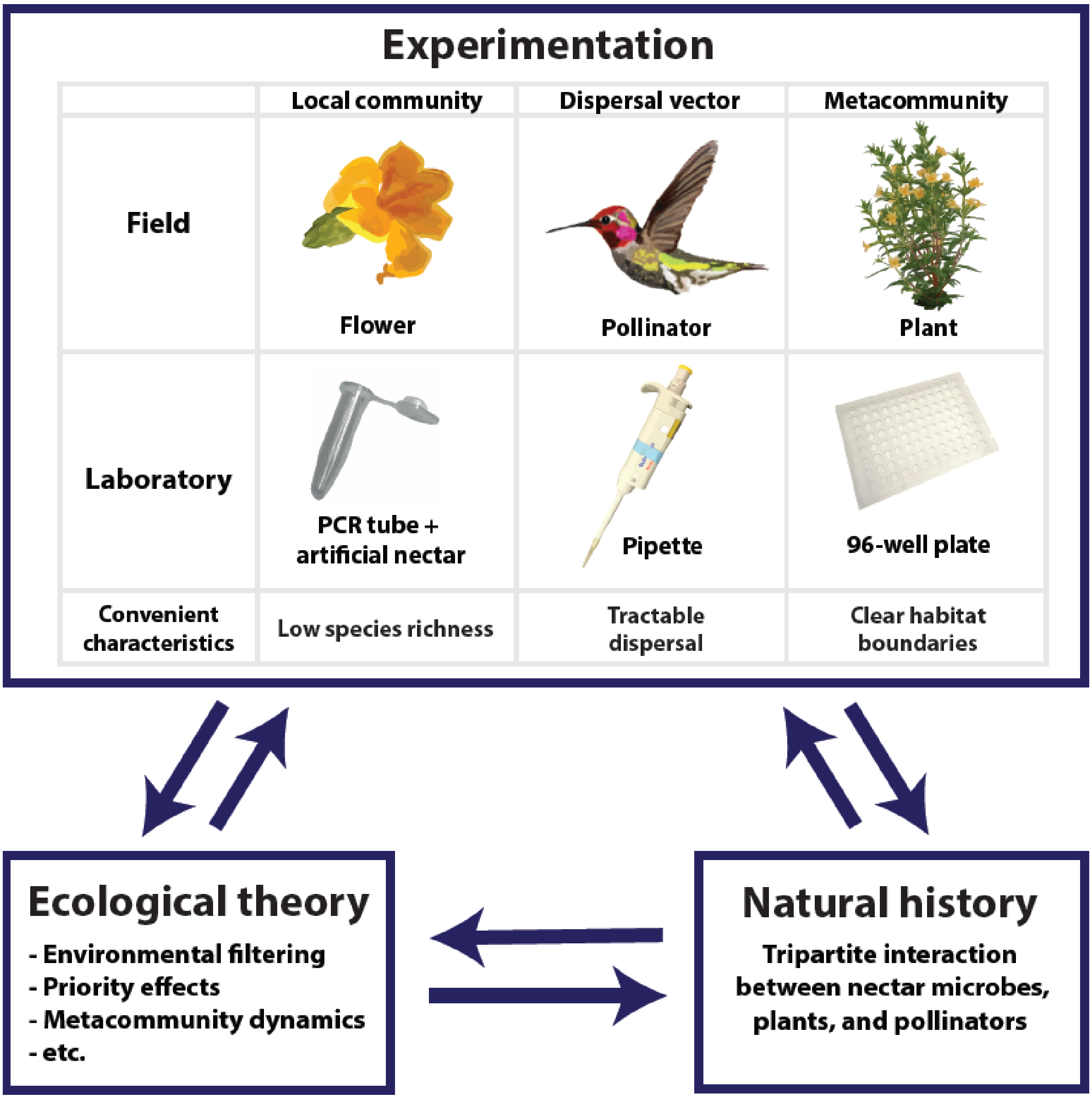


Figure 1. Method integration in nectar yeast research. Nectar yeasts as a natural microcosm facilitate the combined use of multiple methods, including natural history, ecological theory, and experimental approaches. For example, natural history provides insight needed to build ecological theory, which can then be tested by field and laboratory experiments. Field experiments enable hypotheses to be tested in a more natural context, whereas laboratory experiments afford greater experimental control. In this sense, they complement each other. One strength of the nectar yeast system is that it is relatively easy to conduct parallel laboratory and field experiments. Findings from experiments contribute to improving ecological theory and advancing deeper understanding of natural history. Original artwork modified from photographs from Mark Turner (*Mimulus aurantiacus*), Paul Higgins (*Calypte anna*), and Callie Chappell (laboratory materials), reproduced with permission.

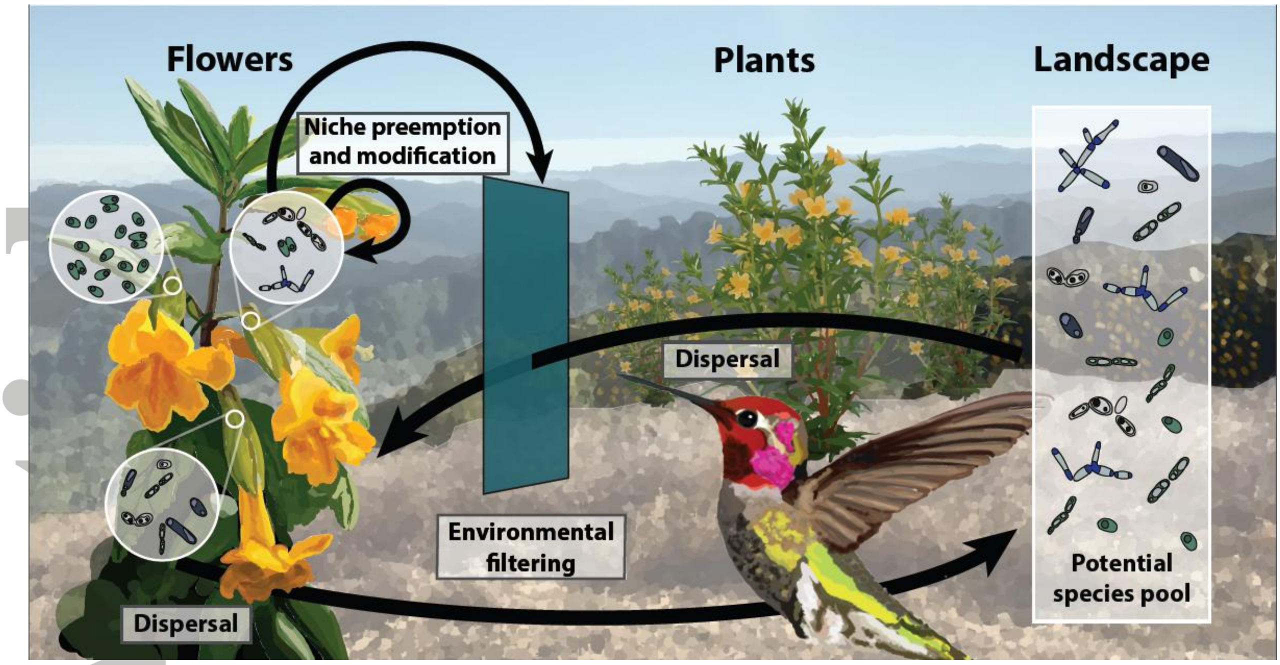
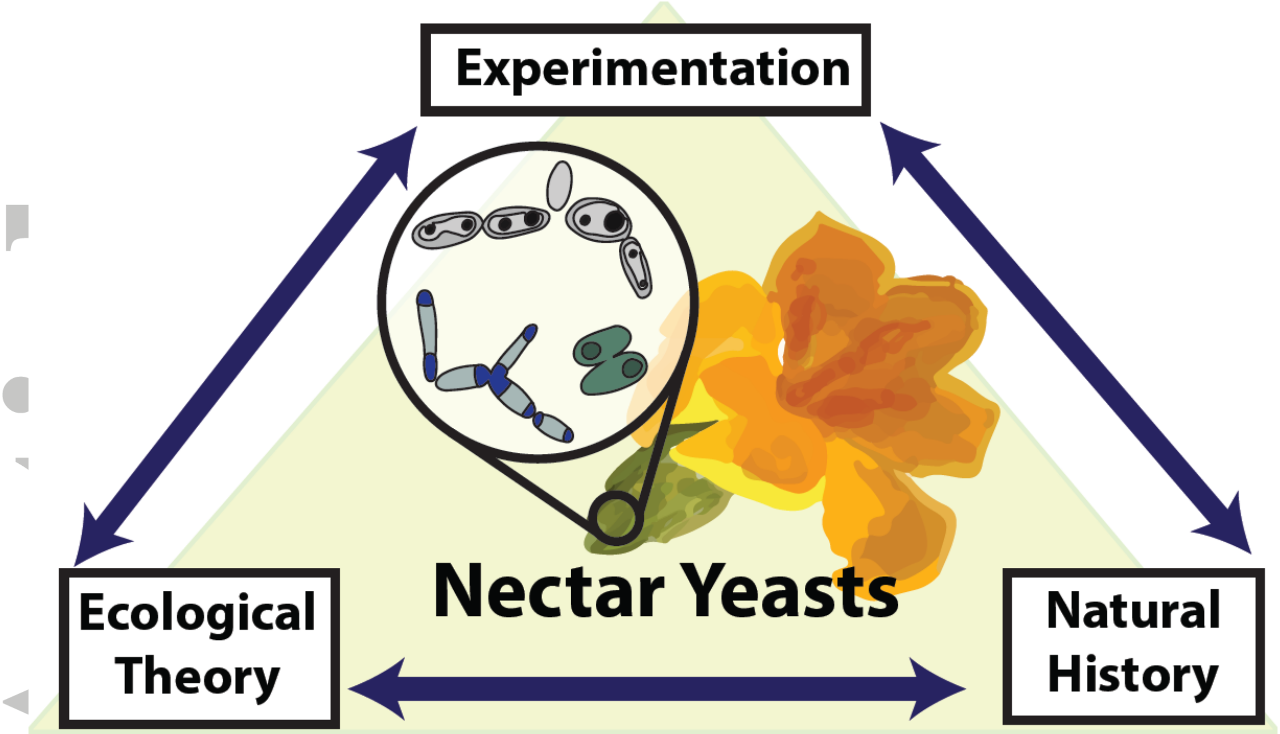


Figure 2. Developing ecological theory using nectar yeasts as a natural microcosm. Nectar yeasts are a powerful study system for testing and refining ecological theory on processes affecting the assembly of ecological communities, including dispersal, environmental filtering, and local species interactions such as niche preemption and modification, operating at different spatial scales. Nectar microbial communities are contained in individual flowers, which are nested within plants, which are themselves nested within plant populations and communities in the landscape. This clear hierarchical structure of the nectar habitats allows researchers to examine how communities are shaped jointly by processes occurring at multiple scales and feedbacks between these processes. For example, priority effects by niche preemption and modification at the flower scale can modify the environmental filtering at the plant scale and the species pool at the landscape scale. The environmental filtering and the species pool will in turn determine which species colonize nectar and interact with one another via priority effects within flowers. Image credit as in Fig 1 with additional artwork modified from photographs by the Herrera lab (nectar yeasts) and Callie Chappell (California landscape).



Graphical Abstract:
Article Title: Nectar yeasts: a natural microcosm for ecology
Authors: Callie Chappell* and Tadashi Fukami

Multi-species assemblages of yeasts that inhabit floral nectar constitute a novel "natural microcosm" that can be used to develop general ecological theory. In this review, the authors describe low species richness, tractable dispersal, and well-replicated community assembly as advantages of using nectar yeasts, discuss environmental filtering, priority effects, and metacommunity dynamics as select topics illustrating these advantages, and identify metagenomics and comparative genomics as new tools that can be applied to nectar yeasts to uncover basic principles of community assembly.