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Presence of yeasts in floral nectar is consistent with the hypothesis of microbial-mediated signaling in plant-pollinator interactions

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Olfactory floral signals are significant factors in plant-pollinator mutualisms. Recently, unusual fermentation odors have been described in the nectar and flowers of some species. Since yeasts are common inhabitants of many angiosperms nectars, this raises the possibility that nectar yeasts may act as causal agents of fermentation odors in flowers and, therefore, as possible intermediate agents in plant signaling to pollinators. A recent field study has reported that nectar yeasts were quite frequent in floral nectar across three different regions in Europe and America, where they reached high densities (up to 10^5 cells/mm^3). Yeast incidence in floral nectar differed widely across plant host species in all sampling sites. A detailed study currently in progress on one of the species surveyed in that study (*Helleborus foetidus*, Ranunculaceae) has detected that, in addition to interespecific differences in yeast incidence, there is also a strong component of variance in yeast abundance that takes place at the subindividual level (among flowers of the same plant, among nectaries of the same flower). If yeast metabolism is eventually proved to contribute significantly to floral scent, then multilevel patchiness in the distribution of nectar yeasts (among species, among individuals within species, and among flowers and nectaries of the same individual) might contribute to concomitant multilevel variation in plant signaling and, eventually, also in pollination success, pollen flow and plant fitness.

Pollinators forage on a wide range of flowers that differ in morphology, colour, scent and quality and quantity of reward. The majority of these floral features are important visual and olfactory cues that are directly related to plant-pollinators signaling and the pollination process.[1-12] Recently, the intriguing possibility has been raised that microbial communities (especially nectarivorous yeasts) inhabiting flowers could explain better than, or in addition to, plant physiology itself, certain floral features that participate in plant-pollinators signaling, like yeasty nectar or floral scent.[13,14] However, some of these suggestions are based on circumstantial or indirect evidence indicative of the presence of microbes in flowers. For example, fermentation odors have been described in a number of Angiosperms,[14-16] in which different compounds found in nectar were not shared with any other floral parts.[13] In addition, yeasty odors (ketones and short-chain alcohols) have only been observed in mature flowers that were already visited by pollinators and thus potentially contaminated with microbes, in contrast, for example, to the sesquiterpenes isolated in immature flowers that are also common in the foliage of many plants.[14] Yeasty odors were found in species whose flowers are long-lived, produce large amounts of nectar, and are visited by flies and beetles, which are known to act as yeast vectors to flowers.[17-19] In spite of these plausible suggestions, studies indicating a potential role of microbes in the origin of floral scents generally have not looked directly for their presence or abundance in floral nectar, which clearly would provide critical empirical evidence in support of the hypothesis of microbial-mediated signaling in plant-pollinator interactions.

Key words: nectar, yeast, scent, plant-animal interaction, plant signaling

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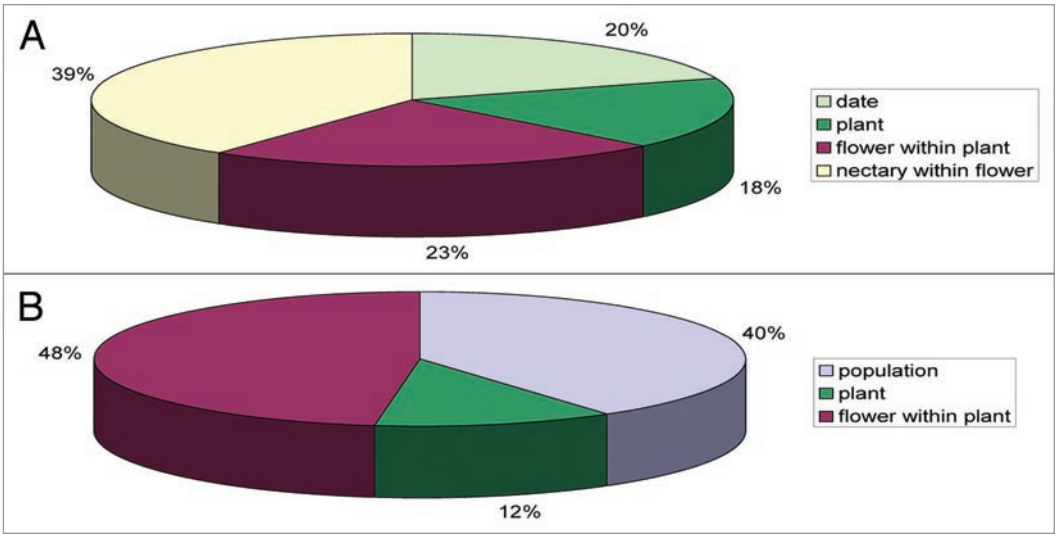

Figure 1. Hierarchical dissection of variance in yeast abundance in single-nectary nectar samples of *Helleborus foetidus*. (A) Temporal patterns. Collection dates and plant, flower within plant and nectary within flower as hierarchical levels of variance analyzed. (B) Spatial patterns. Population, plant within populations and flower within plants as hierarchical levels of variance analyzed.

That yeasts are common inhabitants of floral nectars was well known to microbiologists more than a century ago[20,21] and has been recently corroborated by Herrera et al.[22] This study was conducted at three widely separated areas, which differed greatly in ecological features and biogeographical affinities: two study sites were located in the Southern Iberian Peninsula, about 350 km apart, and one in Yucatán Peninsula, eastern Mexico. Floral nectar samples from 40, 63 and 37 species, belonging to 21, 23 and 21 families, were examined microscopically for yeast cells at these three areas. Yeasts occurred very frequently in floral nectar at all areas, as revealed by the high proportion of nectar samples that contained them (31.8%, 42.3% and 54.4%; samples from all species at each site combined). In addition to being quite frequent in nectar samples, yeast cells often reached extraordinarily high densities in floral nectar at the three areas, which reached roughly 4 x 10^5 cells/mm^3. When plant species, rather than individual nectar samples, were considered as the units for analyses, Herrera et al.[22] found wide variation among species in both the frequency of occurrence and the density of yeasts in nectar samples. A significant fraction of such variation was found to be correlated with differences in pollinator composition, a link between pollination ecology and floral nectar microbiology that has remained unexplored until now. Similar results showing high densities and frequency of occurrence of yeasts in nectar, and interespecific differences in these magnitudes related to variation in pollinator composition, have been also reported by de Vega et al.[23] for 40 South African plant species, which further supports the generality of the phenomenon. In addition to interespecific differences in the prevalence of nectar yeasts, the data examined by Herrera et al.[22] and de Vega et al.[23] revealed also considerable intraespecific variability (i.e., among individuals plants of the same species), although this aspect of results was not explicitly considered in their studies.

A study currently in progress has documented patterns of intraespecific variability in yeast occurrence in the nectaries of *Helleborus foetidus* (Ranunculaceae), a winter-flowering, bumble bee-pollinated perennial herb whose long-lived flowers last for roughly two weeks. Frequency of occurrence and cell density of yeasts in nectar were studied at six populations of this species from Sierra de Cazorla (SE Spain). *Helleborus foetidus* flowers have five separated horn-shaped nectaries hidden at the corolla base, each of which produces up to 5 μl of nectar. This enabled us to study patterns of yeast occurrence also at the within-flower level. At each population, total variance in yeast cell density on a per-nectary basis was partitioned into components due to differences between individual plants, flowers within plants and nectaries within flowers. We found extreme differences concerning the abundance and frequency of yeasts in *H. foetidus* nectar, the magnitude of intraespecific variation being similar or even greater than variation found in interespecific comparisons in the same study area (Pozo MI, et al. unpublished results). Our data suggest that temporal and spatial factors may explain differences regarding yeast abundance in *H. foetidus* nectar, and possibly other species as well. The largest component of intraespecific variance in yeast abundance occurred at the subindividual level, and was mainly accounted for by the variance between nectaries in the same flower (**Fig. 1**). This intraespecific variation in nectarivorous yeast incidence can have some important implications related to plant-pollinators interactions and, more specifically, to plant signaling, as outlined below.

Nectar-inhabiting yeasts modify certain flower characteristics linked to pollinator foraging behavior, such as nectar sugar composition and energetic value, by reducing total sugar concentration and altering the relative proportions of constituent sugars (sucrose, glucose and fructose) and the sucrose:hexose ratio.[23-26] Furthermore, as noted above, yeasts could

be also implicated in floral volatiles emission.[13,14] Consequently, yeast incidence (measured both by frequency and abundance of yeast cells in nectar samples) may have been modifying signaling cues which have been postulated to be intrinsic plant species-specific. Although an empirical connection between yeast presence and fermentation nectar odor is needed, the fact that nectarivorous yeast presence would be as variable as described by our studies could imply the same variability for plant species signaling aspects, along with potential consequences for pollinators, since variance was mainly accounted for by variation below individual plant level. For example, in *H. foetidus* study variance in yeast abundance occurs mainly at the single nectary, which matches with the smallest scale that is perceived by a foraging insect**.** The fact that nectar is an important floral reward that plays a decisive role in the establishment of plant-pollinator mutualisms, together with the recently confirmed ubiquity of nectarivorous yeasts which could be acting as parasites of such mutualisms, open up new and exciting avenues to explore their effect on pollination success and pollen flow[27-30] and finally on plant fitness.[31-35]

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