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# Spatial and temporal distribution patterns of nectar-inhabiting yeasts: how different floral microenvironments arise in winter-blooming *Helleborus foetidus*

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ABSTRACT

Yeasts frequently colonise floral nectar, where they can reach high densities. Recent investigations have further shown that yeast metabolism alters nectar properties by decreasing its total sugar content, modifying sugar composition, or raising nectar local temperature. However, the distribution patterns of nectar yeasts remain poorly investigated at multiple spatial and temporal scales. Here, we study natural variation of the nectar yeasts in a single host plant, *Helleborus foetidus*, in a mountainous region. We quantified spatio-temporal variation in the frequency and abundance of yeast species across six populations located along an altitudinal gradient. Variance partitioning techniques were used to estimate the relative magnitude of variation in yeast abundance between individual plants, flowers within plants, and nectaries within flowers. Although yeast frequency and abundance varied widely across sites and dates, the largest part of total variance occurred at the sub-individual level (i.e., flowers on the same plant). Pollinator composition and activity seemed the main factors explaining the observed patterns of yeast frequency and abundance across floral nectar samples.



## Introduction

The presence of yeast in flowers has been repeatedly addressed by microbiologists, from the late nineteenth century onwards (Boutroux, 1884; Schuster and Ulehla, 1913; Grüss, 1917; Schoelhorn, 1919; Nadson and Krassilnikov, 1927; Capriotti, 1953; Vörös-Felkai, 1957; Sandhu and Waraich, 1985; Lachance et al., 2001; Brysch-Herzberg, 2004). Over the last decade it has become gradually more apparent that yeast metabolism alters the physicochemical properties of nectar, including the sugar concentration and composition (Canto et al., 2007, 2008; de Vega et al., 2009; de Vega and

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Herrera, 2013; Herrera et al., 2008), amino acid profile (Peay et al., 2012) and even flower temperature (Herrera and Pozo, 2010). Some of these changes occur due to the density-dependent action of a species-poor yeast community. Understanding the patterns of yeast prevalence and distribution in natural habitats has, therefore, become a topic of broad ecological interest (Belisle et al., 2012; de Vega et al., 2009; Herrera et al., 2009; Jacquemyn et al., 2013; Pozo et al., 2009).

Yeasts are widespread in floral nectar, occurring in 40–60 % of samples collected in North America, Europe and South Africa (Belisle et al., 2012; de Vega et al., 2009; Herrera et al., 2009). Despite the extensive occurrence of nectar yeasts, yeast frequency and abundance in floral nectar not only varies between regions, but also between different host plant species within a given region (de Vega et al., 2009; Herrera et al., 2009). Interestingly, the nectar yeast abundance data reported by Herrera et al. (2008, 2009) and de Vega et al. (2009) revealed considerable intraspecific variability as well (i.e., amongst individuals of the same plant species), but their sampling method, aimed at uncovering broad-scale patterns, did not explicitly address intraspecific variance in nectar yeast abundance. Further research was, therefore, necessary to determine the main factors contributing to observed variation in yeast abundance at different spatial scales.

The few currently available studies focussing on more detailed nectar yeast distribution patterns point out that yeast presence in a single plant population may be extremely patchy. The presence of yeast in nectar may vary as a result of the availability of nectar, but its occurrence is reliant on its dispersal either by pollinators or by air (Belisle et al., 2012; Golonka and Vilgalys, 2013; Pozo et al., 2009, 2012). As a result, several environmental factors may contribute directly or indirectly to generate heterogeneity in nectar yeast abundance in natural plant populations, including relative air humidity, air temperature, and precipitation (Belisle et al., 2012; Herrera et al., 2009; Lachance, 2006). Relative air humidity, for example, affects nectar secretion rates and concentrations (Corbet et al., 1979), whereas air temperature can have profound effects on plant phenology and floral density (Sánchez-Lafuente et al., 2005), in addition to nectar secretion and concentration (Freeman and Head, 1990). Temperature also influences pollinator composition and visitation rates (Herrera, 1995), as well as yeast growth and survival (Deak, 2006). The frequency of precipitation events can alter insect pollinator foraging patterns (Herrera, 1995), which in turn may affect yeast dispersal (Canto et al., 2008). Besides these abiotic variables, biotic variables such as floral density can also be expected to affect pollinator visitation rates (Belisle et al., 2012) and hence yeast frequency and abundance.

In this paper, we present a multiscale analysis of yeast distribution patterns in the nectar of a single species using a spatially nested design. This method enables variation to be investigated among populations at different altitudes, individuals within populations, flowers within plants, and distinct nectaries within individual flowers of the perennial, winter-flowering herb *Helleborus foetidus*. At the same time, it permits the study of variation at the sub-individual level (Herrera et al., 2006; Herrera, 2009). Nectar yeast prevalence was studied in six *H. foetidus* populations located at different elevations in a mountainous area in SE Spain. More specifically, the purpose of this study is to quantitatively assess the frequency and abundance of nectar yeasts in a single host plant at different locations where there is the likelihood of variation in both biotic (e.g., pollinator composition and activity, or floral density) and abiotic features (e.g., air temperature, rain, relative humidity of the air) linked to changes in altitude in Mediterranean mountainous areas (Giménez Benavides et al., 2006). Because *H. foetidus* has long-lasting flowers (Herrera et al., 2002), temporal variation in yeast prevalence during the flowering period was also investigated. Although the spatial and temporal scope of this study are relatively modest, results provide new insights into the role of biotic and abiotic factors potentially contributing to shape nectar yeast distribution patterns for a single host plant.

## Materials and methods

### *Study species and sites*

*Helleborus foetidus* is a winter blooming herb that is widely distributed in Western Europe. It is quite abundant at our study area (see below), where it is found at a wide range of elevations (Herrera et al., 2001). Each plant produces from one to a few inflorescences each year, and 20–75 flowers will open asynchronously throughout the 1-3 month-long flowering season. The flowers are protandrous and primarily visited by bumblebees (Herrera et al., 2001). Each individual flower lasts for 1–3 weeks, and usually bears five big, horn-shaped nectaries deeply hidden inside a globose, pendant corolla. Each individual nectary may contain up to 5 μl of nectar (see (Herrera et al., 2002) for further floral details). The presence of several nectaries within each flower enables the analysis of yeast variation patterns at the within-flower level.

This study was carried out in 2009 on six *H. foetidus* populations growing in well-preserved mountain forests in the Cazorla, Segura y las Villas Natural Park, Jaén province, SE Spain (see Herrera et al., 2009; for further details of the study region). Pairs of populations were selected along an altitudinal gradient, roughly covering the elevational range of *H. foetidus* in our study area. The two lowest elevation sites (denoted L1 and L2) were located 960 and 1 100 m above sea level (m a.s.l.); the two mid elevation sites (M1 and M2) were located 1 460 and 1 540 m a.s.l.; and the two highest elevation sites (H1 and H2) were 1790 and 1810 m a.s.l. Distances between populations of the same pair ranged from 0.8 to 2.5 km, and distances between pairs between 2 and 10 km (Fig S1). The site elevation, average abiotic conditions (temperature, air relative humidity), pollinator composition and activity, and floral density are given in Table S1 for the 2009 flowering season. In 2008 we detected slight differences in sunlight incidence, and hence in flowering time between the two low-altitude populations. Therefore, for spatial comparison purposes, we selected peak bloom in L2 according to the highest floral density estimates.

The flowering season tends to be shorter at high elevation sites, so we selected one of the low elevation sites (L2) for the temporal monitoring of nectar yeast before and after peak bloom.