

Population growth of the floricolous yeast *Metschnikowia reukaufii*: effects of nectar host, yeast genotype, and host × genotype interaction

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Abstract

Genetic diversity and genotypic diversity of wild populations of the floricolous yeast *Metschnikowia reukaufii* exhibit a strong host-mediated component, with genotypes being nonrandomly distributed among flowers of different plant species. To unravel the causal mechanism of this pattern of host-mediated genetic diversity, this paper examines experimentally whether floral nectars of different host plants differ in their quality as a growing substrate for *M. reukaufii* and also whether genetically distinct yeast strains differ in their relative ability to thrive in nectars of different species (host × genotype interaction). Genetically distinct *M. reukaufii* strains were grown in natural nectar of different hosts under controlled conditions. Population growth varied widely among nectar hosts, revealing that different host plants provided microhabitats of different quality for *M. reukaufii*. Different *M. reukaufii* strains responded in different ways to interspecific nectar variation, and variable growth responses were significantly associated with genetic differences between strains, thus leading to a significant host × genotype interaction. Results of this study provide support for the diversifying selection hypothesis as the underlying mechanism preserving high genetic diversity in wild *M. reukaufii* populations and also suggest that consequences of functional plant-pollinator diversity may surpass the domain of the mutualistic organisms to implicate associated microorganisms.

Introduction

Plant–animal mutualisms have been thoroughly investigated from ecological and evolutionary viewpoints (Herrera & Pellmyr, 2002), but they have been relatively little explored from the perspective of the functional connections between biodiversity components (but see Fontaine *et al.*, 2006; Albrecht *et al.*, 2012; García & Martínez, 2012). In plant–pollinator systems, for example, functional diversity may have positive effects on reproductive success, species composition, recruitment, and persistence of plant communities (Fontaine *et al.*, 2006; Albrecht *et al.*, 2012). Cascading effects of plant–pollinator systems on biodiversity, however, may surpass the boundaries of the mutualistic domain to implicate also nonmutualistic exploiters (*sensu* Bronstein, 2001), a possibility that remains little explored so far. Animal-pollinated

flowers worldwide harbor microbial populations specialized in the exploitation of floral nectar (Brysch-Herzberg, 2004; de Vega *et al.*, 2009; Herrera *et al.*, 2009; Belisle *et al.*, 2012; Álvarez-Pérez & Herrera, 2013), and it has been proposed that the local and regional functional diversity of plant–pollinator interactions may have a positive effect on the genetic diversity of nectarivorous microbial populations (Herrera *et al.*, 2011). Chemical features of floral nectar vary widely between plant species (Nicolson & Thornburg, 2007), a variation that is generally correlated with pollinator composition (Baker & Baker, 1983; Petanidou, 2005; Nicolson & Thornburg, 2007) and may lead to differential microbial growth (Peay *et al.*, 2012; Pozo *et al.*, 2012). If nectars of different plants differ in their suitability for microbial growth and the relative fitness of conspecific microbial genotypes varies across nectars (i.e. there exists a genotype × nectar interaction effect on

population growth), then different genotypes will be favored at flowers of different hosts and floral variety at the plant community level will have a positive effect on microbial genetic diversity (Herrera *et al.*, 2011). A recent study on the specialized nectarivorous yeast *Metschnikowia reukaufii* has demonstrated a strong host-mediated component of genetic and genotypic diversity, with genotypes being nonrandomly distributed among flowers of different species (Herrera *et al.*, 2013a). Such host-related pattern of genetic diversity supports the hypothesis that diversity of plant–pollinator interactions will have a positive effect on the genetic diversity of associated populations of nectar-dwelling microorganisms, but controlled experiments are needed to verify the presumed underlying mechanism. In particular, it is essential to know whether nectars of different host plants differ in their quality as a substrate for *M. reukaufii* growth and also whether genotype and genotype \times host interaction effects on population growth lead to genetically distinct yeast strains to differ in their relative ability to thrive in the nectar of hosts with different pollinators and nectar traits. These two questions were addressed experimentally by growing genetically distinct yeast strains in natural floral nectar of different hosts in the laboratory under controlled conditions, and testing for the effects on population growth of the three relevant sources of variation involved (nectar host, yeast genotype, host \times genotype interaction).

Materials and methods

Metschnikowia reukaufii (Ascomycota, Saccharomycetales) is a microhabitat specialist found in association with flowers and floral nectar (Brysch-Herzberg, 2004). In the study region (Sierra de Cazorla, southeastern Spain), it has been so far recovered from floral nectar of > 80 insect-pollinated species (Pozo *et al.*, 2011; Álvarez-Pérez

& Herrera, 2013; C.M. Herrera, unpublished data). Bulk nectar samples (c. 2 mL per species) were obtained from eight representative host plants during February–July 2012 to be later used as a natural growth medium in laboratory experiments (Table 1). These particular host species were selected because they were abundant and widespread, encompassed a variety of pollinator types, their nectar frequently harbored *M. reukaufii* populations, and per-flower daily nectar secretion rates were high enough to allow gathering sufficient nectar volume for experimentation with a reasonable sampling effort. Inflorescences of all species were bagged at the bud stage, and nectar was subsequently gathered into a single sample per species, filter-sterilized using a polyvinylidene difluoride 0.22- μ m filter (Análisis Vínicos SL, Tomelloso, Spain), and stored at -20 °C until used. Nectar pH was estimated to the nearest 0.5 unit using an universal pH indicator (range 1–14; Panreac, Castellar del Vallés, Spain). Ion-exchange high-performance liquid chromatography (HPLC) was used to determine nectar sugar composition, following the analytical procedures and equipment described in detail by Herrera *et al.* (2006). Only sucrose, glucose, and fructose appeared in the analyses. Separate estimates of the concentration of individual sugars on a percent mass of solute to volume of solution basis were obtained for each sample by integrating areas under chromatogram peaks, and total sugar concentration was then computed by summing partial figures.

Two independent laboratory experiments were conducted to examine the effects of host plant and yeast genotype on *M. reukaufii* population growth. Experiment 1 examined the effects of host plant, yeast genotype, and their interaction on growth. Six *M. reukaufii* strains were used, picked at random from a large strain collection assembled during other studies in the same area (Pozo *et al.*, 2011; strains had been isolated from nectar of

Table 1. Nectar characteristics and main natural pollinators of the eight plant species whose nectar was used in experiments as the growth substrate for *Metschnikowia reukaufii*

| Species | Main pollinators* | pH | Sugar concentration (g/100 mL) [†] | | | |
|----------------------------|-------------------|-----|---|-----------------|----------------|----------------|
| | | | Glucose | Fructose | Sucrose | Total |
| <i>Asphodelus albus</i> | Bb, Sb | 5.0 | 11.9 \pm 1.2 | 12.9 \pm 1.2 | 20.5 \pm 2.3 | 45.3 \pm 2.8 |
| <i>Digitalis obscura</i> | Bb, Sb | 5.0 | 1.6 \pm 0.6 | 14.9 \pm 1.5 | 34.3 \pm 2.1 | 50.8 \pm 2.2 |
| <i>Echium flavum</i> | Bb, Sb | 5.5 | 4.3 \pm 0.6 | 10.9 \pm 0.7 | 37.8 \pm 5.1 | 53.0 \pm 5.5 |
| <i>Gladiolus illyricus</i> | Bu, Sb | 3.5 | 11.9 \pm 1.6 | 9.6 \pm 1.0 | 59.0 \pm 3.7 | 80.5 \pm 6.2 |
| <i>Helleborus foetidus</i> | Bb | 8.0 | 0.2 \pm 0.1 | 0.9 \pm 0.2 | 41.8 \pm 2.0 | 42.9 \pm 2.1 |
| <i>Iris foetidissima</i> | Bb | 7.0 | 0.04 \pm 0.02 | 0.01 \pm 0.01 | 34.8 \pm 1.9 | 34.9 \pm 1.9 |
| <i>Lonicera etrusca</i> | Mo | 5.0 | 4.7 \pm 1.4 | 4.4 \pm 1.3 | 12.2 \pm 3.4 | 21.3 \pm 4.6 |
| <i>Lonicera implexa</i> | Mo | 6.0 | 3.7 \pm 0.2 | 3.2 \pm 0.1 | 20.6 \pm 3.8 | 27.5 \pm 3.6 |

*Bb, bumble bees (*Bombus*); Bu, butterflies; Mo, hawk moths; Sb, solitary bees (mainly large-sized species of Anthophoridae and Megachilidae). Based on observations conducted during 2004–2011 in the Sierra de Cazorla study region (C.M. Herrera, unpublished data).

[†]Mean \pm 1 SE. $n = 4$ independent estimates per species, except for *Echium flavum* ($n = 3$).

Atropa baetica, *Linaria lilacina*, and *Helleborus foetidus*, two strains from each). Genetic distinctness of experimental strains was determined by fingerprinting them with 10 nuclear DNA microsatellite markers (Supporting Information, Table S1). The number of strains included in the experiment was the maximum possible that, given the nectar volume available, allowed for a complete two-way factorial design with replication. A fully-crossed, two-way factorial design was used, with *M. reukaufii* strain ($n = 6$) and host plant ($n = 8$) as main factors, and two replicates per each strain \times plant combination ($n = 96$ experimental units in total). Each experimental unit consisted of a 0.2-mL PCR tube containing 15 μ L of natural nectar from the field-collected bulk samples plus 1 μ L of *M. reukaufii* cell suspension. Two replicated controls were set per strain, each consisting of 15 μ L of 15% lactophenol cotton blue (LCB hereafter) solution plus 1 μ L of *M. reukaufii* cell suspension. As LCB killed and fixed yeast cells immediately after inoculation, cell density estimates in control tubes served to estimate initial cell numbers in experimental units. After inoculation, all tubes were held for 4 days at 25 °C without agitation, after which the experiment was terminated by adding 32 μ L of 15% LCB solution to each tube. Cell densities were then estimated microscopically using standard cell counting procedures.

Experiment 2 aimed to elucidate whether *M. reukaufii* strains found naturally in a given nectar host tended to grow better there than strains that had been found naturally in other nectar hosts. This experiment was conducted using exclusively nectar from *H. foetidus* as the growth medium, as available nectar for the remaining seven species included in Experiment 1 was insufficient for a complete two-way 'reciprocal transplant' design. Twelve distinct *M. reukaufii* strains isolated from field-sampled nectar of *H. foetidus*, *Iris foetidissima*, and *Lonicera implexa* (four strains per host) from the study area were grown in *H. foetidus* nectar. Setup was similar to Experiment 1: Experimental units consisting of 15 μ L of nectar plus 1 μ L of *M. reukaufii* cell suspension, two replicates per strain, and two cotton blue solution controls per strain to estimate starting cell densities.

All isolates included in the experiments were identified as *M. reukaufii* by two-way sequencing the D1/D2 domain of the 26S ribosomal RNA gene, assembling a consensus sequence, and comparing the result with the sequence for the *M. reukaufii* type strain (CBS5834) in GenBank (accession number U44825).

Results of growth experiments were analyzed by fitting linear models to the data using SAS procedure MIXED and restricted maximum likelihood estimation (REML; Littell *et al.*, 1996). The \log_{10} of the ratio of final to initial cell density will be used here as an index of population

growth rate. This index would reflect maximum potential growth rate only if yeasts grew exponentially during the whole 4-day experimental period, i.e. without an initial lag or final plateau stages. More generally, however, the index used here will provide a rough estimate of mean population growth rate averaged over the 4-day experimental period. As mean flower duration of *M. reukaufii* host plants in the study area is *c.* 4 days (C.M. Herrera, unpublished data), growth index figures used here may be taken as meaningful descriptors of the ability of yeast genotypes to build up intrafloral populations over periods commensurate with the mean duration of their ephemeral microhabitats. Model-adjusted, least-squares cell means of the response variable for different factor level combinations were obtained with the LSMEANS statement. Significance of 'simple main effects' (i.e. the effects of a given factor at different levels of the other factor; Pedhazur, 1997) was tested with the SLICE option. To explore the possible genetic basis of differential growth patterns exhibited by experimental strains used in Experiment 1, multilocus strain descriptions based on DNA microsatellite fingerprints (Table S1) were correlated with either the mean growth index of individual strains averaged across nectar types or the dissimilarity of their growth profiles across different nectar hosts estimated using pairwise euclidean distances. Multilocus description of strains was obtained by conducting a principal coordinates analysis on their pairwise genetic distance matrix using GENALEX software (Peakall & Smouse, 2006).

Results

Nectar characteristics

All nectar parameters considered varied widely in the set of eight host species used in the experiments, as denoted by the broad ranges of species means: pH 3.5–8.0, total sugars 21.3–80.5%, sucrose 12.2–59.0%, glucose 0.04–11.9%, and fructose 0.01–14.9% (Table 1). Interspecific variation was associated with pollinator differences. For example, the two species pollinated solely by bumble bees (*H. foetidus*, *I. foetidissima*) had pure-sucrose, weakly alkaline nectars, the two species pollinated by hawk moths were characterized by dilute, weakly acidic nectars, and the four species pollinated simultaneously by bumble bees and solitary bees had hexose-rich, acidic nectars (Table 1).

Nectar and genotype effects on growth: Experiment 1

In Experiment 1, growth index [\log_{10} (final/initial cell density), GI hereafter] of *M. reukaufii* depended significantly

on strain ($F_{5,48} = 33.38$, $P < 0.0001$), host plant ($F_{7,48} = 51.69$, $P < 0.0001$), and their interaction ($F_{35,48} = 1.67$, $P = 0.049$). Mean growth index varied widely across hosts, being lowest in nectar from *L. implexa* (Mean GI \pm SE = 0.453 ± 0.035) and *I. foetidissima* (0.465 ± 0.035), and highest in nectar from *Asphodelus albus* (0.910 ± 0.035) and *H. foetidus* (1.167 ± 0.035). The six strains assayed also differed considerably in mean GI, which ranged between 0.507 ± 0.03 (strain R2) and 1.016 ± 0.03 (R6). The genetic basis of growth rate differences between strains is supported by the close correlation between mean GI and strain score on the first principal coordinate axis from the microsatellite-based genetic distance matrix ($r_s = -1.000$, $n = 6$, $P = 0.0014$; significance tested by 10^3 random permutations; Fig. 1).

As denoted by the significant strain \times host interaction, mean GI in a given nectar host was strain dependent, and differences between strains in mean GI fluctuated across nectar hosts (Fig. 2). For example, strain R3 was the fastest growing one in *Echium flavum* nectar; yet, its rank dropped to fourth when growing in *L. implexa* nectar (Fig. 2). Growth in *Digitalis obscura* nectar was lower than average for all strains except R5, for which *D. obscura* nectar ranked second in quality only to *H. foetidus*, the nectar where all strains grew best (Fig. 2). Nectar variation had a significant effect on the growth of every strain when considered separately ($F_{7,48} \geq 8.19$, $P < 0.0001$, simple main effects). With the exception of *A. albus* ($F_{5,48} = 1.54$, $P = 0.20$), all nectar hosts induced significant heterogeneity among strains in growth ($F_{5,48} \geq 3.88$, $P \leq 0.005$).

Variation between strains in their growth profiles across hosts (Fig. 2) was significantly associated with their genetic differences, as denoted by the significant correlation between pairwise matrices of growth index dissimilarity and microsatellite-based genetic distance

($r_s = 0.756$, $P = 0.014$, Mantel test with 10^3 repetitions; Fig. 3).

Host-of-origin effect on growth: Experiment 2

The host of origin of the 12 *M. reukaufii* strains used in Experiment 2 had a significant effect on growth index when grown in *H. foetidus* nectar ($F_{2,21} = 6.84$, $P = 0.0052$). On average, strains that had been isolated from *H. foetidus* nectar grew the best (Mean GI \pm SE = 1.448 ± 0.058), those isolated from *I. foetidissima* grew the worst (Mean GI \pm SE = 1.161 ± 0.058), and those isolated from *L. implexa* were intermediate (Mean GI \pm SE = 1.399 ± 0.058). The contrast between growth indices of 'native' (i.e. originating from *H. foetidus*

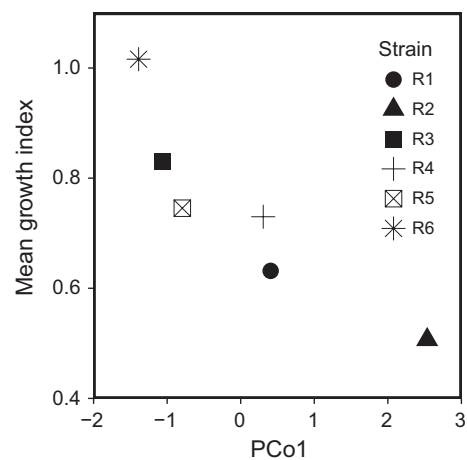


Fig. 1. Relationship between mean growth index [= \log_{10} (final/initial cell density)] in floral nectar of the six *Metschnikowia reukaufii* strains used in Experiment 1 and their multilocus genetic characteristics, as summarized by scores on the first axis from the principal coordinates analysis of the microsatellite-based, pairwise genetic distance matrix.

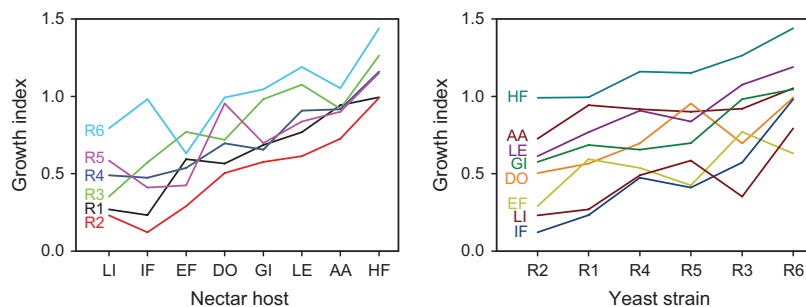


Fig. 2. The two alternative interaction plots depicting the joint effects of nectar host and yeast strain on *Metschnikowia reukaufii* growth index [= \log_{10} (final/initial cell density)] revealed by Experiment 1. Each plot displays the levels of one factor on the horizontal axis; cell means are plotted across the levels of the other factor; and points representing a set of cell means at a given level of this factor are connected. Plotted values are model-adjusted cell means for host-strain combinations. Nectar hosts coded as follows: AA, *Asphodelus albus*; DO, *Digitalis obscura*; EF, *Echium flavum*; GI, *Gladiolus illyricus*; HF, *Helleborus foetidus*; IF, *Iris foetidissima*; LE, *Lonicera etrusca*; LI, *Lonicera implexa*.

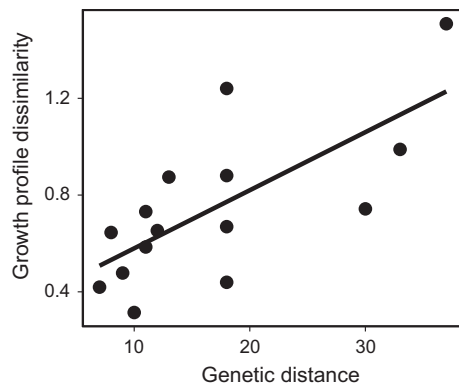


Fig. 3. Relationship between pairwise dissimilarity in growth profile across the eight nectar hosts assayed and the microsatellite-based, pairwise genetic distances, for the six *Metschnikowia reukaufii* strains used in Experiment 1. Least-squares fitted regression is shown only for reference.

flowers) vs. 'foreign' (originating from *I. foetidissima* and *L. implexa* flowers) strains was statistically significant ($F_{1,21} = 5.44$, $P = 0.029$).

Discussion

Functional diversity of pollination in plant communities is associated with variation in structural and functional floral features, including type and characteristics of the reward offered by plants to pollinators (Fenster *et al.*, 2004). Nectar is the commonest type of floral reward (Simpson & Neff, 1983), and interspecific variation in nectar traits is generally correlated with pollinator composition (Baker & Baker, 1983; Petanidou, 2005; Petanidou *et al.*, 2006; Nicolson & Thornburg, 2007). This was also verified by the analytical results for the eight nectar host species used here for the experiments. Nectar varied widely between species in total sugar concentration, sugar composition, and pH, and this variation was associated with differences in pollinators. Variation in nectar pH, as well as its possible association with pollinator type, remains virtually unexplored (Nicolson & Thornburg, 2007). Broad interspecific variation in pH found here (see also Baker & Baker, 1983; Brysch-Herzberg, 2004) may arise from variable concentrations of chemical constituents that act directly on pH, such as organic acids, or pH-buffering agents, such as nectarin proteins (Nicolson & Thornburg, 2007). Given the long-known influence of pH on the growth of microorganisms, future studies on the ecology of nectar microbial communities should pay more attention to that parameter.

Ecological studies on floral nectar have been generally undertaken from the perspective of its animal consumers (but see, e.g. Adler, 2000; Sasu *et al.*, 2010); yet, nectar is

a resource widely exploited also by specialized microbial communities (see references in Introduction). While for animal pollinators floral nectar represents just food, for nectar-dwelling microorganisms it is the vital environment they are immersed in, and natural variation in its chemical properties should influence microbial growth decisively. This is confirmed by the results of experiments reported here, showing that *M. reukaufii* populations grew at broadly different rates in natural floral nectar from different hosts. From the perspective of *M. reukaufii*, this result implies that floral nectars produced by different host plants are not equivalent in terms of their quality as microhabitats, or in other words, that plant communities are heterogeneous with regard to the quality of the nectar resources offered to these specialist nectarivorous yeasts. Differential quality of nectar from different host plants as *M. reukaufii* microhabitats may help to explain, at least in part, the broad natural variation in the abundance of this yeast at the flowers of different plant species (Brysch-Herzberg, 2004; Pozo *et al.*, 2011).

Between-host differences in host quality may stem from variation in any of the nectar parameters considered here, as pH, sugar concentration, and sugar composition are all known to affect yeast growth (e.g. Membré *et al.*, 1999; Peay *et al.*, 2012; Pozo *et al.*, 2012). When cultured in single-sugar artificial media, *M. reukaufii* growth declines with increasing sugar concentration, and also in the direction sucrose–glucose–fructose (Herrera *et al.*, 2012), which supports the interpretation that nectar sugar differences account, at least in part, for between-host variation in growth found in this study. Although sugars are by far the dominant components of floral nectar, other minor chemical constituents contribute also to shape their characteristics, including amino acids, proteins, organic acids, and a broad range of secondary compounds (Nicolson & Thornburg, 2007). Differences between nectar hosts in concentration of these or other constituents not considered here may also have contributed to differences between hosts in growth indices. For example, nitrogen sources are essential to yeast growth, and *M. reukaufii* populations respond to alterations in amino acid composition and concentration in artificial nectars (Peay *et al.*, 2012). Host plants tested here vary widely in the amino acid content of their nectars (Herrera, 1989; Table 1; data for six of the eight hosts tested here), and the marginally significant positive correlation existing between *M. reukaufii* growth index and published amino acid data ($r_s = 0.706$, $n = 6$ species, $P = 0.10$) suggests that high amino acid concentration was perhaps responsible for enhanced growth in certain particularly favorable nectars (e.g. *A. albus*, *H. foetidus*). Interspecific differences in concentration of some of the antimicrobial compounds frequently found in nectar (Adler, 2000; Nicolson &

Thornburg, 2007; Sasu *et al.*, 2010) may also have contributed to between-host variation in *M. reukaufii* growth, but no relevant information is available on their possible occurrence in the nectars considered here.

Different *M. reukaufii* strains responded in different ways to interspecific nectar variation, and differential growth responses were significantly associated with genetic differences between strains as assessed with nuclear microsatellite markers. Although microsatellites are usually treated as neutral markers, microsatellite variation has been often proven to have significant functional effects involving, among other, regulation of gene expression and metabolic processes (Li *et al.*, 2002), which may explain the significant relationships found here between microsatellite fingerprints and growth patterns. These results confirm in an ecological context what has been long known to microbiologists working with artificial culture media, namely that genetically distinct strains of the same yeast species often differ in physiological capacities and growth response to environmental variation (e.g. Lachance & Bowles, 2002; Sumner & Avery, 2002; Nozaki *et al.*, 2003). In the framework of the present study, the finding of a genetically-based genotype \times host interaction on *M. reukaufii* population growth, in combination with the results of Experiment 2 revealing host-of-origin effects on population growth, supports a parsimonious mechanism to account for the nonrandom distribution of genotypes of this species among floral hosts in the study area (Herrera *et al.*, 2013a), as discussed below.

Under stringent culture conditions, *M. reukaufii* may produce asci and ascospores, and reproduce sexually (Giménez-Jurado *et al.*, 1995), but sexual reproduction does not occur naturally in the nectar-living populations of southern Spain, where it is predominantly or exclusively clonal (Herrera *et al.*, 2013a). In contrast to predictions of theoretical models for clonal organisms (Balloux *et al.*, 2003; Bengtsson, 2003), populations of *M. reukaufii* harbor considerable genetic and genotypic diversity (Herrera *et al.*, 2013a). Diversifying selection, in which the fitness of different genotypes differs across microsites, has been proposed as a major factor maintaining genetic variation in clonal organisms (Ellstrand & Roose, 1987; Kassen & Rainey, 2004). The experimental results presented here support the mechanism inherent to the diversifying selection hypothesis (Hedrick, 1986), namely that microhabitat types differ in suitability for microbial growth and that conspecific genotypes vary in their relative fitness across microhabitats. Experiment 2 showed that strains originally isolated from *H. foetidus* nectar significantly outperformed those from *L. implexa* and *I. foetidissima* when all were grown in *H. foetidus* nectar. Although these results support the interpretation that

diversifying selection accounts for host-mediated genetic variation in *M. reukaufii*, reciprocal transplant experiments involving more yeast strains and nectar hosts are needed to confirm the generality of the host-of-origin advantage revealed by Experiment 2.

Taken together, results of the present study and Herrera *et al.* (2013a) support the hypothesis that the biodiversity consequences of functional plant–pollinator diversity can extend beyond the domain of the mutualistic organisms concerned (Herrera *et al.*, 2011). A substantial proportion of total genetic diversity of wild *M. reukaufii* populations in the Sierra de Cazorla study area is accounted for by genetic differences between subpopulations associated with different floral hosts (Herrera *et al.*, 2013a). This pattern most likely arises from genetically distinct strains responding differentially to interspecific variation in nectar features and strains associated with a given host tending to grow better there than elsewhere, as documented here. Genetic and genotypic diversity of *M. reukaufii* populations is therefore expected to increase with increasing diversity of nectar hosts in the plant community. In addition to its key role in evolutionary processes, genetic diversity may have multiple ecological consequences at the population, community, and ecosystem levels (reviewed by Hughes *et al.*, 2008). *Metschnikowia reukaufii* is by far the dominant yeast in all floral nectar communities studied so far in the northern hemisphere (Brysch-Herzberg, 2004; Pozo *et al.*, 2011; Belisle *et al.*, 2012; Álvarez-Pérez & Herrera, 2013). It is possible that the high genetic diversity of its populations (Herrera *et al.*, 2013a) is consequential for the community ecology of plant–pollinator mutualisms with which they are closely associated. For example, differences between *M. reukaufii* genotypes in the composition and concentration of metabolites added to floral nectar (e.g. polyols, fructooligosaccharides; Park *et al.*, 1996; Nozaki *et al.*, 2003) might broaden the natural range of nectar chemical characteristics in plant communities. The latter could in turn affect pollinator composition, diversity, and pollinator-mediated yeast effects on plant reproduction (Herrera *et al.*, 2013b). Host-mediated genetic diversity of *M. reukaufii* populations may also have consequences in the microbial realm. In some host plants, *M. reukaufii* grow in association with *Acinetobacter* and *Leuconostoc* bacteria (Álvarez-Pérez & Herrera, 2013). Insofar as the establishment or maintenance of these microbial consortia in nectar depend on genetic features of *M. reukaufii*, the local and regional genetic diversity of *M. reukaufii* might influence communities of nectar-dwelling bacteria and their associated pollinator-mediated effects on plant reproduction (Vannette *et al.*, 2013). Further research is needed to substantiate these speculations.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Microsatellite DNA fingerprints of *Metschnikowia reukaufii* strains used in Experiment 1.