

Scavengers that fit beneath a microscope lens

If favorite tools of field ecologists had to be ranked in decreasing order of preference, microscopes would undoubtedly lie low on their fondness list, well behind telescopes or binoculars. An obvious reason for such a subordinated rank of microscopes is the unsurmountable difficulty of witnessing the behavior of microorganisms embedded in solid substrates that are impervious to light without prior, inevitably disturbing preparations. This limitation vanishes in liquid environments, drops of which may be watched directly under the microscope without previous preparation. In contrast to marine and freshwater habitats, terrestrial environments offer few opportunities in the way of transparent microbial habitats amenable to direct natural history observations using a microscope. Or so I thought until recently. The floral nectar of animal-pollinated plants are miniature islands of aquatic environments in terrestrial habitats, and they are the abode of a plethora of specialized microorganisms, notably microfungi (“yeasts” hereafter; de Vega et al. 2009, Herrera et al. 2009, Belisle et al. 2012).

For more than one century, microbiologists and pollination biologists alike have been acquainted with the presence of yeasts in floral nectar (Boutroux 1884, Eisikowitch et al. 1990*a, b*). What recent studies have shown is that there is much more to nectar yeasts than an interesting, albeit ecologically inconsequential oddity. Nectar yeasts have been proven to be ubiquitous and to play a third-party, significant influence on the ecology of plant–pollinator mutualisms through their metabolic impact on the chemistry of nectar, the main reward that plants offer to pollinators to entice them to flowers (Herrera et al. 2008, 2013, Schaeffer et al. 2014). A vexing gap remains, however, in our current understanding of the ecology of plant–yeast–pollinator associations. Almost without exception, the floral nectar of animal-pollinated plants consists essentially of a sugary solution that, at best, contains only trace amounts of other substances, including some that are essential for cell maintenance and multiplication such as amino acids (Nicolson and Thornburg 2007). Such severe nutrient limitation and strong compositional bias of nectar, however, specialist floricolous yeasts seem able to easily overcome. As a matter of fact, some species proliferate very quickly in nectar, reaching surprisingly high densities for an

ephemeral habitat, often in the range 10^4 – 10^5 cells/mm³ (Herrera et al. 2008, 2009). This commonplace observation becomes particularly puzzling given the extreme shortage in floral nectar of nutrients other than sugars. How do specialized nectar yeasts acquire the indispensable non-sugar nutrients to fuel their rapid cellular multiplication?

While conducting systematic microscopical observations of nectar drops, I came upon an unreported facet of the natural history of the plant–yeast–pollinator interaction that may elucidate the perplexing observation of fast microbial growth in a liquid medium that lacks the fundamental nutrients needed to achieve profuse cell multiplication. The nectar yeast specialist *Metschnikowia reukaufii*, a cosmopolitan species dwelling in the nectar of many species throughout the world, apparently engages in scavenging the pollen grains that contaminate nectar as a byproduct of the activity of pollinators. Pollen grains are ubiquitous in floral nectar. I examined microscopically 2,000+ nectar drops from 90+ species of southern Spain’s insect-pollinated plants whose flowers had previously been visited by natural pollinators (Appendix S2), and found pollen grains in the nectar of 85 species (93% of total). Frequency of occurrence and density of pollen grains were remarkable in many species: 33 species had pollen in $\geq 50\%$ of nectar samples, and 22 species had ≥ 100 grains/mm³ on average. When nectar drops were handled gently, without stirring them or pressing too hard on coverslips, yeast cells often appeared clustered around pollen grains, as are the *M. reukaufii* cells in Fig. 1, the photograph of the nectar of *Helleborus foetidus* (Ranunculaceae). Many pollen grains were ungerminated and apparently intact, but bursting grains with ejected cell contents on their surface were frequent, as in the grains of the photograph. Germinated grains bearing pollen tubes were not exceptional. Regardless of whether pollen grains were intact or not, yeast cells tended to be intimately associated to them, either as loose, roughly spherical cell aggregates wrapping around grains or as densely packed three-dimensional cell masses closely attached to pollen grains or pollen tubes.

Pollen grains are particularly rich in proteins, and students of floral nectar chemistry have long been aware that they should be particularly cautious about contamination with pollen, for it can increase considerably the amino acid content of nectar (Gottsberger et al. 1984, 1990). Spatial intimacy of yeast cell clusters and pollen grains in floral nectar therefore prompts the parsimonious interpretation that nutrients being leached from pollen grains into the nectar boost the local population growth of yeasts well beyond the levels attainable exclusively with the resources available in pure nectar. Under

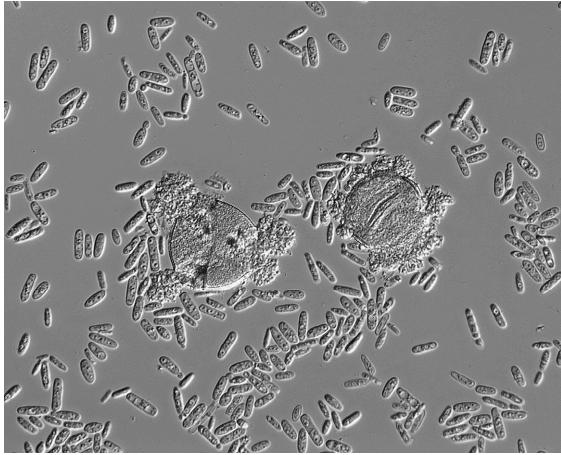


FIG. 1. Two bursting pollen grains surrounded by living cells of the specialist yeast *Metschnikowia reukaufii* in nectar of *Helleborus foetidus* (Ranunculaceae). The three masses of ejected pollen material, each associated with a pollen aperture, are clearly visible on the left grain. Nomarsky interference contrast photomicrograph taken on undisturbed (i.e., undiluted, unstained) floral nectar.

this simple hypothesis, nectar yeasts would play the role of opportunistic scavengers of nectar contaminants accidentally brought into nectar by pollinators. But the odds are that the yeasts' strategy to cope with nutrient scarcity in nectar is somewhat more intricate than simple passive scavenging. Nutrients encased within the pollen grain walls will sooner or later be delivered to the nectar by passive diffusion alone (Erhardt and Baker 1990), but pollen grains bursting as those in Fig. 1 will doubtless make nutrients available at a much faster pace. There are reasons to suspect that grain bursting perhaps does not come about spontaneously, but may sometimes be actively induced by the yeast cells nearby. More than two decades ago, experiments showed that *M. reukaufii* cells possess the intriguing ability to induce the bursting of pollen grains and pollen tubes in nectar (Eisikowitch et al. 1990b). This burst-inducing behavior may be of obvious adaptive value to yeasts, because it will accelerate the nutritional enrichment of the cells' immediate surroundings and, in so doing, will eventually contribute to hasten local population growth. The extracellular proteolytic activity of many *Metschnikowia* yeasts, including *M. reukaufii* (Lachance 2011), fits well into

this hypothesis. Bacteria are often found in nectar, sometimes in association with yeasts (Álvarez-Pérez and Herrera 2013). The possibility also exists that they can contribute to pollen degradation and make the contents available to yeasts. Were one or more of the "simple scavenging," "scavenging by directed bursting," or "bacterially facilitated scavenging" hypotheses verified by future experiments, the complexities involved in the tripartite interaction linking plants, pollinators and yeasts would climb still one step further. In particular, since the protein content of pollen grains varies by about one order of magnitude among species (Roulston et al. 2000), it might eventually turn out that population growth of nectar yeasts depends more on the composition, magnitude, and dynamics of pollen contaminants than on the chemistry of the nectar itself, as currently recognized. At its simplest, a preliminary test of this hypothesis would involve comparing yeast population growth in water sugar solutions with and without experimentally added pollen grains. A more informative experiment, however, should consist of assessing yeast growth in water sugar solution and natural nectar, each with and without a series of different pollen types of known chemical composition. This test would allow to assess the importance of pollen grains for growth relative to non-sugar nectar constituents, and also to evaluate whether compositional differences between pollen types translate into differential yeast growth.

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CARLOS M. HERRERA

Estación Biológica de Doñana
Consejo Superior de Investigaciones Científicas
Américo Vespucio 26
E-41092 Sevilla, Spain
E-mail: herrera@cica.es

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Appendix S1

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Appendix S2. Scavengers that fit beneath a microscope lens. Carlos M. Herrera. *Ecology*. 2017.

Table S1. Occurrence of pollen grains in 2,045 samples of floral nectar from 91 species of southern Spanish insect-pollinated plants examined microscopically.

Species	Family	Nectar samples examined	Percent samples with pollen grains	Mean pollen grain density (grains/mm ³)
<i>Acinos alpinus</i>	Lamiaceae	18	5.6	6.5
<i>Anarrhinum laxiflorum</i>	Plantaginaceae	18	83.3	630.0
<i>Anthericum liliago</i>	Asparagaceae	18	5.6	2.8
<i>Anthyllis vulneraria</i>	Fabaceae	23	4.3	3.5
<i>Antirrhinum australe</i>	Plantaginaceae	18	88.9	201.1
<i>Aphyllantes monspeliensis</i>	Aphyllanthaceae	18	38.9	40.0
<i>Arbutus unedo</i>	Ericaceae	49	65.3	60.4
<i>Asphodelus albus</i>	Asphodelaceae	18	5.6	0.6
<i>Astragalus incanus</i>	Fabaceae	19	42.1	14.2
<i>Atropa baetica</i>	Solanaceae	25	48.0	30.7
<i>Bituminaria bituminosa</i>	Fabaceae	16	18.8	15.7
<i>Calamintha nepeta</i>	Lamiaceae	24	16.7	10.2
<i>Carduus granatensis</i>	Asteraceae	27	11.1	3.5
<i>Carduus tenuiflorus</i>	Asteraceae	16	0.0	0.0
<i>Carlina corymbosa</i>	Asteraceae	19	63.2	76.8
<i>Centaurea calcitrapa</i>	Asteraceae	23	17.4	16.8
<i>Cirsium flavispina</i>	Asteraceae	25	28.0	12.0
<i>Cirsium odontolepis</i>	Asteraceae	21	61.9	39.3
<i>Cirsium vulgare</i>	Asteraceae	25	20.0	12.8
<i>Cleonia lusitanica</i>	Lamiaceae	20	35.0	27.5
<i>Clinopodium vulgare</i>	Lamiaceae	21	4.8	3.3
<i>Cornus sanguinea</i>	Cornaceae	19	73.7	199.9
<i>Digitalis obscura</i>	Plantaginaceae	44	79.5	35.6
<i>Echium flavum</i>	Boraginaceae	20	90.0	202.4
<i>Erinacea anthyllis</i>	Fabaceae	20	5.0	0.5
<i>Erinus alpinus</i>	Plantaginaceae	15	60.0	182.2
<i>Erysimum myriophyllum</i>	Brassicaceae	19	100.0	711.5
<i>Gladiolus illyricus</i>	Iridaceae	65	49.2	45.3
<i>Hedera helix</i>	Araliaceae	23	87.0	399.5
<i>Helleborus foetidus</i>	Ranunculaceae	40	50.0	252.7
<i>Iris foetidissima</i>	Iridaceae	17	17.6	1.8
<i>Iris pseudacorus</i>	Iridaceae	16	50.0	49.1
<i>Iris xiphium</i>	Iridaceae	16	31.3	4.2
<i>Knautia subscaposa</i>	Dipsacaceae	20	25.0	43.0
<i>Lamium amplexicaule</i>	Lamiaceae	19	10.5	11.7
<i>Lavandula latifolia</i>	Lamiaceae	35	5.7	4.3
<i>Linaria aeruginea</i>	Plantaginaceae	20	75.0	291.5
<i>Linaria lilacina</i>	Plantaginaceae	17	88.2	1536.0
<i>Lithodora fruticosa</i>	Boraginaceae	19	94.7	127.7
<i>Lonicera arborea</i>	Caprifoliaceae	21	33.3	25.0
<i>Lonicera implexa</i>	Caprifoliaceae	22	22.7	9.1
<i>Lonicera periclymenum</i>	Caprifoliaceae	19	15.8	6.8
<i>Lonicera splendida</i>	Caprifoliaceae	11	36.4	25.9

<i>Lysimachia ephemerum</i>	Primulaceae	23	91.3	224.8
<i>Lythrum salicaria</i>	Lythraceae	18	88.9	924.4
<i>Marrubium supinum</i>	Lamiaceae	18	72.2	38.1
<i>Narcissus cuatrecasatii</i>	Amaryllidaceae	65	66.2	69.1
<i>Narcissus hedraeanthus</i>	Amaryllidaceae	10	30.0	20.7
<i>Narcissus serotinus</i>	Amaryllidaceae	9	77.8	596.3
<i>Narcissus triandrus</i>	Amaryllidaceae	20	60.0	166.0
<i>Nepeta tuberosa</i>	Lamiaceae	22	27.3	24.4
<i>Onosma tricosperma</i>	Boraginaceae	10	90.0	293.8
<i>Orchis coriophora</i>	Orchidaceae	20	0.0	0.0
<i>Origanum virens</i>	Lamiaceae	21	9.5	5.3
<i>Ornithogalum umbellatum</i>	Asparagaceae	20	80.0	283.7
<i>Orobanche haenseleri</i>	Orobanchaceae	28	42.9	44.6
<i>Phlomis herba-venti</i>	Lamiaceae	28	0.0	0.0
<i>Phlomis lychnitis</i>	Lamiaceae	21	0.0	0.0
<i>Pistorinia hispanica</i>	Crassulaceae	20	5.0	1.6
<i>Platanthera algeriensis</i>	Orchidaceae	20	5.0	0.6
<i>Plumbago europaea</i>	Plumbaginaceae	20	45.0	30.3
<i>Polygala boissieri</i>	Polygalaceae	16	18.8	5.2
<i>Polygonatum odoratum</i>	Ruscaceae	14	57.1	31.1
<i>Primula vulgaris</i>	Primulaceae	37	67.6	505.4
<i>Prunella laciniata</i>	Lamiaceae	20	5.0	1.3
<i>Ptilostemon hispanicus</i>	Asteraceae	24	12.5	4.3
<i>Rosmarinus officinalis</i>	Lamiaceae	75	28.0	6.8
<i>Salvia lavandulifolia</i>	Lamiaceae	22	22.7	43.1
<i>Salvia verbenaca</i>	Lamiaceae	15	46.7	24.4
<i>Saponaria ocymoides</i>	Caryophyllaceae	8	0.0	0.0
<i>Satureja intricata</i>	Lamiaceae	63	23.8	14.5
<i>Scabiosa andryalifolia</i>	Dipsacaceae	21	23.8	39.7
<i>Sideritis incana</i>	Lamiaceae	26	42.3	78.3
<i>Silene alba</i>	Caryophyllaceae	15	60.0	212.5
<i>Silene colorata</i>	Caryophyllaceae	21	14.3	7.2
<i>Silene lasiostyla</i>	Caryophyllaceae	20	65.0	35.9
<i>Silene vulgaris</i>	Caryophyllaceae	13	15.4	20.1
<i>Sisymbrella aspera</i>	Brassicaceae	16	100.0	869.7
<i>Stachys officinalis</i>	Lamiaceae	22	4.5	1.3
<i>Tetragonolobus maritimus</i>	Fabaceae	18	55.6	33.3
<i>Teucrium polium</i>	Lamiaceae	24	25.0	58.6
<i>Teucrium rotundifolium</i>	Lamiaceae	28	17.9	7.9
<i>Teucrium webbianum</i>	Lamiaceae	21	14.3	15.0
<i>Thymus mastichina</i>	Lamiaceae	19	63.2	101.2
<i>Thymus orospedanus</i>	Lamiaceae	18	55.6	77.7
<i>Thymus serpylloides</i>	Lamiaceae	20	35.0	63.2
<i>Urginea maritima</i>	Asparagaceae	20	5.0	1.0
<i>Valeriana tuberosa</i>	Caprifoliaceae	6	50.0	174.6
<i>Vicia onobrychioides</i>	Fabaceae	20	25.0	6.5
<i>Viola cazorlensis</i>	Violaceae	18	27.8	21.9
<i>Viola odorata</i>	Violaceae	14	0.0	0.0