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Yeasts in nectar enhance male fitness in a montane perennial herb

Robert N. Schaeffer[1,2,3] and Rebecca E. Irwin[1,2]

[1]*Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755 USA*
[2]*Rocky Mountain Biological Laboratory, Gothic, Colorado 81224 USA*

Abstract. Floral nectar of many plant species is prone to colonization by microbial organisms such as yeasts. Their presence and metabolism of nectar chemical components have the potential to modify a suite of floral traits important for pollinator attraction, including nectar quality and scent. However, studies on the direct and indirect effects of nectar-inhabiting microorganisms on pollinator behavior and plant reproductive success remain rare. To determine their potential to affect pollinator behavior and plant fitness, we experimentally manipulated the common nectar-inhabiting yeast *Metschnikowia reukaufii* in the nectar of *Delphinium nuttallianum*, a short-lived montane perennial herb. We detected positive, indirect, pollinator-mediated effects of yeasts on male plant fitness measured as pollen donation using powdered fluorescent dyes. However, we detected no direct or indirect effects on components of female fitness. Matching effects on male plant fitness, pollinators responded positively to the presence of yeasts, removing more nectar from flowers treated with *M. reukaufii*. Our results provide evidence of effects of nectar-inhabiting yeasts on male plant fitness and highlight the importance of microorganisms in mediating plant–pollinator interactions and subsequent plant fitness.

Key words; Bombus; Delphinium nuttallianum; *female fitness; fluorescent dyes; male fitness;* Metschnikowia reukaufii; *nectar remaining; nectar yeasts; pollen donation.*

Introduction

Plants participate in myriad interactions with antagonistic and mutualistic species. Such interactions, often mediated by traits of the shared host plant, precipitate plastic trait responses that can alter the nature of subsequent plant–animal interactions (Strauss and Irwin 2004). For example, the ability of plants to successfully attract pollinators can be affected by changes in floral attractive traits (e.g., floral display size, nectar availability, or scent) due to the presence and activity of antagonists, such as herbivores (Strauss et al. 1996), florivores (McCall and Irwin 2006), and nectar-robbers (Irwin et al. 2010). Floral nectar is a resource important for mediating interactions between plants and pollinators. Comprised primarily of sugars and amino acids (Baker and Baker 1973), nectar can attract a suite of non-pollinating floral visitors who exploit nectar for their own benefit at the potential expense of the plant and competing legitimate flower visitors (Adler and Bronstein 2004, Herrera et al. 2008, Irwin et al. 2010).

Considering its nutritional value, it is not surprising that nectar is prone to colonization by microorganisms such as yeasts and bacteria. Recent surveys demonstrate that nectar-inhabiting microorganisms are ubiquitous among plant species (Brysch-Herzberg 2004, Herrera et al. 2008, Álvarez-Pérez et al. 2012, Fridman et al. 2012). Nectar-inhabiting yeasts (NIYs) have the potential to modify a variety of nectar traits important for pollinator attraction and visitation, including nectar quality and scent (Raguso 2004, Herrera et al. 2008, Peay et al. 2012), through their presence and metabolism of nectar chemical components. Such modifications of nectar may have important consequences for plant fitness mediated through changes in pollinator behavior. NIYs are capable of decreasing nectar sugar concentrations and altering sugar ratios (Herrera et al. 2008), altering amino acid content and concentrations (Kevan et al. 1988, Peay et al. 2012), and increasing floral temperature (Herrera and Pozo 2010), all traits that can influence foraging decisions by pollinators (Waller 1972, Alm et al. 1990, Dyer et al. 2006). Moreover, ethanol production through fermentation of sugars can alter the liquid and volatile chemical profile of nectar, which could affect the attractiveness of flowers through changes in nectar taste or scent (Ehlers and Olesen 1997, Raguso 2004, Wiens et al. 2008). Finally, NIYs may also have direct effects on plant health or reproduction through a variety of mechanisms. For instance, consumption of resources (e.g., carbohydrates) may stress the host plant if the cost of replenishing depleted resources is high. Alternatively, NIYs have the potential to directly interfere with floral reproductive processes, as demonstrated in *Asclepias* sp. where NIYs disrupt pollen tube growth and fertilization of ovules (Eisikowitch et al. 1990*a*, *b*). Despite these hypothesized mechanisms, we

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[3] Present address: Department of Biology, Tufts University, 163 Packard Avenue, Medford, Massachusetts 02155 USA. E-mail: Robert.Schaeffer@Tufts.edu

know surprisingly little about how NIYs and their metabolism of nectar directly or indirectly affect plant–pollinator interactions, pollen flow, and plant reproduction (but see Herrera et al. 2013, Vannette et al. 2013).

We experimentally tested the degree to which the cosmopolitan NIY *Metschnikowia reukaufii* affected pollinator behavior and components of reproduction in the montane larkspur *Delphinium nuttallianum* (Ranunculaceae). *Delphinium* nectar regularly harbors yeasts, especially *M. reukaufii* (see *Methods: Study system*). *Delphinium* relies on a community of bee and bird pollinators to transfer pollen and facilitate subsequent plant reproduction, and previous research has shown that the foraging behavior of this pollinator community is sensitive to variation in at least one nectar trait, volume (Zimmerman 1983). Changes in other measures of nectar reward quality as a function of NIY metabolism may similarly affect pollinator foraging and subsequent *Delphinium* fitness. To test this hypothesis, we manipulated *M. reukaufii* densities to determine the effects of this NIY on pollinator foraging and male and female components of plant fitness.

Methods

Study system

We conducted this study using the montane larkspur *Delphinium nuttallianum* (hereafter *Delphinium*), near the Rocky Mountain Biological Laboratory (RMBL), in the Elk Mountains of Colorado, USA. Studies were carried out in 2010–2013 in four populations of *Delphinium*: Brush Creek (BC, 38°53′45.99″ N, 106°53′19.91″ W), Deer Creek (DC, 38°56′48.34″ N, 106°58′46.86″ W), Kettle Ponds (KP, 38°56′45.28″ N, 106°58′33.57″ W), and Spring Creek (SC, 38°51′53.36″ N, 106°39′49.22″ W). Around the RMBL, *Delphinium* is a short-lived perennial that flowers from late May to early July. The nectar-spurred, purple flowers of *Delphinium* are protandrous, zygomorphic, and last approximately 6 days, each producing 0.5–1.5 μL of nectar over 24 hours with 51.2% ± 0.7% (mean ± SE) sugar concentration (Waser 1978). Plants typically produce a single raceme with 1–15 flower buds that mature in a bottom-up fashion. Although the flowers of *Delphinium* are weakly self-compatible (Price and Waser 1979), protandry prevents autogamous self-pollination and very few seeds are produced in the absence of pollinator visitation (Waser 1978). Thus, pollinators are required to transfer even self-pollen within flowers and plants (Waser and Price 1991), and seed set is often limited by pollinator visitation (Waser and Price 1991), with greater pollinator visitation increasing stigma pollen loads and seed set as a saturating function (Bosch and Waser 1999). The most common flower visitors to *Delphinium* around the RMBL are queen bumble bees, *Bombus appositus*, *B. balteatus*, *B. californicus*, *B. flavifrons*, *B. frigidus*, and *B. nevadensis* (Apidae) and one hummingbird, *Selasphorous platycercus* (Trochilidae; Waser 1978).

Nectar surveys completed around the RMBL indicate that *Delphinium* flowers are prone to colonization by nectar yeasts, with inoculation likely occurring through dispersal of yeasts by flower-visiting insects (Brysch-Herzberg 2004, Herrera et al. 2010). Flowers bagged from insect visitors remain yeast-free, whereas flowers left open to floral visitors become inoculated with yeasts (R. N. Schaeffer and R. E. Irwin, *unpublished data*). NIYs occurred in 60% and 74% of nectar samples from individual *Delphinium* flowers spanning all ages inspected in 2009 ($N = 55$ flowers) and 2010 ($N = 80$ flowers), respectively. Yeast densities reach an average (± SE) of $3.1 \times 10^4 \pm 6.6 \times 10^3$ cells/mm^3 (range = 0–1.5×10^5 cells/mm^3), with the most frequently observed species being *Metschnikowia reukaufii* (R. N. Schaeffer and R. E. Irwin, *unpublished data*), a cosmopolitan, ascomycetous yeast of the Metschnikowia clade commonly associated with floral nectar and pollinators (Lachance et al. 2001, Herrera et al. 2008, 2009, Belisle et al. 2012).

Nectar yeast manipulations

To determine the effects of NIYs on pollinator foraging and reproduction of *Delphinium*, we randomly assigned plants to yeast-inoculation or control treatments. In the yeast-inoculation treatment, we manipulated yeast density by adding 2 μL of *M. reukaufii*-inoculated 50% (on a mass basis) sucrose solution (initial density 1×10^4 cells/mm^3, incubated for five days) to open flowers using a 25-μL Hamilton syringe (Hamilton Company, Reno, Nevada, USA). Control plants received sterilized 50% (on a mass basis) sucrose solution of the same volume, using a separate syringe to prevent cross-contamination. We wiped the syringe tips clean of pollen between plants using 70% ethanol to avoid any accidental pollen transfer. Addition of 2 μL of nectar falls within the range of lifetime floral nectar production and so can be experienced by foragers naturally, as there is no resorption of nectar by nectaries (Waser and Mitchell 1990). Yeast-inoculated solutions were incubated for five days because this time period falls within an average flower's time span. Cells of an isolated *M. reukaufii* strain local to the RMBL were obtained from cultures maintained on YM agar supplemented with the antibiotic chloramphenicol to inhibit bacterial growth on the plates. Upon visitation by pollinators, control plants were no longer free of yeasts as pollinators can serve as vectors for yeast dispersal (Brysch-Herzberg 2004, Herrera et al. 2010). Thus, application of these solutions reflected an augmentation and dilution of yeast cells, respectively, not unlike studies that have manipulated secondary compounds in floral nectar through augmentation or dilution of secondary compound concentrations (e.g., Adler and Irwin 2005). Treatments were applied at the whole-plant level in the morning (07:00–09:00) before peak pollinator activity.

To confirm that the addition of control and yeast-inoculated solutions resulted in lower and higher yeast

densities respectively, we sampled the standing crop of nectar from control and yeast-inoculated flowers that were open to natural pollinator visitation and assessed densities through cell counts approximately six hours after treatment additions. Cell density of flowers treated with yeasts ($1.7 \times 10^4 \pm 3.7 \times 10^3$ cells/mm^3) was significantly higher than cell density of controls ($4.7 \times 10^3 \pm 4.5 \times 10^3$ cells/mm^3; $t_{55} = 2.18$, $P = 0.04$), confirming that we could successfully manipulate yeast densities and that treatments persisted throughout much of the pollinator foraging activity period during a day.

Effects of nectar yeasts on male and female components of plant reproduction

Male reproduction.—We estimated male plant fitness in 2010 and 2011 by measuring pollen donation using powdered fluorescent dyes as pollen analogues (JST-300, Radiant Color, Richmond, California, USA). We chose pollen (dye) donation as our surrogate for male fitness for three reasons. First, powdered fluorescent dyes successfully serve as pollen analogues in *Delphinium* and both transfer distance and amount of material transferred closely resembles that of natural *Delphinium* pollen (Price and Waser 1979, Waser 1988). Second, pollen dispersal in *Delphinium* is a function of pollinator foraging behavior. Thus, estimating pollen (dye) dispersal allows us to assess indirect effects of NIYs on pollinator foraging behavior and the implications of such effects for mating patterns. Third, a number of studies on a variety of flowering species have successfully used powdered fluorescent dyes to compare estimates of male reproductive success measured as pollen (dye) donation among flower or nectar treatments and to estimate pollen transfer dynamics (Rademaker et al. 1997, Irwin and Brody 1999, Adler and Irwin 2005), and the effects of flower treatments on pollen (dye) donation and seeds sired have shown similar patterns in other systems (i.e., compare Irwin and Brody 1999, 2000). Thus, powdered fluorescent dyes have proven as a reliable, economic means for estimating male plant reproduction in natural plant populations (e.g., Campbell 1989, Rademaker and De Jong 1998). Measuring the effects of nectar yeasts on seeds sired was beyond the scope of this study but can be assessed in future research. In the SC, DC, and KP populations, we chose *Delphinium* individuals of similar height and floral display size to serve as donor plants. Individuals were randomly assigned to one of the two treatments, control or yeast, and treated at the whole-plant level.

In the first experiment in 2010 at SC, we used 20 plants per treatment with individuals at least 3 m apart. To estimate pollen (dye) donation, treatments were each randomly assigned one of two dye colors (blue and pink), with dye particles applied to dehiscing anthers of all male-phase flowers using flat-head toothpicks. Nectar treatments were applied in the morning (07:00–09:00) followed by application of the dyes. Forty-eight hours after dye application, we collected stigmas from all female-phase *Delphinium* flowers within a 1 m radius of donor plants to score dye donation. We measured dye donation within a 1 m radius because approximately 90% of pollen is transferred within this radius (Waser 1988). We counted dye particles of each color on stigmas under a dissecting microscope (Nikon SMZ1000, Melville, New York, USA). We standardized dye donation by dividing the number of particles on each stigma by the number of flowers dyed in each treatment to control for differences in the number of male-phase flowers dyed on donor plants ([mean number of flowers dyed]/[donor plant] = 5.2 flowers; range is 2–10 flowers). For each donor plant, we calculated the mean number of dye particles donated per stigma per flower dyed (hereafter referred to as dye donation). In this experiment, we applied dye particles twice during the flowering season, with one week between dye applications (hereafter referred to as "rounds"). Individual *Delphinium* flowers are typically only open for up to six days; thus, having at least one week between dye applications ensured that the dye from the first application was no longer in the population. The yeast treatment-by-color combination was reversed in the second round to control for potential color bias in pollinator visitation and dye donation.

In 2011, we repeated the experiment at SC, in addition to two other *Delphinium* populations, DC and KP. In each population, we randomly assigned 15 donor plants to each treatment. We performed dye applications as described, except treatments were only applied for a single round.

Female reproduction.—We first examined the direct effects of *M. reukaufii* on female plant reproduction in the absence of potential changes in pollinator behavior. At DC in 2010, we chose 60 plants with all flowers in bud. Plants were bagged with bridal veil throughout the entire experiment to exclude all flower visitors who could potentially vector yeasts. We randomly assigned 30 plants each to control and yeast treatments. We added treatment solutions daily to all open flowers throughout blooming. On all plants, flowers in female phase were hand pollinated with flat-head toothpicks every other day using a mixture of pollen from at least three haphazardly chosen donor plants growing at least 10 m away. We monitored plants for fruit abortion, collected expanded fruits in late July, and counted seeds per fruit. We calculated three measures of female reproduction in each plant: proportion fruit set (expanded fruits/[expanded + aborted fruits]), mean seeds per expanded fruit, and total seeds per plant.

We tested for combined direct and pollinator-mediated indirect effects of *M. reukaufii* on components of female plant reproduction with the following experiment. In 2010, in the same population (DC), we randomly assigned 30 plants each to control and yeast treatments. Nectar treatments were added daily over the course of the flowering season and flowers were left available for natural pollinator visitation. Upon floral senescence, we collected stigmas from three haphazardly

chosen flowers per plant. Collecting stigmas at this stage does not affect fruit or seed set (Waser and Price 1991, Bosch and Waser 1999). Stigmas were stained with basic fuchsin dye (Kearns and Inouye 1993), and we counted the number of conspecific pollen grains received per stigma under a compound microscope (Nikon Eclipse E400). We then monitored plants for fruit abortion, collected expanded fruits in late July, and counted seeds per fruit. We calculated four measures of female reproduction per plant: mean number of pollen grains received per stigma per plant, proportion fruit set, mean seeds per fruit, and total seeds per plant.

Effects of nectar yeasts on pollinator foraging

Given that we found effects of the yeast-inoculation treatment on some components of plant reproduction (see *Results*), we then tested the degree to which yeast inoculation affected pollinator foraging in 2013. To estimate pollinator foraging, we measured the amount of nectar remaining in treated flowers following exposure to pollinator visitation. Less nectar remaining can signify increased nectar removal from flowers by pollinators. Increased nectar removal can be a function of the number of times a flower is visited, as well as time spent per flower (Thomson and Plowright 1980, Mitchell and Waser 1992), both components of pollinator foraging behavior. During peak bloom, we tested for effects of yeasts on artificial nectar remaining on 6–9 June. Each day, we haphazardly chose 50 plants at BC, with one-half assigned to the control and one-half assigned to the yeast-inoculated treatments. On each plant, the bottom three flowers were treated with 3 μL of solution between 07:00 and 09:00, before peak pollinator activity. As pollinators tend to forage on *Delphinium* in a bottom-up fashion, this approach ensured that the flowers treated and examined had a high probability of being visited. The nectar standing crop of flowers was negligible, often $\ll 1$ μL of nectar, before treatment application. Plants were left exposed to pollinator activity for six hours, after which all control and yeast-inoculated flowers were collected. We measured nectar remaining in treated flowers using calibrated microcapillary tubes and calculated the mean volume of nectar remaining across treated flowers for each plant.

Statistical analyses

All analyses were performed using R version 2.15.3 (R Core Development Team 2013). We tested for effects of NIYs on male fitness estimated through dye donation using linear mixed effects models. For both analyses (2010 and 2011), nectar treatment was a fixed effect, while round and site were treated as random effects for each year respectively. Analyses were performed using the nlme package (Pinheiro et al. 2013). We arcsine-square-root transformed dye donation (mean dye particles donated·stigma^{-1}·[number of flowers dyed]$^{-1}$) to improve normality of residuals.

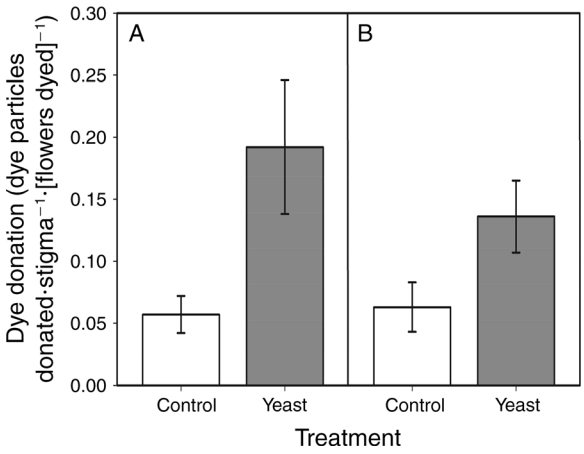

FIG. 1. Dye donation of *Delphinium* plants treated with control (white bar) or yeast (gray bar) sucrose solutions in (A) 2010 and (B) 2011. Values are mean ± SE.

Direct effects of yeast-treatment on estimates of female reproduction were tested using a one-way MANOVA with proportion fruit set, mean seeds per fruit, and total seeds per plant as response variables. To test for combined direct and indirect effects of yeast treatment on estimates of female reproduction in naturally pollinated plants, we performed a one-way MANOVA with proportion fruit set, mean seeds per fruit, and total seeds per plant as response variables. For the first analysis, mean seeds per fruit and total seeds per plant were $\log(x+1)$-transformed to improve normality. We analyzed pollen receipt separately using a two-sample t test, as we were unable to obtain pollen receipt measures for all plants for which we had fruit and seed estimates ($N = 48$ plants).

To examine effects of NIYs on nectar remaining, we performed a nested ANOVA with nectar treatment nested within treatment date.

RESULTS

Effects of nectar yeasts on male and female components of plant reproduction

Male reproduction.—Opposite to our initial prediction, NIYs enhanced pollen (dye) donation (Fig. 1). In 2010 at SC, yeast-treated plants donated approximately three times more dye to neighboring plants in comparison to controls ($t_{37} = 3.79$, $P = 0.0005$). We detected a similar pattern in 2011, as yeast-treated plants again had enhanced dye donation to neighboring plants relative to the control treatment ($t_{70} = 2.60$, $P = 0.011$), with yeast-treated plants donating two times more dye to neighboring plants than controls.

Female reproduction.—We found no evidence that the yeast inoculation directly or indirectly affected female reproduction. We detected no significant difference between control and yeast-treated plants for female plant reproduction when flowers were hand-pollinated (Appendix: Table A1, Wilks' lambda = 0.96, $P = 0.57$).

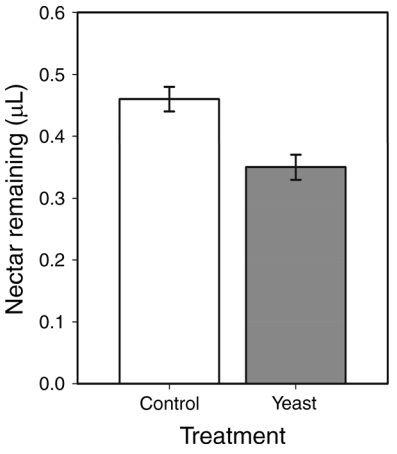

FIG. 2. Nectar remaining (mean ± SE) in control (white bar) and yeast (gray bar) treated *Delphinium* flowers.

Similarly, we found no evidence for combined direct and indirect effects of NIYs on components of female plant reproduction, measured through pollen receipt ($t_{47} = 0.79$, $P = 0.43$) or fruit and seed production (Appendix: Table A2, Wilks' lambda = 0.97, $P = 0.65$).

Nectar-inhabiting yeasts affect pollinator foraging

Pollinator foraging responded positively to the presence of NIYs. We found that 27% less nectar remained in flowers treated with yeasts in comparison to controls (Fig. 2: $F_{1, 182} = 13.04$, $P = 0.0004$). Nectar remaining also varied significantly with date ($F_{6, 182} = 10.97$, $P < 0.0001$).

DISCUSSION

Yeasts frequently colonize floral nectar, making interactions between them and plants and pollinators potentially common. Conventional wisdom has suggested that yeasts should be detrimental to plant fitness, as reductions in sugar concentrations through yeast metabolism should deter pollinators (Herrera et al. 2008). Our findings are contrary to this notion, supporting instead recent findings suggesting pollinator preference for NIYs (Herrera et al. 2013). We detected positive effects of the nectar-inhabiting yeast *M. reukaufii* on pollen (dye) donation, a component of male plant reproduction, and on nectar remaining in flowers, which is a function of pollinator foraging. Moreover, our findings highlight the context-dependent nature of the effects of species interactions on male vs. female components of plant reproduction, as male and female estimates of fitness responded differently to the presence of NIYs. These results add to the increasing evidence that nectar-inhabiting microorganisms are capable of affecting plant–pollinator mutualisms.

To our knowledge, only a handful of studies to date have examined the effects of NIYs on components of plant fitness. Eisikowitch et al. (1990*a*, *b*) found direct inhibitory effects of *M. reukaufii* on pollen germination and tube growth in *Asclepias syriaca*. In *A. syriaca*, the nectaries are located in the stigmatic chamber, and the presence of NIYs negatively impacted the maternal environment, inhibiting pollen germination and tube growth, with drastic consequences for ovule fertilization success. Similarly, Herrera et al. (2013) found negative effects of NIYs on multiple estimates of female fitness of the bumble-bee-pollinated plant *Helleborus foetidus*, observing reduced measures of pollen tube number, fruit set, seed set, and seed mass. However, Vannette et al. (2013) found no detrimental effects of *M. reukaufii* on estimates of female fitness in the hummingbird-pollinated plant *Mimulus aurantiacus*. Our study highlights the importance of considering both female and male estimates of plant reproduction, as they may respond differently to the same species interaction (Strauss et al. 1996, Schaeffer et al. 2013). We likely failed to detect effects of NIYs on female fitness for two reasons. First, *Delphinium* nectar, located in spurs, is separated spatially from the stigma where pollen is received and germinates. Direct effects on female fitness may be unique to flowers like *Asclepias* spp. where the nectaries are located in the stigmatic chamber and nectar is required for successful pollen germination (Kevan et al. 1989, Eisikowitch et al. 1990*a*, *b*). Second, female reproduction in our study was likely not limited by pollen receipt, as pollen receipt was high in both nectar treatments (Appendix). Based on the studies that have manipulated NIYs thus far, the effects of NIYs on plant fitness may be a function of flower morphology, plant mating system, component of reproduction measured, how pollinators respond to NIYs, and both the extent and nature of potential pollen limitation experienced by species (Herrera et al. 2013).

Though we observed positive effects of *M. reukaufii* on nectar remaining and male plant fitness, the proximate cues and mechanisms driving these patterns remain unknown. We provide three potential non-exclusive mechanisms, although others may also be plausible. First, yeasts may play an important role in honest signaling of nectar presence through the production of volatiles during fermentation (Raguso 2004, Goodrich et al. 2006, Wiens et al. 2008). Second, changes in amino acid, vitamin, or other metabolite availability as a consequence of yeast metabolism may be driving changes in pollinator foraging decisions (Herrera et al. 2013). Third, yeast metabolism may affect pollinator foraging behavior by increasing flower and nectary temperature (Herrera and Pozo 2010). *Delphinium* initiates flowering after snowmelt and serves as an important resource for queen bumble bees and hummingbirds that establish territories at the start of the breeding season. Such temperature increases, albeit small on average, may serve as an additional metabolic reward in addition to nectar (Seymour et al. 2003, Dyer et al. 2006). Careful dissection of the effects of NIYs on nectar traits and their relative role in driving observed

patterns of plant–pollinator interactions warrant further investigation.

At least three caveats are important to the interpretation of our study. First, we only manipulated the cosmopolitan yeast *M. reukaufii*; however, other yeast and bacteria species can occur in floral nectar both individually and simultaneously (Herrera et al. 2010, Álvarez-Pérez et al. 2012, Fridman et al. 2012, Peay et al. 2012). Thus, there may be variable outcomes depending on microbe identity, as well as potential non-additive effects of multiple microbes (Herrera et al. 2013, Vannette et al. 2013). Second, our use of pollen (dye) donation as a measure of male fitness may not capture the full scope of effects of NIYs on male reproductive success in *Delphinium*. Male plant fitness is susceptible to gain or loss during both pre- and post-pollination events (Schaeffer et al. 2013). However, this study provides exciting evidence suggesting the effects of NIYs on components of male plant fitness, and measuring seeds sired is warranted in future research. Finally, we presented results on the effects of yeast on pollen (dye) donation within 1 m of donor plants; however, these donation events fall short of the optimal out-crossing distance (10 m) where seed set and survivorship is higher in *Delphinium* (Price and Waser 1979, Waser and Price 1991, 1994). Although we attempted to measure pollen dispersal at greater distances from donor plants (up to 10 m away), we observed too few dye particle movements from control or yeast-inoculated plants for analysis (data not shown). Thus, our pollen donation distances match work demonstrating the short spatial scale over which most pollen flow occurs in this system (Price and Waser 1979).

In conclusion, we found that NIYs are capable of affecting both pollinator foraging and subsequent estimates of plant fitness, though effects on fitness may be dependent upon the component measured. Our results add to the growing call for consideration of microorganisms as potentially important drivers of plant–pollinator interactions, with consequences for mutualism strength and outcomes that may be context-dependent in nature. Finally, as consumers of NIYs and NIY-modified nectar, future research should consider the role of NIYs not only on plant fitness but also on pollinator nutrition and their potential effects on pollinator fitness.

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Supplemental Material

Appendix

Summary of results on the direct and indirect effects of yeasts on *Delphinium* female fitness (*Ecological Archives* E095-158-A1).