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VARIABILITY IN NECTAR PRODUCTION AND YIELD, AND THEIR RELATION TO POLLINATOR VISITS, IN A MEDITERRANEAN SHRUB

by

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Variability in nectar production and yield, and their relation to pollinator

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ABSTRACT

Nectar yields (standing crops) in flowers within an individual plant are often highly variable. This variability may be a by-product of the foraging activity of insect pollinators. Alternatively, plants may be selected to produce highly variable rewards to reduce consecutive visitation by risk-averse pollinators, thus diminishing within-plant pollen transfer. This study evaluated the roles of pollinator control vs. plant control over nectar variability in the bee-pollinated shrub *Rosmarinus officinalis* L. We sampled nectar production, yield and pollinator visits in three shrubs of one population over 17 days during one blooming season. Nectar production rates were highly variable (CV=1.48), and increased after rainy days. Nectar yields were even more variable (CV=2.16), and decreased with increasing temperatures. Pollinator visit rates decreased with variability in nectar yields, increased with flower number per shrub, and were unaffected by variability in nectar production rates. Repeated sampling of marked flowers revealed no correlation between their nectar yields and production rates. These findings support the role of reward variance in reducing pollinator visits, but suggest that plants are not in complete control of this variability. Rather, plant-generated variability can be modified by intensive foraging activity of pollinators. Such pollinator control over nectar variability is likely to reduce the selective advantage of plant-generated reward variation. Plant-controlled variability may provide evolutionary advantage when pollinator activity is insufficient to generate reward variation.

INTRODUCTION

Insect-pollinated plants that are visited by a large number of pollinator individuals gain reproductive benefits, due to increased import and export of pollen. Repeated visits of the same pollinator to a plant, on the other hand, increase within-plant pollen transfer (geitonogamy), which is genetically equivalent to self-pollination. Self-incompatibility mechanisms prevent geitonogamous fertilization in some cases, and lead to lower-quality offspring in others, thereby decreasing female fitness. Geitonogamy can also decrease male fitness because of reduced export of pollen to other plants. It has been therefore suggested that plant traits that reduce geitonogamous pollination would be selectively advantageous (de Jong et al. 1993). When pollinators are abundant, the optimal situation for plants would be to receive visits from a large number of pollinator individuals, but have each of them visit only a small number of flowers in succession (Klinkhamer et al. 1994).

Pollinators often visit only a small fraction of the flowers available to them, before shifting to a different individual (Klinkhamer and de Jong 1990; Ohashi and Yahara 2001). Encounters with low or zero rewards have been shown to promote patch shifts by bees in laboratory situations, and in natural settings (Kadmon and Shmida 1992; Keasar et al. 2002). Furthermore, bees often forage in a risk averse-manner, i.e. prefer food sources with low variance over high-variance sources with equal mean rewards (Kacelnik and Bateson 1996; Shafir 2000). These observations underlie the hypothesis that within-plant variation in nectar production rates may reduce geitonogamy levels, so that such variation is favored by selection (Rathcke 1992; Biernaskie et al. 2002; Pappers et al. 1999). This hypothesis predicts that variable within-plant nectar production rates would generate variability in nectar

yields (standing crops). This variability, in turn, would reduce visitation by insect pollinators.

Considerable within-plant variability in nectar production rates was measured in a number of species (Feinsinger 1983; Zimmerman and Pyke 1986; Herrera and Soriguer 1983; Marden 1984; Boose 1997). Nectar yields within plants are variable as well, and show a patchy spatial distribution (Shmida and Kadmon 1991). Nectar yields are affected both by the plant's rate of nectar production, and by nectar consumption by pollinators. Bees forage for nectar in an area-restricted manner, i.e. fly shorter distances after visiting nectar-rich flowers, and longer distances after visiting flowers with low rewards (Keasar et al. 1996; Burns and Thomson 2005). This movement pattern leads to patchy exploitation of the nectar, and generates patchiness in nectar yields even in the absence of variability in nectar production (Motro and Shmida 1995). It is not straightforward, therefore, to deduce variability in nectar production from measuring variability in nectar yields. The existence of such variability may be due to nectar production by the plant, to pollinator activity, or to a combination of both factors. Combined measurements of nectar production, nectar yields, and pollinator activity are needed to understand whether variability in nectar secretion by plants can play a role in restricting pollinator visitation and geitonogamy.

In the present study we assessed nectar production, yield and insect visits in *Rosmarinus officinalis* (Lamiaceae). This Mediterranean bee-pollinated shrub simultaneously produces several hundred protandrous, self-compatible flowers, and suffers inbreeding depression due to geitonogamous pollination (Hidalgo and Ubera 2001). We recorded the number of open flowers per shrub on each observation day, because plant size may affect nectar production (Pleasants and Chaplin 1983) and pollinator visit rates (Brody and Mitchell 1997; Goulson et al. 1998). In addition, we

recorded rainfall prior to sampling sessions, and temperature during sampling, since these variables may affect nectar production (Wyatt et al. 1992; Carroll et al. 2001; Leiss and Klinkhamer 2005).

We asked the following questions regarding this model plant:

1.   What is the extent of within-shrub variability in nectar production and yield?

2.   Does within-plant variability in nectar production and yields affect pollinator visitation?

3.   How do plant size and weather conditions affect nectar production, yield and insect visits?

MATERIALS AND METHODS

We repeatedly measured nectar production and pollinator visits in three shrubs of *Rosmarinus officinalis* in Kibbutz Hatzor, Central Israel. Plants in our study populations were in bloom from October to March. Corolla length and width were 1.37±0.18 (SD) and 0.50±0.07 cm, respectively, and floral tube length was 1.04±0.25 cm (n=50). Nectar and insect visit data were collected between 8 am and 2 pm on 17 days during October-December 2002. On the start of each observation session (8 am), we recorded nectar content (yield) in 10 flowers per plant using 1-µl micropipettes. To characterize within-shrub patchiness, we haphazardly selected five flowers per plant, and their nearest neighbors, for this sampling. We bagged the sampled, depleted flowers with bridal-veil netting (Wyatt et al. 1992), and harvested them again after 3 h (11 am), 6 h (2 pm) or 24 h (8 am on the following morning). We sampled 10 flowers (3-4 per shrub) at each time point. The nectar that accumulated in the sampled flowers represents the plant's 3 h, 6 h or 24 h nectar production. We divided the produced nectar volume by the covering time to obtain nectar production rates. We determined

sugar concentration in nectar samples that exceeded 1/3 µl with a Bellingham-Stanley hand-held refractometer. This was done for samples taken for nectar yields, as well as for samples taken for 3-, 6- and 24 h nectar production. We noted the time of concentration measurements, since nectar concentrations may change during the day. We were not able to determine sugar concentrations in nectar volumes of less than 1/3 µl.

We counted the number of insect visits on a sample of 100 flowers in each shrub during a 10-minute observation period. Counts were performed during peak pollinator activity hours, between 10 am and 12 pm, on the same plants that were used for nectar measurements. We performed one count per shrub on each observation day, totaling 51 counts. We classified the pollinators into the following functional groups: honeybees, large bees (larger than honeybees), small bees (smaller than honeybees), flies, butterflies and beetles.

Data analysis

We used ANOVA to test for the effect of bagging duration on hourly nectar production rates. We then tested for the effects of sampling date, plant, and pair within plant on nectar production rates using a general linear model. Sampling date (17 possible values) and plant (3 possible values) were treated as fixed factors. Flower pair (5 possible values) was considered as a nested factor within a given plant and sampling date. Bagging duration was treated as a covariate, and the variance between flowers within a pair was treated as the residual error term. We tested for correlations in nectar production between pair members using Pearson's correlations.

Since nectar yield data were obtained in unbagged flowers, we only considered the effects of sampling date, plant and flower pair on nectar yields. As

with the nectar production analysis, we used a general linear model with date (17 possible values) and plant (3 possible values) defined as fixed factors, and flower pair (5 possible values) defined as a nested factor within date and plant.

We used stepwise forward regression models (entry criterion: 0.05, exclusion criterion: 0.1) to evaluate the contributions of the following environmental variables on nectar yield and production: recent rainfall (mm rain on the last rain event before observation), the number of days elapsed since this rain event, minimal and maximal temperatures during observation, and the number of flowers per shrub. We calculated the mean nectar yield and production rate, and their standard deviations, corresponding to each of the 51 observations (17 days×3 plants) insect visits. We then regressed the number of visits per observation against the means and SDs of nectar yields and production rates, in addition to flower number, rainfall and temperature parameters. We used a stepwise forward regression model as above.

Nectar production and yield data were normalized through logarithmic transformation prior to analysis. Visitor count data were square-root transformed. We used SPSS version 14.0 for statistical analyses.

RESULTS

Nectar production

Mean (±SD) total nectar volumes produced during 3, 6 and 24 hours of covering were 4.81±7.11 (CV=1.48), 5.34±7.55 (CV=1.41) and 12.55±17.45 (CV=1.39) μl, respectively. Accordingly, the volume produced per hour was highest for flowers covered for 3 hours (8 am-11 am), and lowest for the 24-hour covering period (8 am-8am) (Fig. 1). The effect of covering period on nectar production was highly significant ($F_{2, 335}=45.90$, $p<0.001$). Hourly nectar production rates varied

significantly among sampling dates, and among flower pairs, but not among plants. The interaction between sampling date and plant was significant, however. (GLM: $F_{16}=2.142$, p=0.010 for date; $F_2=0.280$, p=0.756 for plant; $F_{29}=2.098$, p=0.003 for date×plant interaction; $F_{156}=1.536$, p=0.005 for flower pair; n=273; covering duration treated as a covariate).

For the remaining analyses we used only the nectar production data that were obtained from flowers covered for three hours. We report mainly on the 3-hour covering period for two reasons: First, nectar production pattern observed in Fig. 1 may be due to higher production rates between 8-11 am than later in the day. Since peak pollinator activity occurred during these hours as well, these rates seem the most relevant for explaining pollinator visit rates. Second, the effect of covering time on hourly nectar production rates may reflect inhibitory effects of nectar accumulation within the nectaries on further production (Castellanos et al. 2002). The high rate of pollinator visits to *R. officinalis* (see below) probably prevented nectar accumulation in flowers of our study population. Hourly nectar production rates based on 6-hour or 24-hour netting may therefore underestimate production rates in flowers exposed to pollinators.

The hourly nectar production rate (based on 3-h measurements) was characterized by a high coefficient of variation (CV=1.48, n=170 flowers). Mean sugar concentration in the nectar was 70.6±8.5 (CV=0.12, n=65 flowers). Thus, the variability in nectar concentration was much lower than the variability in volumes. Nectar production rates were significantly correlated between flower-pair members within an individual (n=85 flower pairs, Pearson's correlation=0.39, P<0.001). They were significantly affected by the amount of the last rain before sampling (stepwise regression, $r^2=0.13$, p=0.014). The number of days elapsed since the rain, the

minimum and maximum temperatures on the day of sampling, and the number of open flowers per shrub did not significantly affect nectar production rates.

Nectar yields

Mean nectar yield was 0.32±0.69 (SD, n=510 flowers, CV=2.16) µl, thus nectar yields were highly variable. 56% of the sampled flowers contained no measurable nectar, while 1.4% of the samples contained more than 3 µl nectar, reflecting an extremely skewed distribution of yields. The variance in nectar yields was significantly higher than the variance in hourly nectar production rates (Levene's test, $F_{1, 676}$=83.408, p<0.001). Nectar yields were positively correlated between flower-pair members, similarly to nectar production rates (n=255 flower pairs, Pearson's correlation coefficient = 0.42, p<0.001). Nectar yields varied significantly among sampling dates, but not among plants or flower pairs (GLM: F_{16}=12.36, p<0.001 for date; F_2=0.864, p=0.424 for plant; F_{24}=1.56, p=0.074 for date×plant interaction; F_{104}=1.176, p=0.226 for flower pair; n=211). Nectar yields and hourly nectar production rates, measured in the same flowers, were not correlated (Pearson correlations coefficients: -0.05, p=0.49; -0.06, p=0.45; 0.07, p=0.34 for flowers covered for 3, 6 and 24 hours respectively, n=170 flowers for each covering period). Nectar yields decreased as maximal air temperatures increased (stepwise regression, r^2=0.64, p<0.001), but were not affected by the amount of rain, days since rainfall, and the number of flowers per shrub.

Pollinator visits

We observed 63.4±8.1 (mean±SE) flower visits by insects on 100 flowers during the 10-minute observation periods (n=51 observations). This is equivalent to

an average of 3.8 visits per flower per hour. We did not record the number of

pollinator approaches to the shrubs, and lengths of visit bouts within them. Honeybees

were the most abundant visitors (57.09% of all visits). Small bees accounted for

36.25% of the visits, and the remaining groups of pollinators visited the flowers at

low frequencies (Fig. 2). The number of insect visits significantly decreased with the

SD of nectar yields, and increased with increasing number of flowers per shrub

(stepwise regression: coefficient for yield variability -0.39, p=0.014; coefficient for

number of flowers 0.33, p=0.028; r^2=0.26, p=0.004 for the whole model, Figs. 3, 4).

Mean and SD of nectar production, mean nectar yields, rainfall and temperature did

not significantly affect pollinator visitation, and were excluded from the regression

model.

DISCUSSION

Our study combines data on variability in nectar production and yields, and

their relations with pollinator visits, in a single set of field observations. Our

observations of *R. officinalis* demonstrate high within-plant variation in nectar

production rates. Within-plant variability in nectar yields was even higher, as

indicated by a larger coefficient of variation. Pollinator visitation was negatively

affected by the variation in nectar yield, but was not related to the variability in nectar

production. These findings suggest that the plants' nectar production patterns were not

the only source for variability in our study. Rather, the foraging activity of pollinators

increased the plant-generated variability in nectar yields. Such increased variability, in

turn, reduced further pollinator visits.

Under this scenario, can variability in nectar production be considered an

adaptive plant strategy to reduce geitonogamy? If pollinators mainly react to nectar

variability created by their own foraging activity, then plant-generated variability in nectar production should not be favored by selection. Such variability would then merely reflect between-flower differences in sexual phase (Carlson and Harms 2006), flower age (Pleasants 1983; Zimmerman and Pyke 1986) or microhabitat conditions. For example, since blooming in *R. officinalis* advances along the inflorescence from bottom to top, neighboring flowers that were sampled at different heights along the inflorescence may differ in age and sexual phase, and thus also in nectar production rates. Alternatively, plant-generated variability in nectar production may be selectively beneficial at lower pollinator densities than observed at our study site. If insect activity is insufficient to generate variability in nectar yields, then plant-generated variability would affect pollinator activity more strongly. It is important to note, though, that the selective advantage of avoiding geitonogamy may decrease at low pollinator densities. This is because plant fitness may be higher with some degree of selfing (due to geitonogamy) than with no pollination at all (due to lack of insect visits). This may, in turn, reduce the selective advantage of plant-generated nectar variability when pollinators are very rare.

Neighboring flowers within a plant were positively correlated in nectar production rates as well as in nectar yields, but we found no correlation between production rate and yield within a single flower. In other words, the flowers that had the highest production rates when bagged did not necessarily contain the highest yields when exposed to pollinators. A possible interpretation of this finding is that nectar production rate in each flower is variable over its lifetime, and that measurement of production and yield at different points in time reflects this variability. An alternative interpretation is that the variability in yield is partly generated by pollinator activity, while the variability in production is only due to the

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plant. Repeated measurements of nectar production along the blooming duration of individual flowers are needed to distinguish between these interpretations.

Our study corroborates previous findings of environmental effects on floral nectar traits (Wyatt et al. 1992; Carroll et al. 2001; Leiss and Klinkhamer 2005). Interestingly, nectar production rates and yields were affected by different environmental parameters: rainfall was the most important predictor of nectar production rates, while temperature best predicted nectar yields. Increased temperatures may have allowed higher pollinator activity, leading to increased nectar consumption and reduced yields. A second possible effect of elevated temperature is increased nectar evaporation from flowers, leading to reduced yields. We consider this explanation more likely, since pollinator visit rates were not directly affected by temperatures. Contrary to some previous work (Pleasants and Chaplin 1983), nectar parameters were not affected by the number of blooming flowers in our study system. Flower number significantly influenced pollinator attraction, on the other hand, as previously observed for other plant species (Robertson and McNair 1995; Goulson et al. 1998).

Our pollinator sampling protocol did not include separate recording of the number of insect approaches to each shrub, and the number of consecutive visits (bout length) after each approach. We therefore cannot determine whether nectar yield variability diminished the number of insect approaches, their bout lengths, or both. A reduction in bout lengths is expected to serve the interests of the plants by reducing geitonogamy. A reduction in pollinator approach frequency, on the other hand, is expected to lower plant fitness by reducing pollen import and export. Thus, the implications of reward variability for *R. officinalis*' fitness are still unclear. Detailed

observations of approach frequency, bout lengths and seed sets, at different levels of reward variance, are needed to address this question.

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FIGURE LEGENDS

Fig. 1: Mean (+SE) hourly nectar production in flowers that were covered to exclude insect visits for 3, 6 or 24 hours. Data were collected from 170 flowers for each covering period.

Fig. 2: Mean (+SE) numbers of visits by various groups of pollinators to 100 *R. officinalis* flowers. Data are based on 51 observation periods of 10 minutes.

Fig. 3: The effect of the number of open flowers per shrub on the number of pollinator visits in a 100-flower sample during a ten-minute observation period. Data were log-transformed to linearize the exponential function between flower number and visit number. The slope of the linear regression line corresponds to the exponent (de Jong & Klinkhamer, 2005). N=51 observations, Y=0.663x+36.17, r^2=0.09.

Fig. 4: The effect of the within-shrub nectar yield variability, expressed as the standard deviation of the mean reward volume, on the number of pollinator visits. Visits were recorded in a 100-flower sample during ten-minute observation periods. N=51 observations, Y=-3.98x+72.48, r^2=0.08.
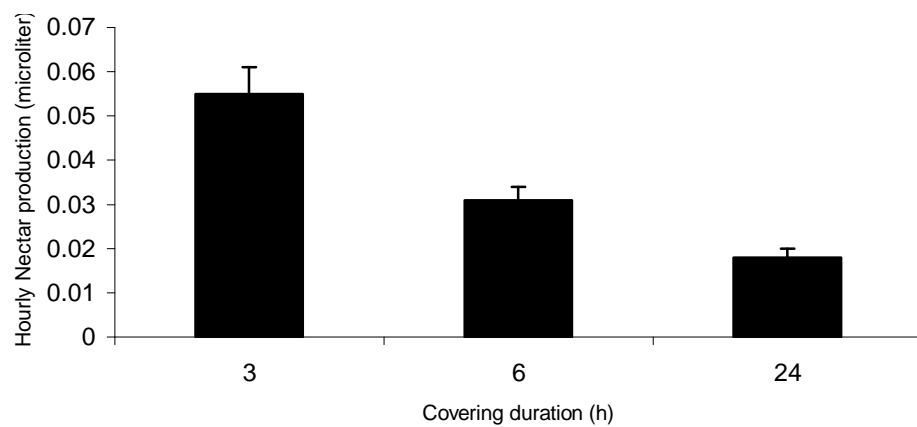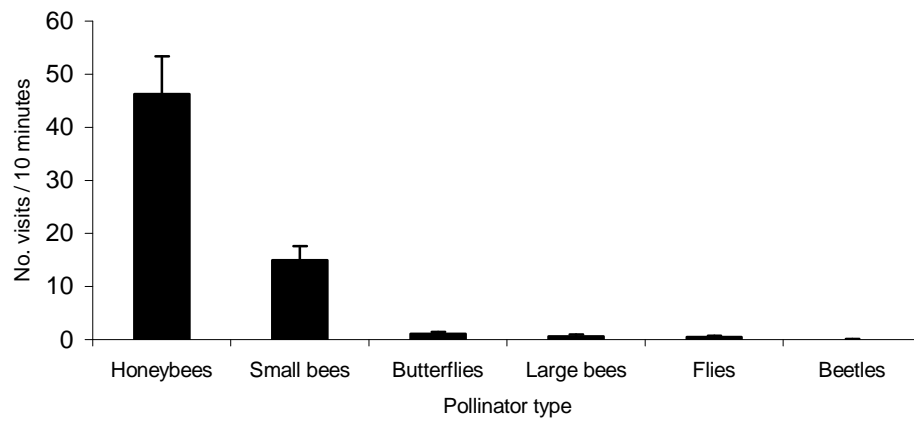
Fig. 1

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