Surplus Nectar Available for Subalpine Bumble Bee Colony Growth

SUSAN E. ELLIOTT[1,2]

ABSTRACT Mutualisms may cause coupled population expansion or decline if both partners respond to variation in the other's abundance. Many studies have shown how the abundance of animal mutualists affects plant reproduction, but less is known about how the abundance of plant mutualists affects animal reproduction. Over 2 yr, I compared reproduction of the bumble bee, *Bombus appositus*, across meadows that varied naturally in flower density, and I compared reproduction between fed colonies and unfed control colonies. Colony reproduction (gyne, worker, and male production) was constant across meadows that varied naturally in flower density. Forager densities per flower did not vary among meadows, and daily nectar depletion was consistently low across meadows, suggesting that bees had ample nectar in all meadows. However, colonies directly fed with supplemental nectar and pollen generally produced over twice as many gynes as control colonies. Feeding did not affect male or worker production. Although colonies may benefit from food supplementation at the nest, it is possible that they may not benefit from additional flowers because they have too few workers to collect extra resources.

KEY WORDS *Bombus appositus*, bumble bee, *Delphinium barbeyi*, food limitation, flower density

Mutualisms are often considered from a unilateral perspective, emphasizing the more sessile or larger partner, whose fitness is often easier to measure (e.g., the plant in a plant-pollinator mutualism: Cushman and Beattie 1991). Knowing how a visitor affects a host mutualist can help explain the ecology and evolution of the host, but if the visitor responds to changes in its partner species (e.g., by an increase or decrease in abundance), this could alter the nature (and our interpretation) of their relationship (Bronstein 1994b, Thompson 2005). For example, little is known about how the fitness or population growth of mutualistic mycorrhizal fungi, nitrogen-fixing microbes, and photosynthetic zooxanthellae vary with the frequency of their mutualistic partners (Bever 1999, Simms and Taylor 2002, Hay et al. 2004). In plant–pollinator mutualisms, flowering plant reproduction often varies with the number and diversity of pollinators (Pellmyr and Thompson 1996, Gomez et al. 2007, Sahli and Conner 2007). However, the sensitivity of pollinator reproduction to changes in floral resources or other environmental variables is poorly understood, especially in North America (National Resource Council 2006).

The outcomes of mutualisms are often highly context dependent (Bronstein 1994a). The benefits of mutualistic associations can vary with partner quality, resource availability, the presence or absence of predators, competition for access to a mutualistic partner, and the costs of partner exploitation (Johnson and Steiner 1997, Herrera 2000, Morales 2000, Egger and Hibbett 2004, Ness et al. 2004, Rudgers and Strauss 2004). In plant–pollinator mutualisms, unless the pollinator also acts as a seed predator, there is likely little to no cost of extra pollinator visits for seed production (but see Young and Young 1992). Similarly, there is probably no cost to bees for living in areas where there are surplus flowers unless bee parasites are positively correlated with flower density (Carvell et al. 2008). The benefits of pollinator abundance to female plant reproduction and changes in population growth (through effects on seed production) may saturate when abiotic resources become limiting for seed production (Burd 1994, Ashman et al. 2004). Plants may compensate for pollinator shortages if they can self-pollinate without inbreeding costs, reproduce asexually, or endure unfavorable periods via longevity or dormancy by living longer or having seed banks (Pake and Venable 1996, Morgan et al. 2005). In contrast, most insect pollinators are short-lived organisms, so they must reproduce even when resources are scarce. With very few exceptions, bee pollinators cannot reproduce without harvesting pollen and nectar to feed themselves and their offspring (Michener 2007). However, is pollinator per-capita reproduction food-limited?

Positive correlations between bee and flower densities could indicate that bees have higher reproduction, recruitment, or survival in areas with more flowers (Steffan-Dewenter et al. 2002, Westphal et al. 2006), but these relationships could also be influenced by other limiting factors such as parasitism and nest

[1] Corresponding author: Pinyon Publishing, Montrose, CO 81403 (e-mail: susanelliott@pinyon-publishing.com).
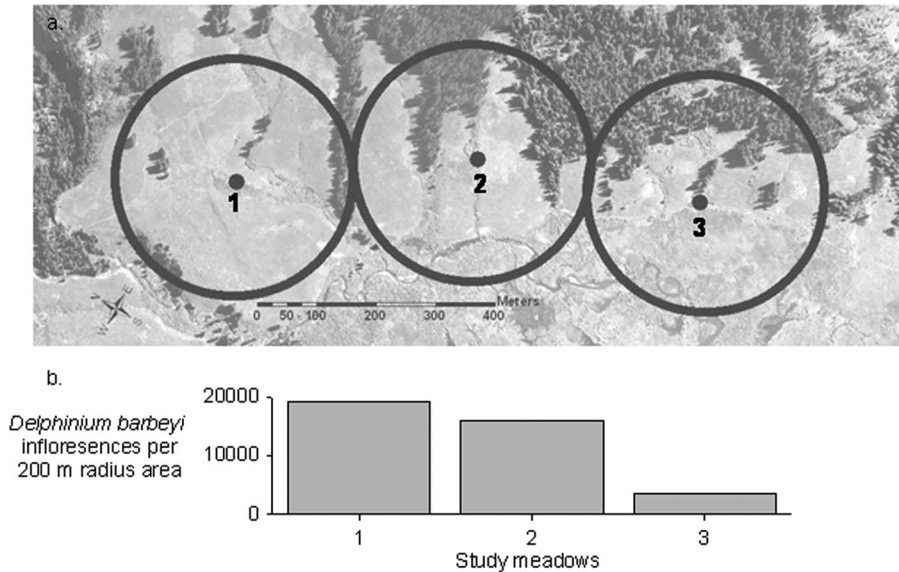[2] Dartmouth College, Biology, Hanover, NH 03755.

Fig. 1. Study meadows (a) in the East River Valley, Gunnison National Forest, CO, and total number of *D. barbeyi* inflorescences (b) in 200-m radii around each captive colony location (1–3; GPS coordinates = 1: 2°56′8″ E, 38°49′33″ N; 2: 2°56′12″ E, 38°49′21″ N; 3: 2°56′17″ E, 38°49′13″ N).

site availability. Recent studies from low-elevation areas (<200 m) confirm that bee reproduction of solitary and colonial bee species is greater in areas with higher natural or experimental levels of floral resources (Goulson et al. 2002, Pelletier and McNeil 2003, Greenleaf 2005, Williams and Kremen 2007, Carvell et al. 2008). However, because bees at high elevations have shorter growing seasons and consequently less time to establish nests, collect resources, and produce offspring, their reproduction may instead be time-limited (Pyke 1982). To determine whether subalpine bumble bees have surplus flowers for colony reproduction, I measured bumble bee reproduction across meadows that varied naturally in flower density and in response to supplemental feeding at the nest. Time limitation of bumble bee reproduction was not addressed experimentally but is discussed within the context of the experimental findings of this study.

Although bumble bees visit a variety of flowers, they may be functionally specialized if they derive most of their resources from the most rewarding flower species (Dramstad and Fry 1995, Stenstrom and Bergman 1998, Goulson and Darvill 2004). The focal bumble bee in this study, *Bombus appositus* L. (Apidae) may visit at least 20 flower species throughout the season in the study area (Pyke 1982), but it collects much of its pollen and nectar from the abundant perennial wildflower, *Delphinium barbeyi* Huth (Ranunculaceae) (Elliott 2009). *Delphinium barbeyi*, the second most abundant flower species in these meadows, is in bloom in the middle of season while *B. appositus* colonies are provisioning and producing adult workers, gynes, and males. During this time, *D. barbeyi* accounts for the majority of pollen collection and flower visitation by *B. appositus* (described below). I tested the hypothesis that *B. appositus* reproduction (i.e., worker, gyne, and male offspring production) was limited by floral resources, by asking the following questions.

1. Does colony reproduction vary across meadows that vary naturally in flower density? If bee colony density is independent of flower density and if colony reproduction is food-limited, colonies in meadows with more flowers should produce more offspring, assuming that flower number represents pollen and nectar availability (Tepedino and Stanton 1982). Alternatively, if meadows with more flowers have disproportionately more colonies, flower availability per colony might be lower in meadows with more total flowers. If flower availability per colony is greater in some meadows, flowers in those meadows should have lower per-flower pollinator visitation rates, and nectar in those meadows should be depleted less quickly than in meadows with more flowers per colony.

2. Is bee colony reproduction food-limited? If bee reproduction is food-limited, colonies that are fed at the nest with supplemental nectar and pollen should have higher reproduction than unfed control colonies. Also, fed colonies may reduce foraging to avoid costs associated with foraging outside the colony (Dukas and Morse 2003) or to benefit the colony by devoting more time to brood care or colony defense (Cartar 1992).

Materials and Methods

In 2006 and 2007, I studied reproduction in the long-tongued bumble bee, *B. appositus* (Apidae), in subalpine meadows in the East River Valley near the Rocky Mountain Biological Laboratory in Gothic, CO (2,952- to 2,969-m elevation; Fig. 1). *B. appositus* is one of the three most abundant bumble bee species in this

study area (Pyke 1982, Elliott 2009). Inseminated bumble bee queens emerge from hibernation in early spring (late May to early June) and establish new nests, where they incubate their first brood. About 6 wk later, the first worker cohort emerges from their cocoons, and forager density increases sharply. During this increase in bee abundance, the common perennial wildflower, *Delphinium barbeyi* (Ranunculaceae), accounts for 91.2% of the pollen collected by *B. appositus* and 94.6% of the floral visits made by *B. appositus* (median contributions, S1).

In the study area, *D. barbeyi* occurs in meadows and forest clearings in distinct patches, and *D. barbeyi* flower density varies over three orders of magnitude among 0.5-ha meadow plots (0.8–26.2 flowers produced/m²; Elliott 2008). With 13.6 ± 0.5 *D. barbeyi* inflorescences per plant (mean $\pm$ SE, $n = 420$ plants), each bearing 25.4 ± 0.8 flowers per inflorescence ($n = 372$ inflorescences), *D. barbeyi* accounts for $14.0 \pm 4\%$ of all flowers produced (mean $\pm$ SE, $n = 6$ meadows; Elliott 2008). On average, flowers contain 1.8 ± 0.05 μl of nectar per flower in the morning (before pollinator visits begin, $n = 512$ flowers) with $36.1 \pm 0.7\%$ sugar concentration ($n = 34$ flowers; Elliott 2008). Flowers are protandrous, with anthers dehiscing over a period of ~3 d (unpublished data).

## 1. Does Colony Reproduction Vary Across Meadows That Vary Naturally in Flower Density?

I compared *B. appositus* colony reproduction in three meadows that varied naturally in *D. barbeyi* density. The meadows were separated by ≈400 m (Fig. 1). Although large-bodied bees like *B. appositus* are physiologically able to fly up to 9.8 km (Goulson and Stout 2001), in this study system, bumble bees forage primarily within a 100-m radius (Elliott 2009). Therefore, bees were unlikely to fly among focal meadows, each separated by >300 m. In addition, all bees in this study that were marked at the colony and relocated in foraging meadows were found in the meadows nearest their respective colonies even though all meadows were sampled equally, suggesting that they did not fly among study meadows.

Flower Density. In 2007, I counted the number of *D. barbeyi* flowering inflorescences in a 200-m radius around each shelter that housed captive bee colonies (one shelter per focal meadow; Fig. 1). Flower number per inflorescence did not vary with inflorescence density ($r = 0.09$, $P = 0.8$, $n = 10$ meadows, 60 inflorescences averaged per meadow). This analysis and all subsequent analyses were performed with JMP v. 4.04 (SAS Institute 2001). Although *D. barbeyi* density is correlated with total flower density in these meadows, the most abundant flower species, *Potentilla pulcherrima* (Rosaceae) only contributed to 2.6% of all *B. appositus* visits and therefore probably does not contribute strongly to colony reproduction (Elliott 2008, 2009). In 2007, I compared corbicular pollen loads carried by *B. appositus* in each meadow. *Potentialla pulcherrima* pollen is bright orange and easily distinguished from *D. barbeyi's* cream-colored pollen. Pol-

len collection by *B. appositus* is dominated by these two species (S1). I used a one-way analysis of variance (ANOVA) to compare the proportions of cream and orange pollen loads carried by foragers returning to the colonies (described below) among the three study meadows, using colony as the unit of replication.

Bumble Bee Colonies. In 2006 and 2007, I monitored captive *B. appositus* colony reproduction in the three focal meadows. To obtain colonies, I set out ≈100 wooden nest boxes in the study meadows, providing each box with cotton for nest insulation. Each year, ≈10% of boxes succeeded in attracting emerging *B. appositus* queens from the study meadows to the nest boxes. I also started colonies in the laboratory using nest-searching queens collected from the study meadows (2006: three colonies, one per meadow, 2007: one colony, used in one meadow only). As soon as field or laboratory colonies laid their first brood, I distributed the colonies evenly among the three study meadows. In each study meadow, all colonies were placed in the same fenced and covered shelter (to reduce damage from porcupine, bear, and rain). Therefore, this study tests the effects of flower density on colony growth and reproduction (as in Goulson et al. 2002, Pelletier and McNeil 2003, Thomson 2004, Greenleaf 2005) and not on colony establishment. The queens in the captive colonies fed their offspring with nectar and pollen obtained in the meadows. The study colonies contributed to a small proportion of ambient bee density (S2). The shelters were fenced enclosures that reduced, but did not completely eliminate, porcupine and bear disturbances. After excluding disturbed colonies, there were seven (2006) and eight (2007) control colonies (Fig. 2). Once per week at night, I counted the number of individuals of each caste present in each colony.

Colony Reproduction. After colonies were abandoned at the end of the season, I quantified three aspects of offspring production per colony: number of gyne cocoons, number of worker cocoons, and number of males. I counted all cocoons produced (which remain intact after adult individuals emerge), and I measured the diameter of each cocoon to the nearest 0.1 mm. Cocoons that had housed gynes were roughly three times larger than worker or male cocoons, as with most *Bombus* species that feed offspring individually (Goulson 2003). Gyne cocoons ranged from 9.6 to 18.8 mm in diameter and nonqueen cocoons ranged from 5.6 to 9.9 mm in diameter. For intermediate sizes, I assigned the caste based on the cocoon size, relative to the other cocoons in the colony. I measured gyne cocoon diameter as a proxy for gyne body size, which can affect diapause survival (Beekman et al. 1998). I quantified male production as the sum of all males seen in the colonies during weekly night censuses. This is a conservative estimate of male production because males leave the colonies 2–4 d after emerging from their cocoons (Kearns and Thomson 2001), and I only visited colonies once per week. I estimated worker production as the number of small (nongyne) cocoons minus the number of males observed during night censuses. Therefore, worker production is
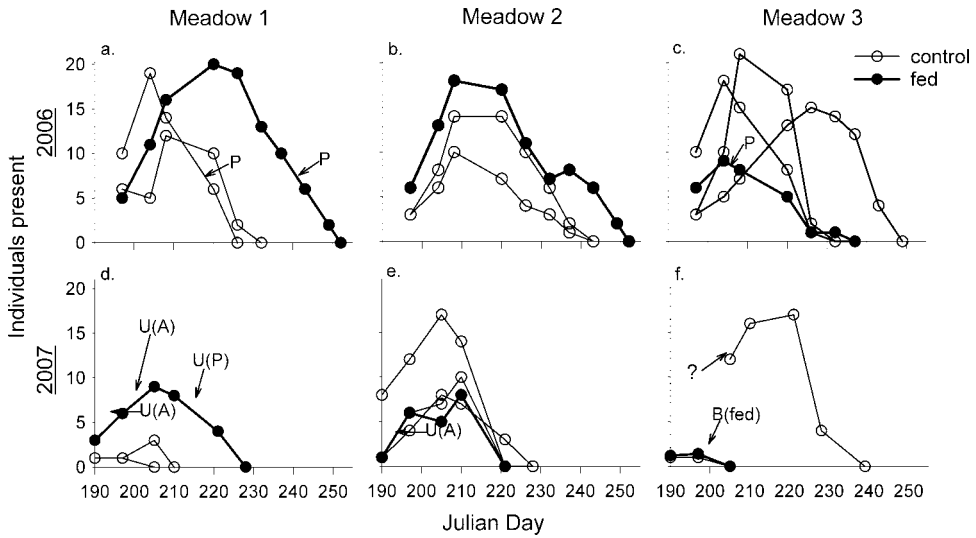
Fig. 2. Number of live *B. appositus* individuals observed per colony during night censuses over the season in three study meadows (see Fig. 1) for control colonies (open) and fed (closed) colonies (a–f). Arrows point to disturbances [P = porcupine, U = nest usurpation by U(A) (*B. appositus*) or U(P) (*Psithyrus* sp.), and B = bear damage]. The ? points to a colony that was found after the start of the study so its starting size is unknown. In 2007, the fed colony with bear damage had the same colony size and is thus plotted over a control colony without damage.

slightly inflated because I probably missed seeing some males if they dispersed between censuses. With the exception of successful social nest parasitism, bumble bees that enter colonies that are not their own are killed or chased out by resident bees (Kearns and Thomson 2001). Thus, all workers, males, and new gynes observed in the colony were most likely produced by that colony.

I used multivariate analyses of variance (MANOVAs; one for each year) to test whether the number of gynes, workers, and males per colony varied among meadows. I did not use flower density as a covariate because by using meadow as a categorical predictor variable, I could detect differences among meadows that may not vary linearly with flower density. I used a MANOVA because reproduction among castes may be correlated (Pelletier and McNeil 2003). Given a marginally significant effect in 1 yr, I used separate one-way ANOVAs to test how the number of gynes, workers, and males per colony varied among meadows. I could not test for meadow effects on average gyne cocoon diameter because some meadows had only one colony that produced gynes. I first separated analyses by year and then combined years to assess overall effects.

Foraging Behavior and Nectar Availability. In 2007, to determine whether foragers were distributed in proportion to flower availability, I calculated flower visitation rates in ≈20-m² patches (12 15-min observation bouts between 0900 and 1500 hours, spread over 3 wk in the middle of the *D. barbeyi* blooming period). I recorded visitation rates in the three focal meadows plus nine adjacent meadows. However, because I never saw marked (captive colony) bees in the surrounding meadows, I only report visitation rates in the three focal meadows. A sample size of 12 observation bouts per meadow provides sufficient power to detect among-meadow variation in visitation rates ($F_{1,11}$ = 2.9, P = 0.002, n = 12 meadows). Before each 15-min observation period, I recorded the number of open inflorescences I was observing. I also counted the number of open flowers per inflorescence from 20 inflorescences per meadow to calculate per-flower pollinator visitation rates. I recorded visits by all bee and bird species, although *B. appositus* made up the majority of all flower visits (S3). For each visitor, I recorded the species and the number of flowers they visited. I used a one-way ANOVA to test whether *B. appositus* per-flower visitation rates (i.e., percent of open flowers visited by *B. appositus* per minute) varied among the three study meadows, using observation period as the unit of replication.

In 2007, to determine whether per-flower nectar availability varied among meadows, I compared pollinator visitation rates per flower (all pollinator species) and daily nectar depletion per flower across the three meadows. I collected flowers to measure nectar availability and depletion on 3 d (5, 18, and 25 July) in the middle of the *D. barbeyi* blooming period. Each day, I collected flowers from different plants spread throughout each meadow in the mornings (before 0800 hours when *D. barbeyi* pollinators become active) and in the late afternoons (after 1600 hours when most foraging on *D. barbeyi* has slowed or stopped). I collected 20 flowers per meadow at each time period on each day, but I excluded flowers that were damaged so total sample size per meadow varied from 32 to 48 morning flowers and 24–38 afternoon flowers per meadow (total n = 217 flowers). I picked flowers from the middle of inflorescences to standardize flower age. I kept the flowers in a cooler and extracted the nectar (from the two nectar spurs per

flower) within 24 h, using 2-μl capillary tubes. Nectar reabsorption in unvisited flowers in *D. barbeyi* has not been reported in published studies. To determine whether meadows varied in nectar availability and whether nectar availability dropped over the course of a day, I used a two-way ANOVA to test the effects of meadow and time period (morning versus afternoon) on nectar volume per flower. I also tested whether nectar depletion varied among meadows (meadow × time period interaction), using flower as the unit of replication.

2. Is Bee Colony Reproduction Food-Limited?

In 2006 and 2007, to test whether colonies were food-limited, I fed two colonies (one from each of the first two meadows) and compared their reproduction and activity levels with the control (unfed) colonies (described above). Because of a limited sample size, I did not feed multiple colonies to maximize control colony sample size to establish baseline variation in reproduction across meadows and to avoid inflating forager number (and resource depletion) by feeding multiple colonies. I also fed one colony in the third meadow each year, but they were destroyed by porcupine and bear (2006 and 2007, respectively) early in their development and were therefore excluded from analyses. Each week, I fed the colonies with 50 ml of sugar-water (50:50 sucrose and water, with 1 ml of honey mixed in) with two plastic pipette feeders. The colonies typically drained the feeders in 3 d. I added Colorado wildflower honey (Ambrosia Honey, Parachute, CO) so that natural nonsugar constituents would also be present (Baker and Baker 1982). The 50-ml nectar volume is comparable to the total amount of nectar held within one third of all of the *D. barbeyi* flowers in an average meadow (given an average of 3,856 inflorescences per meadow × 25 flowers per inflorescence × 1.8 μl of nectar per flower). In 2007, to determine whether colonies were limited by both nectar and pollen, I also gave the two fed colonies supplemental pollen. Each week, I gave the colonies ≈500 mg of honey bee–collected pollen moistened with artificial nectar (Kearns and Thomson 2001), which is slightly more pollen than all of the workers in a colony would bring in on an average day (19 ± 5 corbicular pollen loads per day; unpublished data; 21 mg pollen per load, Heinrich 1979). I used pollen collected from honey bees that were foraging in similar subalpine habitats in Colorado. The food was provided inside the colonies so individuals from other colonies could not collect or consume it.

I compared four metrics of offspring production between fed and control colonies: number of gyne cocoons, worker cocoons, and males censused, and gyne cocoon diameter (described above). First, I used MANOVAs (one for each year) to test whether the number of gynes, workers, and males per colony varied among meadows. Given significant differences in both years, I tested the effect of feeding on these four variables with separate two-tailed *t*-tests for each year and for both years combined. For males and gynes, I used pooled estimates of variance for the two treatments (Levene's test of the null hypothesis that equal variance are equal were all nonsignificant at α = 0.05),

but for worker production, I could not assume equal variances, so I did not pool variances.

To determine whether individuals in fed colonies foraged more frequently (e.g., because of increased energy to fuel foraging efforts) or less frequently (e.g., to reduce foraging costs) than unfed control colonies, I compared foraging activity of fed and control colonies. In 2006, I recorded foraging activity for 30 min per colony on 1 d toward the end *D. barbeyi's* blooming period. In 2007, I increased observation time to 12 30-min observation periods per colony, spread over 2 wk at peak *D. barbeyi* bloom. For each colony, I divided the total number of entries into and exits out of the colony by the number of individuals and by the 30 min of observation time to calculate an activity rate per individual per minute. I used two-tailed *t*-tests to compare activity rates between fed and control colonies, with colony as the unit of replication, analyzing each year separately.

Results

1. Does Colony Reproduction Vary Across Meadows That Vary Naturally in Flower Density?

Despite five-fold variation in flower abundance (Fig. 1), bee reproduction was relatively constant among meadows (2006: F = 0.4; df = 6,4; P = 0.9; 2007: F = 5.7; df = 6,4; P = 0.056; Fig. 3; Table 1). During both years, control colonies produced 0–12 gynes (median = 0 gynes per colony), and average gyne production did not vary among meadows (2006: F = 0.4; df = 2,4; P = 0.7; 2007: F = 0.5; df = 2,5; P = 0.6; both years: F = 0.5; df = 2,12; P = 0.6; Fig. 3a; Table 1). Colonies produced 0–27 workers (median = 11 workers per colony), and average worker production did not vary across meadows for both years combined (F = 0.5; df = 2,12; P = 0.6; Fig. 3b; Table 1) and in 2006 alone (F = 0.1; df = 2,4; P = 0.9; Fig. 3b; Table 1). However, in 2007, colonies in the meadow with intermediate-high flower density (Fig. 1) produced marginally more workers than colonies in the other two meadows (F = 6.1; df = 2,5; P = 0.06; Fig. 3b; Table 1). Males were seen in 5 of 16 control colonies during night censuses from the 2 yr, with a maximum of five males observed per colony (median = 0 males per colony). In 2006, very few males were seen in any of the meadows, but in 2007, males were seen more frequently in the meadow with intermediate-high flower density (2006: F = 0.1; df = 2,4; P = 0.9; 2007: F = 7.0; df = 2,5; P = 0.04; both years: F = 2.7; df = 2,12; P = 0.1; Fig. 3c; Table 1).

In 2007, *B. appositus* flower visitation rate (i.e., percent of *D. barbeyi* flowers visited by *B. appositus* per minute) did not vary among meadows (F = 1.1; df = 2,33; P = 0.4; Fig. 4). Similarly, flower visitation rate of all visitor species combined (S3) did not vary among meadows (F = 0.9; df = 2,33; P = 0.4; Fig. 4). Although nectar volume per flower was highest in the meadow with the fewest *D. barbeyi* flowers (F = 7.6; df = 2,211; P = 0.02; Fig. 4), nectar depletion did not vary among meadows (meadow × time period: F = 1.7, df = 2,211; P = 0.2; Fig. 4). Available nectar decreased by 28.4%
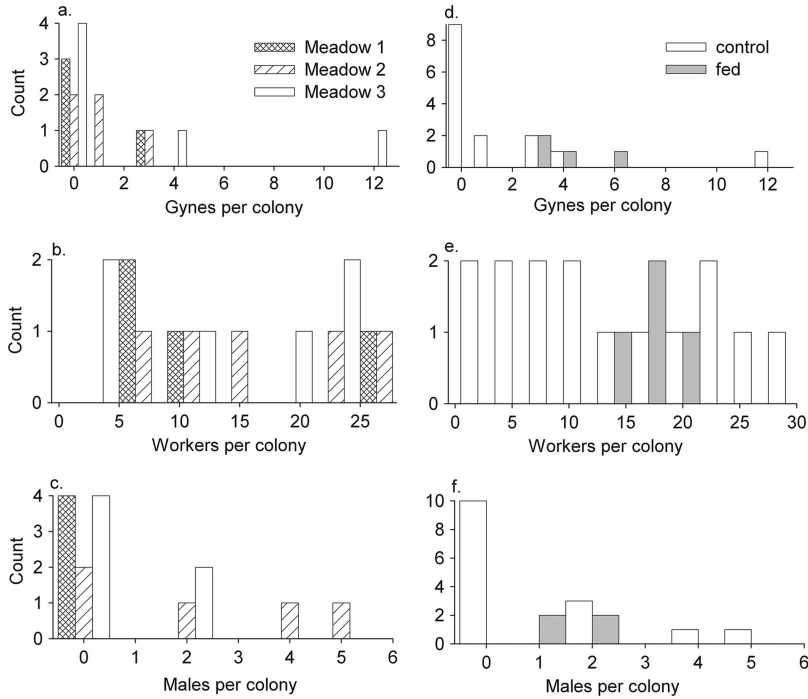
Fig. 3. *Bombus appositus* colony reproduction across meadows (unfed control colonies; a–c) and between control and fed treatments (d–f) in terms of queens (a and d), workers (b and e), and males (c and f) produced per colony.

over the course of the day (time effect: $F = 14.5$; df = 1,211; $P = 0.0002$; Fig. 4).

The proportion of cream-colored pollen loads brought back by foragers, indicating *D. barbeyi* pollen use instead of orange *P. pulcherrima* pollen, varied among meadows ($F = 13.3$; df = 2,6; $P = 0.006$). The lowest *D. barbeyi* use was in the meadow with the most *D. barbeyi* flowers, which was also the meadow with intermediate *P. pulcherrima* density in 2004 (mean ± SE percent cream pollen loads per colony: meadow 1 = 42.7 ± 7.5%, meadow 2 = 85.6 ± 6.5%, meadow 3 = 96.4 ± 9.2%).

2. Is Bee Colony Reproduction Food-Limited?

In individual years, there were some significant differences in reproduction between control and fed colonies (2006: $F = 8.0$; df = 3,5; $P = 0.02$; 2007: $F = 8.5$; df = 3,5; $P = 0.02$; Fig. 3; Table 1). Although all four fed colonies produced gynes, only 8 of the 15 control colonies produced gynes (Fig. 3d). In 2006, fed colonies produced five times more gynes than control colonies ($t = 3.0$; df = 7; $P = 0.02$; Table 1). However, in 2007, feeding did not significantly affect gyne production ($t = 0.3$; df = 9; $P = 0.8$; Table 1). The

Table 1. Mean ± SE *B. appositus* offspring production (queen cocoons, worker cocoons, and male sightings) compared across three subalpine meadows (1 = high density, 2 = medium-high density, 3 = low density; see Fig. 1) and between fed and control (unfed) colonies

| Factor	Year	Meadows or feeding	N colonies	Queen cocoons	Worker cocoons	Male sightings
Meadows	2006	1	2	1.5 ± 1.5	18.0 ± 9.0	0.0 ± 0.0
		2	2	0.0 ± 0.0	13.0 ± 8.0	0.0 ± 0.0
		3	3	1.3 ± 1.3	15.0 ± 3.6	0.7 ± 0.7
	2007	1	2	0.0 ± 0.0	5.0 ± 1.0	0.0 ± 0.0
		2	3	1.7 ± 0.7	16.3 ± 4.4	3.7 ± 0.9
		3	3	4.0 ± 4.0	7.3 ± 7.3	0.7 ± 0.7
	Both years	1	4	0.8 ± 0.8	11.5 ± 5.3	0.0 ± 0.0
		2	5	1.0 ± 0.5	15.0 ± 3.6	2.2 ± 1.0
		3	6	2.7 ± 2.0	11.2 ± 4.0	0.7 ± 0.4
Fed versus control	2006	F	2	5.0 ± 1.0	14.0 ± 1.0	1.5 ± 0.5
		C	7	1.0 ± 0.7	15.3 ± 3.1	0.3 ± 0.3
	2007	F	2	3.0 ± 0.0	16.5 ± 1.5	1.6 ± 0.7
		C	8	2.1 ± 1.5	10.1 ± 3.4	1.5 ± 0.5
	Both years	F	4	4.0 ± 0.7	15.3 ± 1.0	1.5 ± 0.3
		C	15	1.6 ± 0.8	12.5 ± 2.3	1.0 ± 0.4
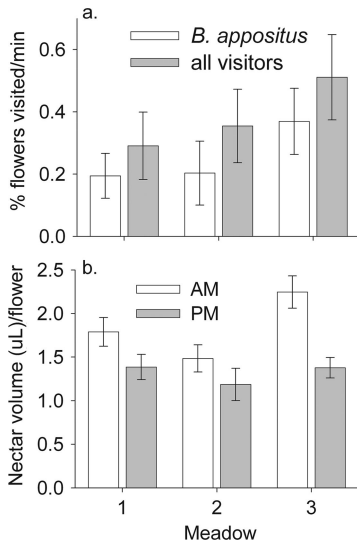
Fig. 4. Comparison of pollinator abundance and nectar availability per flower for *D. barbeyi* growing in three subalpine meadows in Gunnison National Forest, CO (in decreasing order of flower abundance, see Fig. 1) showing (a) flower visitation rate (percent of flowers visited per minute) and (b) ambient nectar volume per flower measured before (AM) and after (PM) pollinator foraging. Error bars represent ±SE around meadow averages. Significance levels for among-meadow comparisons are reported in text.

nonsignificant effect of feeding on gyne production for both years combined was caused by a control colony in 2007 with very high reproduction (i.e., 12 gynes; with this outlier excluded, $t = 3.9$; df $= 16$; $P = 0.001$; Fig. 3d). Feeding did not affect gyne cocoon diameter in either year (2006: control $= 10.9 \pm 0.5$ mm, fed $= 11.9 \pm 0.6$ mm, $t = -1.2$; df $= 5$; $P = 0.3$; 2007: control $= 11.7 \pm 0.2$, fed $= 11.5 \pm 0.3$ mm, $t = 0.4$; df $= 4$; $P = 0.7$; both years: control $= 11.3 \pm 0.3$ mm, fed $= 11.7 \pm 0.4$ mm, $t = 0.9$; df $= 9$; $P = 0.4$). Worker production also did not vary between control and fed colonies in either year (2006: $t = 0.4$; df $= 6.9$; $P = 0.7$; 2007: $t = 1.8$; df $= 7.9$; $P = 0.1$; Fig. 3e; Table 1). Males were found in the colonies at night in all four fed colonies, yet they were only observed in one third of the control colonies (Fig. 3f). However, there were no statistically significant differences in the number of males censused between control and fed colonies in either year (2006: $t = 2.0$; df $= 7$; $P = 0.08$; 2007: $t = 0.8$; df $= 7$; $P = 0.9$; Table 1). With both years combined, there were no statistically significant effects of feeding on gyne, worker, or male production (gynes: $t = 1.4$, df $= 17$; $P = 0.2$; workers: $t = 1.1$; df $= 17$; $P = 0.3$; males: $t = 0.6$, df $= 17$; $P = 0.6$; Fig. 3d–f; Table 1).

In 2006, individuals in fed colonies made more entries and exits in and out of their nest boxes than individuals in control colonies (mean $\pm$ SE activity per individual: fed $= 0.38 \pm 0.06$, control $= 0.06 \pm 0.03$, $t = 4.8$; df $= 7$; $P = 0.0021$). However, in 2007, individual activity rates in fed colonies did not differ from control colonies (fed $= 0.60 \pm 0.36$, control $= 0.57 \pm 0.18$, $t = 0.08$, df $= 8$; $P = 0.9$).

Discussion

In contrast to the wealth of studies that test for pollen limitation of plant reproduction, very few studies have tested for flower limitation of pollinator reproduction, creating a skewed understanding of plant–pollinator mutualisms. A handful of studies from low elevations suggest that bumble bee pollinator reproduction is limited by floral resources (Goulson et al. 2002, Pelletier and McNeil 2003, Greenleaf 2005). However, in subalpine meadows, although bumble bee reproduction was marginally food-limited (i.e., fed colonies generally produced more gynes), there was no evidence that reproduction was flower-limited (i.e., nectar was not depleted to a large degree and reproduction did not vary across a natural gradient in flower density). One explanation for this pattern may be that unless bumble bee colonies have ample workers, they may not be able to take full advantage of abundant flowers.

Natural flower density may not have affected colony reproduction if colony density increased proportionally with flower density, resulting in areas with more flowers harboring more foragers (Steffan-Dewenter et al. 2002, Westphal et al. 2006). However, relationships between forager and flower densities may not hold if pollen and nectar production per flower varies across habitats (Cartar 2004, Goulson et al. 2007). In 2007, forager abundance per *D. barbeyi* flower was constant across meadows, confirming that forager density was proportional to flower density. Also, although recapture rates were low overall, marked bees from the captive colonies contributed to a greater proportion of flower visitors in the meadow with the fewest *D. barbeyi* flowers (S2). Therefore, there may have been fewer natural bee colonies where there were fewer flowers. However, more extensive mark–recapture studies are needed to test the hypothesis that natural bumble bee colony densities are proportional to flower densities.

Colony reproduction also may have been constant across meadows if all meadows had similar nectar (and pollen) availability. Because daily *D. barbeyi* nectar depletion was minimal, it is unlikely that bees exhausted the available nectar in 2007. For example, in 2005, bees depleted *D. barbeyi* nectar from a starting level of 0.68 ± 0.03 μl per flower down to 0.23 ± 0.04 μl per flower over the course of the day (Elliott 2008). Therefore, the average 1.3 ± 0.1 μl of nectar remaining in *D. barbeyi* flowers at the end of the day in 2007 was probably not too deep within the nectar spur for *B. appositus* to reach. The three nectar sampling dates included the period of colony growth up to peak colony size (Fig. 1, first three census dates). Later in the season, if less nectar was available, it would have primarily affected eclosion, but not provisioning, of gynes and males. However, a greater proportion of gynes did not emerge from their cocoons from than control colonies (95% confidence interval for the mean percent of gynes that did not eclose per colony: control $= -13.1$–41.6%, fed $= -23.7$–48.7%). Therefore, there was probably ample nectar available for

colony growth and after the period of colony growth, bee colonies may have needed less nectar.

Food limitation for bumble bee colonies depends on both nectar and pollen availability. In 2006, foragers in fed colonies were six times more active than foragers in control colonies (i.e., more movements in and out of the hive, scaled by colony size). Fed colonies may have used the extra nectar to increase foraging efforts, enabling them to harvest even more resources, to produce more gynes. In contrast, in 2007, colonies that were fed with extra nectar and pollen, instead of nectar only, were not more active than control colonies. If foraging activity increases when colonies need more pollen (Plowright et al. 1993, Rasheed and Harder 1997), this could also explain why bees in fed colonies in 2007 were not more active (entry/exit rate) than bees in control colonies—i.e., they may have already had plenty of pollen so they did not need to leave the colony and forage more (Cartar 1992, Weinberg and Plowright 2006). Future studies could compare ambient pollen and nectar availability and the relative pollen and nectar requirements for colony reproduction.

In the feeding experiment, there was conflicting evidence for nectar (2006) or nectar and pollen (2007) limitation of bee reproduction. In 2006, gyne (but not worker or male) production increased with nectar additions. However, in 2007, supplemental nectar and pollen additions did not affect reproduction. The feeding effect size was so low that 80% power to detect a significant effect of feeding on gyne production would require a sample size of 73 colonies, which would require many years of study, given the low success rates of rearing captive colonies (Kearns and Thomson 2001). Adding few nonambient colonies per meadow is ideal because ambient colony densities are unknown and increasing captive colony number per meadow could inflate natural levels of resource depletion. However, future work could increase sample sizes by studying colonies in more meadows and/or more ambient colonies per meadow. It is important to note that supplemental food could have had a stronger effect on natural colonies if my captive colonies had been smaller than natural colonies. Small colony sizes (i.e., few workers per colony) may prevent colonies from taking advantage of extra flowers and reduce the overall demand for food. Also, additional floral resources might have had a larger effect on colony establishment than on colony growth, because flower availability per bee is lower early in the season when colonies are becoming established than later during colony growth (Elliott 2009).

The few studies that have tested whether bumble bee colony productivity in the wild is sensitive to food supplementation or natural variation in floral resources suggest that at lower elevations (<200 m), bumble bees are food-limited (Goulson et al. 2002, Pelletier and McNeil 2003, Thomson 2004, Greenleaf 2005, Carvell et al. 2008). For example, in the only other feeding experiment with field colonies, fed colonies of *Bombus impatiens* and *B. ternarius* colonies in Quebec, Canada, produced more gynes, workers, and

males, than unfed control colonies (Pelletier and McNeil 2003). Reduced resource availability in conventional agricultural habitats versus natural habitats or flower rich suburban habitats also reduced colony reproduction of *B. vosnesenskii* in California (Greenleaf 2005) and *B. terrestris* the United Kingdom (Goulson et al. 2002). In another United Kingdom study, worker production was higher when *B. terrestris* colonies were placed near flower-rich oil-seed rape fields versus flower-poor wheat fields, but rich resources were also associated with higher social parasitism (Carvell et al. 2008). Because Carvell et al. (2008) terminated the colonies before they reached gyne production, it is not known if the social parasitism would have outweighed the positive effects of floral resources on overall colony reproduction. Finally, in California, proximity to honey bee competitors reduced *B. occidentalis* gyne and male production (Thomson 2004). In all of these systems, colonies had longer foraging seasons and grew to be larger than the colonies in this study.

Subalpine bees may be at one end of a food limitation gradient, being less food-limited than other bees. As the length of the growing season increases at lower elevations (and as summer daylength increases at higher latitudes), colonial bees have more time to build large colonies, and multivoltine solitary bees have more time to produce additional generations (Minckley et al. 1994, Goodwin 1995, Thiele 2005, de la Hoz 2006, Packer et al. 2007). Because the growing season at high elevation is shorter than at low elevation, high elevation bumble bee reproduction may be time-limited (Pyke 1982) instead of food-limited. For example, in this study, colonies produced a maximum of only 21 workers (Fig. 2), whereas the average number of workers alive at any given time in the Quebec study ranged from 30 to 49 workers per control colony (Pelletier and McNeil 2003). Small colonies may never have enough foragers to collect extra resources even if they are readily available in nearby flowers. Food supplementation not only provides more total resources, but they are also resources that workers can use while staying in the colony and maintaining colony defense and brood care (Cartar 1992). Therefore, the benefits of food additions for reproduction may not necessarily mirror the benefits of flower addition for bee reproduction.

Recent pollinator shortages have heightened awareness that pollinator declines may be linked to declines in plant populations (Buchmann and Nabhan 1996, Kearns et al. 1998). For example, both bees and plants that require pollinators for outcrossing have declined in Britain and The Netherlands (Biesmeijer et al. 2006). However, this study suggests that natural floral resources (in particular, nectar resources) are not the sole driver of subalpine bumble bee reproduction. Although bumble bee colonies that were fed with supplemental nectar generally produced more offspring than control colonies, colonies in meadows with more flowers (which contained surplus nectar at the end of the day) did not produce more offspring than colonies in meadows with fewer flowers. Also,

supplemental nectar feeding did not increase worker production, and these subalpine colonies stayed very small compared with lower elevation colonies. Thus, areas with small colonies may not be able to use surplus natural floral resources because they have a limited number of foragers per colony. This study highlights the need for more studies that examine food and flower limitation of pollinator reproduction in multiple locations and habitats.

Acknowledgments

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