Interpopulation Variation in Nectar Production in *Aconitum columbianum* (Ranunculaceae)

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Summary. In *Aconitum columbianum* there are extreme interpopulation differences in rates of nectar secretion per flower. Since nectar sugar concentration varies little among populations, increased nectar secretion results in a greater mass of sugar per flower for pollinator attraction. These differences in the amount of reward offered per flower account at least in part for observed higher levels of pollinator activity in populations with high nectar production. Nectar production is correlated also with nectary depth, i.e., flowers in populations with deep nectaries have higher rates of nectar secretion than those with shallow nectaries. Nectary depth differences adapt populations to different pollinator-types. Populations with deeper nectaries are adapted to pollination by bumblebees with longer tongues and more specialized foraging behaviors. In conclusion, there are basic differences in pollination ecology among geographical races of *A. columbianum*, which are indicated by correlated interpopulation differences in (1) nectar production, (2) level of pollinator activity, (3) nectary depth, and (4) pollinator-type.

Introduction

Aconitum columbianum Nutt. occurs in the mountains of western North America from British Columbia, Canada to Chihuahua, Mexico. Geographical races of this species are distinguished, in part, on differences in nectary depth, and populations with deeper nectaries are adapted to pollination by bumblebees with longer tongues and more specialized foraging behaviors (Brink 1980).

It was suspected that nectary depth differences might be associated with other differences in pollination ecology as well. In *A. columbianum* populations with deep nectaries there is a high level of nectar foraging activity, with scores of bumblebees and often several moths and hummingbirds foraging for the nectar. In contrast, there is little foraging activity in populations with shallow nectaries. Birds and moths were not observed in these populations, and bumblebee pollinators were seen only occasionally (personal observation).

This suggested that nectary depth and nectar production are correlated characters. This paper presents the results of an investigation designed to test whether there is a trend toward greater nectar production per flower with increased nectary depth. Interpopulation differences in the amount of nectar produced per flower could explain observed differences in levels of pollinator activity. Correlation of characters which affect pollinator-type and activity would indicate considerable variation in pollination ecology among geographical races of *A. columbianum*.

Methods

Populations which were likely to represent a wide range of nectary depths were chosen from herbarium specimens. We chose study locations we had not previously visited so that correlations between nectary depth and nectar production could be investigated in populations other than those which had originally suggested the hypothesis. These populations were studied during summer 1978 (Table 1), and nectar production of flowers over a 24 h period was determined.

After a bumblebee extracted nectar from flowers of an inflorescence, the pedicels of visited flowers were marked. Each inflorescence was enclosed in a cheesecloth pollinator excluder bag, and labelled with the time of the bumblebee visit. Twenty-four hours later, quantities of nectar which had accumulated were determined by squeezing nectar out of nectaries into micropipettes (Drummond "microcaps").

In one population with shallow nectaries (Table 1, CA 1) few bumblebees were present to remove nectar from the flowers, however, nectar quantities available in unbagged flowers were very small (Table 4), and it was deemed unnecessary that the nectaries be emptied before bagging the plants, in this one population.

Since nectar production varies with the age of the flower (Pyke 1974, 1978 a), "flower age" was determined as percent anther dehiscence (dehisced anthers/total number of anthers) when nectar volume was measured.

Nectar quantity measurements were divided into the following five "flower age classes" based on percent anther dehiscence: 0–20%, 21 bis

Table 1. Field study locations. AZ (Arizona), CA (California), CO (Colorado), UT (Utah). Populations are listed in order of increasing nectary depth

CA 1. Bulbiferous population. El Dorado Co., Edge of Lily Lake, South Lake Tahoe vicinity, sw of Fallen Leaf Lake. Aug. 12–14, 1978. Brink 1469.

CA 2. Plumas Co., Plumas National Forest, vicinity of Cape Lake. Aug. 18–21, 1978. Brink 1470.

CO 1. Jackson Co., Routt National Forest, ca. $2^{1}/_{2}$ mi nw Willow Creek Pass. Jul. 17–20, 1978. Brink 1457.

CO 2. Mineral Co., San Juan National Forest, Wolf Creek Pass. Jul. 24–26, 1978. Brink 1462.

CO 3. Pitkin Co., w of Independence Pass, near Lincoln Creek, off Lincoln Creek Rd., head of New York trail and vicinity. Jul. 21–23, 1978. Brink 1461.

AZ 1. Coconino Co., n of Flagstaff, San Francisco Mountains, e side of Humphrey's Peak, Flagstaff Springs, n side of Inner Basin. Aug. 4–6, 1978. Brink 1465.

UT 1. Piute Co., w of Angle, Pole Creek Canyon. Aug. 7–9, 1978. Brink 1466.

AZ 2. Apache Co., sw of Springerville, s edge of Greer, w fork Little Colorado River. Jul. 28-Aug. 2, 1978. Brink 1464.

40%, 41–60%, 61–80%, 81–100%. The nectar quantities in the two nectaries of each flower were averaged. These nectar quantities for single nectaries of individual flowers were regressed on mean population nectary depths (Table 2), for each of the five flower age classes (Fig. 1, Table 5). Linear regression ($Y = a + b\bar{X}$) was used as it produced higher correlation coefficients (r) than the power function ($Y = a\bar{X}^b$). Nectary depth was determined by gluing nectaries from pressed inflorescences within folded strips of clear acetate film, and projecting them at 10X life size using a standard photographic enlarger. A line was drawn, corresponding to nectary depth on the projected image, a copper wire was bent to conform to this line, and the wire was then straightened and measured with a millimeter ruler. *Aconitum* voucher specimens were deposited at the herbarium of California State University, Chico (CHSU).

In the present study we assumed that the energy gain per flower of a bumblebee depends upon nectar volume and sugar concentration. Interpopulation differences in nectar volume per flower should not be compared directly if sugar concentrations are different. Therefore, concentrations of nectar sugars were estimated using a refractometer (Atago, 0–90%) calibrated in sucrose equivalence (percent sucrose as g of sucrose/ 100 g of solution). Nectar from several flowers was pooled to obtain a reading of sugar concentration in populations with low nectar secretion per flower. Mean or pooled population sucrose equivalence (Table 4) was used to calculate mean ug of sugar per ul of nectar for each population (Bolten et al. 1979, p. 303), and nectar quantities per flower were rescaled as ug of sugar per flower (ug sugar/ul X ul nectar/flower = ug sugar/flower).

Linear regressions on nectary depth, of nectar volume per flower (in ul) and mass of sugar per flower (in ug) are not comparable directly, due to scaling differences. Consequently, volume of nectar and mass of sugar per flower were rescaled in common units, as decimal fractions of their respective grand means (computed over all populations and flower age classes). Using these scaled data there were no significant differences ($p > > 0.5$) between slopes and intercepts of regressions on nectary depth, of nectar volume and mass of sugar per flower.

Table 2. Nectary depths of study populations (mm)

Population locations (Table 1)	$\bar{X} \pm 1$ SD (range) sample size
CA 1	4.13 ± 0.44(3.5– 5.0) 20
CA 2	4.62 ± 0.43(3.6– 5.4) 26
CO 1	5.69 ± 0.91(3.8– 7.5) 60
CO 2	5.83 ± 0.73(4.5– 7.6) 31
CO 3	6.37 ± 0.99(4.7– 9.0) 23
AZ 1	7.27 ± 0.82(5.9– 9.1) 10
UT 1	8.76 ± 1.28(6.0–12.0) 60
AZ 2	8.78 ± 0.76(7.1–10.5) 26

Table 4. Nectar sugar concentrations and nectar available in "unbagged" flowers

Population locations (Table 1)	Nectar sugar concentrations $\bar{X} \pm 1$ SD (range) sample size or p = pooled sample (sucrose equivalence, %)	Nectar available in "unbagged" flowers (ul) $\bar{X} \pm 1$ SD (sample size)
CA 1	51.5 ± 8.0 (39–64) 11	0.07 ± 0.09(16)
CA 2	64 p	0.01 ± 0.04(42)
CO 1	46 p	
CO 2	56.5 ± 4.0 (55–65) 10	
CO 3	56.0 ± 2.5 (52–59) 6	
AZ 1	54.6 ± 4.4 (46–61) 9	0.12 ± 0.24(15)
UT 1	54.3 ± 6.9 (40–66) 18	
AZ 2	45.2 ± 3.8 (35–51) 21	

Nectar volume data are compared directly herein since (1) sugar concentration measurements were fewer than volume measurements, (2) interpopulation differences in nectar sugar concentration were not large, and (3) scaling nectar volume data to account for sugar concentration differences had almost no effect on the results.

Results

There is a pronounced trend toward increased nectar production with increased nectary depth. Also, old flowers secrete more nectar than young flowers, as reported by Pyke (1978 b). Both these trends can be seen in the data in Table 3, in spite of variation within populations and flower age classes.

Mean nectary depth of *A. columbianum* populations varies from < 4 mm to > 9 mm (Brink 1980). The populations included in the present study span most of the known range of variation in this character, and also a variety of intermediate nectary depths (Table 2).

The results of regressions of nectar volumes (from single nectaries of individual flowers), on mean population nectary depths (Table 2), are shown in Fig. 1 and Table 5. The positive slopes of these regressions, and of regressions of mass of sugar per nectary on mean nectary depths (not included), are significantly different from zero in two-sided tests. Therefore we reject the null hypothesis of no interpopulation differences in nectar volume or mass of sugar per flower.

In populations with deep nectaries, much more nectar is secreted per flower than in populations with shallow nectaries. Since nectar sugar concentrations vary little among populations, increas-

Table 3. Mean volumes of nectar produced per nectary in 24 hours. Populations are listed in order of increasing nectary depth. For each flower age class the mean ± 1 SD (in ul) and sample size (in parentheses) are indicated

Population location (Table 1)	Flower age (as % anther dehiscence)				
	0–20%	21–40%	41–60%	61–80%	81–100%
CA 1	0.31 ± 0.39 (16)	0.64 ± 0.32 (16)	0.62 ± 0.37 (11)	0.56 ± 0.31 (6)	0.95 ± 0.53 (9)
CA 2	0.14 ± 0.13 (15)	0.11 ± 0.14 (11)	0.04 ± 0.06 (14)	0.07 ± 0.06 (8)	0.18 ± 0.13 (13)
CO 1	0.57 ± 0.10 (5)	1.54 ± 0.55 (4)	1.40 ± 0.54 (7)	1.44 ± 0.88 (10)	1.45 ± 1.03 (18)
CO 2	(0)	2.04 ± 1.16 (10)	2.06 ± 0.51 (5)	1.20 ± 0.55 (10)	1.50 ± 0.54 (30)
CO 3	1.31 (1)	0.90 ± 0.08 (3)	0.81 ± 0.51 (4)	1.67 ± 0.03 (2)	1.28 ± 0.61 (14)
AZ 1	1.17 ± 0.55 (2)	1.59 ± 0.91 (6)	1.37 ± 0.54 (6)	2.87 ± 1.25 (3)	2.81 ± 1.29 (12)
UT 1	(0)	1.45 ± 0.75 (5)	1.56 ± 0.99 (6)	2.28 ± 0.70 (9)	2.20 ± 0.99 (18)
AZ 2	1.64 (1)	1.64 ± 0.44 (3)	2.62 ± 1.23 (6)	2.06 ± 0.46 (3)	2.76 ± 1.29 (10)

Table 5. Probabilities that flowers with deep nectaries secrete more nectar than those with shallow nectaries. Determined by standard analyses of variance of linear regressions of nectar volumes secreted per 24 h by single nectaries of individual flowers (average of the two nectaries of each flower), on mean population nectary depths, for each of five flower age classes (n=sample sizes; $Y=a+bX$; r=correlation coefficients; p=two-sided significance levels of the tests, i.e., p (b=0))

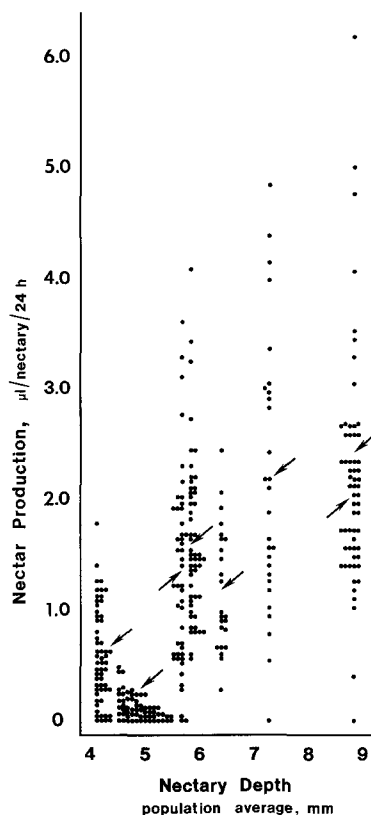| Flower Age	n	a	b	r	p
0– 20%	40	-1.07	0.30	0.70	≪0.001
21– 40%	57	-0.47	0.27	0.47	<0.001
41– 60%	59	-1.17	0.38	0.64	≪0.001
61– 80%	51	-1.38	0.44	0.70	≪0.001
81–100%	124	-1.10	0.42	0.56	≪0.001
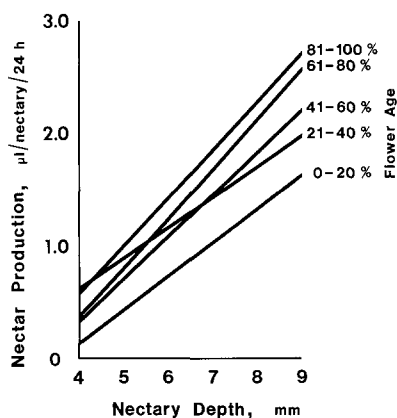
Fig. 1. Regressions of nectar volumes (secreted in a 24 h period after bee visitation, by single nectaries of individual flowers), on mean population nectary depths, for each of five flower age classes ($Y=a+bX$). Multiply nectar volumes by two for nectar produced/flower/24 h, since there are two nectaries per flower

Fig. 2. Volumes of nectar secreted in a 24 h period after bee visitation by single nectaries of individual flowers, plotted against mean population nectary depths. Arrows indicate average nectar secretion/nectary for each population. Multiply nectar volumes by two for nectar produced/flower, since there are two nectaries per flower. There is a high correlation (r=0.89, p<0.005) between mean population nectar secretion and mean population nectary depth, which emerges in spite of high intrapopulation variation in nectar production

ed rate of nectar secretion results in more sugar secreted per flower in populations with deep nectaries.

The average rate of nectar secretion of flowers determines the general level of pollinator activity that a population can sustain, and in Fig. 2, the average nectar production (per nectary) within each population is indicated by an arrow. This figure is provided to show the considerable intrapopulation variation in nectar production, and to illustrate that a significant correlation between nectary depth and nectar production emerges in spite of high intrapopulation variation (linear regression of mean nectar production/nectary/24 h for each population, on mean population nectary depths, $\bar{Y}= -1.21+0.41\bar{X}$, r=0.89, two-sided p<0.005, n=8, regression line not plotted).

Discussion

The pollination syndrome of *A. columbianum* was investigated from several perspectives. Pyke (1974, 1978a, 1978b) documented patterns of bumblebee behavior that optimize foraging efficiency on *A. columbianum*. Inouye (1976, 1978) demonstrated experimentally that competition for nectar results in partitioning of short-. and long-tongued bumblebees between *A.columbianum* and a *Delphinium* species with deeper nectaries, and this niche partitioning is important in bumblebee community structure (Inouye 1977).

Within the *A. columbianum* species complex there is a wide range of interpopulation variation in characters associated with vegetative and sexual reproduction. Nectary depth differences are useful in distinguishing geographical races of *A. columbianum*, and these races are adapted to utilize different pollinator-types (Brink 1980).

Nectary Depth and Pollinator Constancy. Populations with deep nectaries are adapted to pollination by long-tongued bumblebees with specialized foraging behaviors. The deeply located nectar is beyond the reach of many short-tongued foragers, making this a very lucrative nectar resource for long-tongued bees, due to reduced competition. In this way, pollinators with different tongue lengths are "partitioned" among simultaneously flowering plant species with nectar available at different depths (Inouye 1976, 1977, 1978).

Pollinator partitioning probably increases pollinator constancy and reduces interspecific pollen transfer, and this may be beneficial for the plants for several reasons. If pollen, pollinator visitation or receptive stigma surfaces are potentially limiting, interspecific pollinator movement could decrease seed set due to (1) "wastage" of plant gametes on foreign stigmas of co-occurring plant species, (2) loss of effective pollinator visits themselves, an (3) occupation of limited stigmatic surfaces by foreign pollen (Waser 1978a, 1978b).

Nectar Production and Pollinator Activity. Bumblebees are opportunistic and they utilize foraging strategies which maximize their net rate of enery gain (Pyke 1974, 1978a, 1978b; Inouye 1976, 1978). Bumblebees will forage constantly on *Aconitum* only if it is a relatively lucrative nectar resource in relation to other co-occurring plant species.

In the present study we have documented a trend toward increased nectar production with increased nectary depth. Populations with deep nectaries have higher rates of nectar secretion per flower than those with shallow nectaries. High nectar production replenishes flowers quickly, resulting in a high level of constant pollinator activity. This probably increases effective pollination although it sometimes attracts nectar robbing bees, moths and birds.

Increased nectar production is an adaptation which enhances ability to compete for pollinators. This is only useful if it results in higher seed set, which suggests that high nectar production is associated with high reproductive output per flower. This could be tested in future investigations by determining whether nectary depth and nectar production are correlated with the number of seeds and amount of pollen produced per flower.

Conclusions. In the *Aconitum columbianum* complex there are correlated interpopulation differences in (1) volume of nectar and mass of sugar secreted per flower, (2) levels of pollinator activity, (3) nectary depth, and (4) pollinator-type, which indicates that the evolutionary diversification of this group into geographical races involved basic shifts in pollination ecology.

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