Nectar Characteristics of Interspecific Hybrids and Their Parents in Aesculus (Hippocastanaceae) and Iris (Iridaceae)

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BURKE, J. M. (Department of Biology, Indiana University, Bloomington, IN 47405), R. WYATT (Highlands Biological Station, P.O. Box 580, Highlands, NC 28741), C. W. DEPAMPHILIS (Department of Biology, Pennsylvania State University, State College, PA 16802) AND M. L. ARNOLD (Department of Genetics, University of Georgia, Athens, GA 30602). Nectar characteristics of interspecific hybrids and their parents in *Aesculus* (Hippocastanaceae) and *Iris* (Iridaceae). J. Torrey Bot. Soc. 127:200–206. 2000. We studied nectar production characteristics (volume, sugar concentration, and sugar output) of parental and interspecific hybrid plants of the dicot *Aesculus* and the monocot *Iris*. In *Aesculus*, the bird-pollinated *A. pavia* produced significantly larger volumes of significantly less concentrated nectar than did the bee-pollinated *A. sylvatica* (7.81 ± 0.46 μl vs. 0.99 ± 0.08 μl, mean ± SE, and 28.9 ± 0.4% vs. 50.3 ± 0.5%); sugar output for *A. pavia* was also significantly higher than in *A. sylvatica* (3.53 ± 0.33 mg vs. 0.84 ± 0.04 mg). Plants sampled from a natural hybrid swarm were intermediate between the parental species with respect to nectar volume (5.85 ± 0.14 μl) and sugar output (1.76 ± 0.04 mg), but they produced nectar with significantly lower sugar concentration than both parental species (24.3 ± 0.2%). In *Iris*, the bird-pollinated *I. fulva* produced significantly smaller volumes of significantly less concentrated nectar than did the bee-pollinated *I. brevicaulis* (20.4 ± 2.5 μl vs. 56.6 ± 6.0 μl, and 18.3 ± 0.6% vs. 23.6 ± 0.3%). The net result was significantly lower sugar output for *I. fulva* as compared to *I. brevicaulis* (4.3 ± 0.6 mg vs. 14.7 ± 1.7 mg). Regardless of which parental species served as the maternal parent, experimentally generated F_1 interspecific hybrids were almost perfectly intermediate between their parental species with respect to nectar volume (36.6 ± 4.2 μl and 36.2 ± 2.1 μl) and sugar output (9.8 ± 0.7 mg and 9.8 ± 1.4 mg), but their mean concentration was more similar to that of *I. brevicaulis* (23.3 ± 0.3% and 24.0 ± 0.4%). It is unclear whether or not pollinator-mediated selection has shaped and/or maintained the large differences in nectar characteristics between these species.

Key words: *Aesculus*, buckeyes, hybridization, *Iris*, Louisiana irises, nectar.

Over the years, the view of pollination systems as specialized interactions between plants

and their pollinators has been widely accepted in pollination biology (e.g., Grant 1949; Baker 1963; Grant and Grant 1965; Stebbins 1970). An example of this view is the theory of "pollination energetics" (Heinrich and Raven 1972). Under this model, features such as the timing of nectar secretion, volume and concentration of nectar produced, and presence of other constituents in the nectar (such as amino acids) are adaptively linked to the behavior of the principal pollinator(s) (e.g., Grant and Grant 1965; Baker and Hurd 1968; Faegri and van der Pijl 1979; Baker and Baker 1983; Richards 1986; Dafni 1992; Proctor et al. 1996). An alternative view is that pollination systems typically remain more generalized (e.g., Waser et al. 1996). According to this view, pollination systems are temporally and spatially dynamic interactions between a va-

riety of plant and pollinator species and, as such, floral characteristics cannot necessarily be predicted on the basis of pollinator types.

Studies of many species have revealed that flowers of predominantly bird-pollinated plants typically produce large volumes of dilute nectar, averaging 25.4% sugar concentration for hummingbirds, whereas flowers of predominantly bee-pollinated plants produce smaller volumes of more concentrated nectar, averaging 41.6% (Pyke and Waser 1981). Such patterns fit the general predictions of "pollination energetics" theory, but these broad correlations between animal pollinators and floral traits of the plant species on which they feed emerge at a very coarse scale. On a finer scale, it is apparent that there is considerable variation in nectar production among populations, among plants, and even among flowers on a single plant (e.g., Cruden et al. 1983; Richards 1986; Kearns and Inouye 1993). Moreover, it is often unclear to what extent these differences are environmental versus genetic. In some cases, it has been shown that temperature and relative humidity have strong effects on nectar volumes and concentrations (e.g., Corbet 1978; Corbet et al. 1979; Wyatt et al. 1992). In other cases, however, authors have argued that changes in nectar characteristics are adaptive and may reflect active regulation by plants (e.g., Stephenson and Thomas 1979; Cruden et al. 1983). Indeed, there is some evidence that nectar production rates may be quantitatively inherited (e.g., Pedersen 1953; Hawkins 1971; Hodges 1993; Mitchell and Shaw 1993; Campbell 1996). Definitive data regarding the inheritance of nectar concentration are, however, lacking.

An interesting complication for the view of pollination systems as highly specialized interactions between plants and their pollinators is posed by those situations in which two plant species, apparently adapted for pollination by dramatically different animal vectors (e.g., birds versus bees), hybridize. Given our incomplete understanding of the inheritance of nectar characteristics, it is difficult to predict the volume and concentration of nectar in F_1 interspecific hybrids, much less in later-generation segregants. Interestingly, there appear to be numerous cases of hybridization between plant species that are pollinated by very different kinds of animals (e.g., hawkmoth versus hummingbird-pollinated *Aquilegia*: Grant 1952, 1976; Chase and Raven 1975). Two examples that we have recently studied intensively are the dicot *Aesculus* (Hip-

pocastanaceae) and the monocot *Iris* (Iridaceae). Evidence from morphology (Hardin 1957a, 1957b) and genetic markers (dePamphilis and Wyatt 1990) shows a broad hybrid zone in the southeastern United States between red buckeye (*A. pavia* L.) and yellow buckeye (*A. sylvatica* Bartram). Pollinator observations by dePamphilis and Wyatt (1989) confirmed that the red-flowered *A. pavia* is regularly visited by ruby-throated hummingbirds (*Achilochis colubris* L.), as postulated by James (1948) and Austin (1975) and directly observed by Wyatt and Lodwick (1981) in eastern Texas and Bertin (1982) in southeastern Missouri. dePamphilis and Wyatt's (1989) observations also established that the yellow-flowered *A. sylvatica* is regularly visited by bumblebees (*Bombus*). It is important to note, however, that these preferences are not absolute. Rather, visits by hummingbirds to *A. sylvatica* and bumblebees to *A. pavia* are relatively frequent (dePamphilis and Wyatt 1989).

Hybridization in the Louisiana irises is well-documented (e.g., Riley 1938; Anderson 1949; Arnold et al. 1990a, 1990b; Arnold et al. 1991; Cruzan and Arnold 1993, 1994; Arnold 1994; Hodges et al. 1996), and these cases generally involve a red-flowered iris that has crossed with blue-flowered congeners. For example, *Iris fulva* Ker-Gawler has small, brick-red flowers and is regularly pollinated by ruby-throated hummingbirds, whereas *I. brevicaulis* Raf. has larger, blue flowers with yellow nectar guides and is pollinated primarily by bumblebees (Viosca 1935; Cruzan and Arnold 1993, 1994; Wesselingh and Arnold 1999). Once again, however, it is important to note that these preferences are not absolute; *I. fulva* is often visited by bumblebee workers (queens are too large for these flowers) and *I. brevicaulis* is often visited by hummingbirds (Wesselingh and Arnold 1999).

Our goal was to characterize nectar production in the bird- versus bee-pollinated parental species and their hybrids. To this end, we measured nectar volume, sugar concentration and sugar output in parental individuals and natural (in the case of *Aesculus*) or experimental (in the case of *Iris*) hybrids. These data allowed us to evaluate predictions of nectar characters based on the traditional view of pollination syndromes.

Materials and Methods. In the spring of 1981, we chose three study populations of *Aesculus* in which to measure nectar characteristics. Our population of *A. pavia* was located at Shell Bluff on the Savannah River in Burke County,

Georgia. This Coastal Plain population of red buckeye was subsequently included in dePamphilis and Wyatt's (1989, 1990) electrophoretic and pollinator studies as "population 9." Our study population of *A. sylvatica,* the Piedmont yellow buckeye, was located at the University of Georgia Botanical Garden in Clarke County, Georgia. It corresponds to "population 28" of dePamphilis and Wyatt (1989, 1990) and was also included in Hardin's (1957b) earlier morphological studies. Our sample of hybrid plants came from an unusually large, complex hybrid swarm at Panola Mountain State Conservation Park in Henry County, Georgia. This population includes plants that range from yellow to red in flower color and which appear to recombine all of the species-diagnostic morphological characters commonly used to distinguish *A. pavia* and *A. sylvatica* (Hardin 1957a). Genetic markers suggest that, overall, this population predominantly contains alleles of *A. sylvatica* (dePamphilis and Wyatt 1990). For our purposes, however, we selected plants that appeared to be morphologically intermediate and, hence, were more likely to represent early-generation hybrids. This population corresponds to "population 17" of dePamphilis and Wyatt (1989, 1990).

Nectar measurements were done in the field on the following dates in 1981: *A. pavia,* 4–5 April (12 individuals); *A. pavia* × *sylvatica,* 11–12 April (31 individuals); and *A. sylvatica,* 13–14 April (20 individuals). We bagged whole inflorescences with bridal veil (Wyatt et al. 1992) at dusk and returned to measure nectar 12 hr later at dawn. Because complete extraction of the nectar required destruction of the flower, we were not able to measure individual flowers repeatedly. We used only freshly opened flowers whose nectar guides were in the "yellow-stage," which dePamphilis and Wyatt (1989) have shown are selectively visited by pollinators in preference to older, "red-stage" flowers. Nectar was extracted from individual flowers using 10-μL microcapillary pipettes, and the length of the column of nectar was measured in mm. Sugar concentrations were determined using a hand-held refractometer (Bellingham and Stanley, Tunbridge Wells, United Kingdom). Three components of nectar production were measured or calculated: nectar volume in μL, sugar concentration (m/v), and sugar output in mg. Bolten et al. (1979) have pointed out that determination of the amount of sugar in nectar requires conversion of the refractometer reading of percentage mass of sucrose equivalents per mass of solution to concentration in mass of solute per volume of solution.

Our studies of nectar production in *Iris* were carried out in the Botany Greenhouses at the University of Georgia. Nectar production was measured between 1 and 9 May 1996. The *I. fulva* plants were collected in the spring of 1989 from a natural population in Terrebonne Parish, Louisiana (11 individuals). Plants of *I. brevicaulis* were collected in the spring of 1993 from St. Martin Parish, Louisiana (7 individuals). The F_1 interspecific hybrids were produced by reciprocal hand-pollinations in the spring of 1994. Hereafter, we refer to F_1 hybrids produced by placing pollen of *I. brevicaulis* on stigmas of *I. fulva* as "*I. fulva* × *brevicaulis*" (19 individuals) and to F_1 hybrids produced by placing pollen of *I. fulva* on stigmas of *I. brevicaulis* as "*I. brevicaulis* × *fulva*" (18 individuals). Each morning, freshly opened flowers were collected from plants of each class and dissected open to allow access to their nectar reservoirs. Nectar was extracted from each flower using a 200-μL micropipettor (Pipetman, Gilson, France). The nectar was then transferred to a 50-μL microcapillary pipette and measured as described for *Aesculus.* Sugar concentration was determined using a refractometer, and sugar output was calculated as described for *Aesculus.*

Nectar volume, sugar concentration (arcsine-square root transformed), and sugar output in *Iris* were analyzed as dependent variables in mixed-model ANOVAs, using the GLM procedure of SAS (SAS Institute 1988). The two parental and two hybrid types were used as classes to estimate the treatment effect, and plants within each of these four classes were considered to be a random effect nested within the treatment. No significant differences were detected between sampling days, so these data were pooled. Multiple-comparisons among class means were performed using Tukey's Studentized Range Test (Sokal and Rohlf 1995). Unfortunately, the original data for *Aesculus* were misplaced after calculation of means and variances, and ANOVAs could not be performed. Means among the three classes were, therefore, compared using pairwise t-tests, and the resulting significance levels were adjusted for multiple comparisons using the sequential Bonferroni procedure of Holm (1979).

Results. In *Aesculus,* the bird-pollinated *A. pavia* produced significantly larger volumes of

Table 1. Sample sizes, means, and standard errors for nectar characteristics of parental and hybrid classes of *Aesculus* and *Iris*. All values are presented as mean ± SE. Means with the same letter are not significantly different (*P* > 0.05) based on pairwise *t*-tests adjusted for multiple comparisons (*Aesculus*) or Tukey's Studentized Range Test (*Iris*).

Class	N	Volume (μL)	Conc. (%)	Sucrose (mg)
A. pavia	12	7.81 ± 0.46 a	28.9 ± 0.42 a	3.53 ± 0.33 a
A. pavia × *A. sylvatica*	31	5.85 ± 0.14 b	24.3 ± 0.23 b	1.76 ± 0.04 b
A. sylvatica	20	0.99 ± 0.08 c	50.3 ± 0.51 c	0.84 ± 0.04 c
I. fulva	11	20.4 ± 2.5 a	18.3 ± 0.6 a	4.3 ± 0.6 a
I. fulva × *I. brevicaulis*	19	36.6 ± 4.2 b	23.3 ± 0.3 b	9.8 ± 0.7 b
I. brevicaulis × *I. fulva*	18	36.2 ± 2.1 b	24.0 ± 0.4 b	9.8 ± 1.4 b
I. brevicaulis	7	56.6 ± 6.0 c	23.6 ± 0.3 b	14.7 ± 1.7 c

significantly less concentrated nectar than did the bee-pollinated *A. sylvatica* (Table 1). Overall, this pattern led to significantly higher sugar output in *A. pavia* than in *A. sylvatica*. Plants sampled from the hybrid population were intermediate in terms of nectar volume, but they had significantly lower sugar concentration than both parental species. In terms of sugar output, the hybrids were intermediate to, and significantly different from, both parental species.

The ANOVAs for *Iris* revealed significant variation among classes, as well as among plants within each class, for all three measures of nectar production (Table 2). The bird-pollinated *I. fulva* produced significantly smaller volumes of significantly less concentrated nectar than did the bee-pollinated *I. brevicaulis* (Table 1). The net result was significantly lower sugar output in *I. fulva* as compared to *I. brevicaulis*. The reciprocal F₁ hybrids were almost identical to each other in their nectar characteristics. Moreover, they were intermediate to their parents with respect to nectar volume and sugar output, but their mean sugar concentrations were statistically indistinguishable from *I. brevicaulis*. The sugar concentrations of all these greenhouse-grown plants were remarkably similar and quite low. The significant differences in sugar output were, therefore, mainly a result of differences in volume, rather than concentration, of nectar.

Discussion. The two species of *Aesculus* that we chose to study appear to be representative of

temperate-zone hummingbird-pollinated (*A. pavia*) and bee-pollinated (*A. sylvatica*) species with respect to their nectar characteristics. The mean volume of nectar produced by flowers of red buckeye (7.81 μL) is very close to the mean of 9.77 μL reported by Cruden et al. (1983) for 16 North American hummingbird-pollinated species. Similarly, the mean concentration of 28.9% is close to the means of 25.4% and 23.8% reported for hummingbird-pollinated species by Pyke and Waser (1981) and by Cruden et al. (1983), respectively. The mean volume of nectar produced by yellow buckeye (0.99 μL) also falls within the range of values for 12 North American bee-pollinated species studied by Cruden et al. (1983), although the overall mean from that study was somewhat higher (2.51 μL). Concentrations for *A. sylvatica* (50.3%) also ranged somewhat higher than the means of 41.6% and 34.9% reported by Pyke and Waser (1981) and Cruden et al. (1983). Overall, hummingbird-pollinated *A. pavia* produced larger volumes of less concentrated nectar than did bee-pollinated *A. sylvatica*.

Hummingbird-pollinated *I. fulva* produced significantly less concentrated nectar than did bee-pollinated *I. brevicaulis*, although the absolute difference was small (18.3% versus 23.6%, respectively). Nectar concentrations of all *Iris* plants studied were quite low. It is possible that these values were lower than might be expected in the field because the plants were maintained in a greenhouse and watered regularly. As Wyatt

Table 2. Mixed-model nested analysis of variance for nectar characteristics of parental and hybrid classes of *Iris*. Plants are nested within each class.

Source	df	Volume			Concentration			Sugar output		
		MS	F	P	MS	F	P	MS	F	P
Class	3	6780	6.47	0.0008	0.0321	23.7	0.0001	542.3	5.44	0.0024
(Plant)	54	1047	7.26	0.0001	0.0014	2.7	0.0001	99.6	7.46	0.0001
Error	82	144			0.0005			13.4		

et al. (1992) have shown, watering can cause dramatic changes in nectar production. Emms and Arnold (unpublished data), however, found that nectar concentrations of another bee-pollinated *Iris* (*I. hexagona* Walter) were quite low even under natural conditions (26.6 ± 1.17%; mean ± SE), suggesting that low concentrations may be common in these species. The very large volumes of nectar produced by the *Iris* plants may be explained by the unusually large size of individual flowers of these species, especially those of *I. brevicaulis* (Carney and Arnold 1997). A few bird-pollinated species studied by Cruden et al. (1983) approached the volumes we recorded for *I. fulva,* but *I. brevicaulis* far outstrips any bee-pollinated species on their list. This discrepancy can be reconciled to some extent by noting that individual flowering stalks of *Iris* typically produce only about 5 to 6 flowers (Bennett 1989; Viosca 1935), whereas single inflorescences of *Aesculus,* for example, typically consist of 50 to 100 or more flowers (Hardin 1956; Wyatt 1982). Production of more flowering stalks per plant by *I. fulva* versus *I. brevicaulis* may further equalize the disparity in nectar production.

Our study population of *A. pavia* × *sylvatica* was a complex hybrid swarm that electrophoretic markers subsequently showed was genetically more similar to *A. sylvatica* (dePamphilis and Wyatt, 1990). Nevertheless, the plants that we selected as morphologically intermediate also proved to be intermediate to their parental species with respect to nectar volume and total sugar output. It is interesting to note, however, that the hybrids produced nectar with significantly lower sugar concentration than both parental species. Although this finding could be a result of localized habitat differences, the production of extreme hybrid phenotypes, known as transgressive segregation, has been documented in a wide variety of segregating hybrid populations (reviewed by Rieseberg et al. 1999).

In contrast to the complex field situation in *Aesculus,* we studied known F_1 interspecific hybrids that were grown under uniform greenhouse conditions in *Iris.* Both hybrid types were almost perfectly intermediate to their parents with respect to nectar volume, but their sugar concentration did not differ significantly from that of the bee-pollinated *I. brevicaulis.* It is worth noting that the crosses in the present study did indeed behave reciprocally (i.e., there were no significant differences between the two classes of F_1s), indicating that there are no strong maternal

effects on nectar characteristics in these two species of *Iris.*

The clear differences between *A. pavia* and *A. sylvatica* are somewhat surprising. While it is true that data from pollinator observations have allowed us to characterize red buckeye as more "bird-pollinated" and yellow buckeye as more "bee-pollinated," the data also demonstrate that bee visits to the former and hummingbird visits to the latter are quite frequent. In fact, across all populations studied, dePamphilis and Wyatt (1989) reported that bumblebees (*Bombus* spp.) were the most common visitors. Two species (*B. bimaculatus* Greeson and *B. pennsylvanicus* DeGeer) visited both *A. pavia* and *A. sylvatica* and their hybrids, as did ruby-throated hummingbirds. The situation is similar for the bird-pollinated *I. fulva* and the bee-pollinated *I. brevicaulis,* in which visits to the former by bumblebees and to the latter by hummingbirds are common. Moreover, Wesselingh and Arnold (1999) found that hummingbirds favor F_1 hybrids over *I. brevicaulis* and that bumblebees favor F_1 hybrids over both *I. fulva* and *I. brevicaulis.* Clearly, these observations fail to support the view that hybrids between these species will necessarily be discriminated against by pollinators. Rather, it seems that the pollination systems of these species are sufficiently generalized to allow successful pollination of hybrids. If the present situation with respect to pollinators reflects that which has prevailed in the past, it is unclear how such a sharp divergence in nectar characteristics could have evolved. It is even more difficult to discern what factors might contribute to the maintenance of these differences. Finally, with respect to the *Iris* species and their hybrids, it is unclear what has driven the evolution of large flower size, especially with regard to *I. brevicaulis,* which actually produces more nectar than the hummingbird-pollinated *I. fulva.*

This study is one of the first to examine nectar characteristics of hybrids between plant species with widely divergent floral traits. Meléndez-Ackerman (1997) found that hybrids between hummingbird-pollinated *Ipomopsis aggregata* and hawkmoth-pollinated *I. tenuituba* resembled the latter more closely in producing smaller volumes of slightly more concentrated nectar. We included representatives of two very different groups in our comparisons: the dicot *Aesculus* and the monocot *Iris.* In some respects, the parental species fit the general expectations for bird- and bee-pollinated species, but with some interesting exceptions in both cases. Similarly,

the hybrids proved to be intermediate in some respects, but equivalent to one parent, or even transgressive, in others. Many more examples of hybrids between plants with divergent pollination syndromes are needed before generalizations about their nectar characteristics can be made.

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