

# Nectar production dynamics and sugar composition in two *Mucuna* species (Leguminosae, Faboideae) with different specialized pollinators

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**Abstract** Nectar is secreted in particular rhythms throughout the lifespan of a flower, which allows determining the nectar production dynamics. This paper compares nectar features in *Mucuna japura* and *Mucuna urens* describing: dynamics of nectar production, floral response to nectar removal, resorption, nectar sugar composition, and variation in nectar sugar composition. *M. japura* inflorescence bears 12–21 yellow flowers, which are in anthesis for 7 days, whereas *M. urens* inflorescence bears 36–54 greenish flowers, but only 1–3 flowers are in anthesis simultaneously that last one night. Nectar volume and sugar concentration were measured, and the amount of sugar was estimated. Qualitative and quantitative nectar sugar composition was determined. Both species had a constant nectar sugar concentration (ca. 10% for *M. japura* and ca.

16% for *M. urens*) and secreted high volumes of nectar (ca. 340 µl per flower for *M. japura* and 310 µl per flower for *M. urens*), during 5 days for *M. japura* and 6 h for *M. urens*, but after the first removal, i.e., when flower opening mechanism is triggered, nectar production stops immediately. Nectar resorption occurred in both species. Nectar sugar composition showed some similarities between the species. Variation in nectar sugar composition occurred in both species. The *Mucuna* species are dependent on their pollinators to produce fruits and seeds, and they have different strategies to promote the necessary interaction with birds or bats, especially related to nectar and flower characteristics.

**Keywords** *Mucuna japura* · *Mucuna urens* · Nectar features · Nectar resorption · *Glossophaga soricina* · *Cacicus haemorrhous*

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## Introduction

Plants with biotic pollination attract their pollen vectors by generally offering rewards (Willson 1983). Of the diverse floral rewards offered by plants, nectar is the most important. Sugars dominate the total solute in floral nectar: these are generally sucrose, fructose, and glucose. Sugar proportions in nectar can vary according to the groups of floral visitors (Baker and Baker 1983; Freeman et al. 1991; Stiles and Freeman 1993), or according to plant evolutionary constraints (e.g., Galetto and Bernardello 2003; Nicolson 2007).

Nectar variability is determined by many traits related to each plant species (Galetto and Bernardello 2005). These include nectar volume, concentration, production rate, composition, removal effects, active resorption,

flower age, as well as other extrinsic traits such as pollinator behavior, air temperature and humidity, and the presence of nectar robbers (e.g., Baker and Baker 1983; Torres and Galetto 1998; Galetto and Bernardello 2004). Further, intra-plant variation in nectar sugar composition was also pointed out as an additional important source of variability (Herrera et al. 2006; Canto et al. 2007).

Nectar production dynamics can be determined throughout the lifespan of a flower because nectar is secreted in particular rhythms in each plant species. Knowledge of nectar production dynamics is fundamental to the understanding of the plant–animal relationship. Without such knowledge, aspects, such as the plant's strategy of offering nectar, the activity patterns, frequency and diversity of pollinators of a plant species, the rate of nectar consumption by animals, among others, could not be understood (Galetto and Bernardello 2004, 2005).

Nectar removal by floral visitors may have a pronounced effect on the total amount secreted by a flower. Although removal in some species does not modify nectar production (e.g., Galetto and Bernardello 1993, 1995; Galetto et al. 2000); in others, the total amount of sugar in the nectar may increase (e.g., Pyke 1991; Galetto and Bernardello 1995; Castellanos et al. 2002) or decrease (e.g., Galetto and Bernardello 1992; Bernardello et al. 1994; Galetto et al. 1997). Predictions for these patterns are not straightforward because they may be related to pollinators, environmental factors, plant resource allocation, or other factors (Ordano and Ornelas 2004).

Although there are papers relating nectar composition with flower biotypes, main pollinator guilds, and pollinator preferences (e.g., Biernaskie and Cartar 2004), little research has been done on the nectar secretion patterns and their relationship with pollinators or plant reproduction (Musicante and Galetto 2008). In addition, considering that variation in nectar sugar composition is little studied and deserves more attention (Herrera et al. 2006; Canto et al. 2007), the comparison of two co-occurring species with different specialized pollinators would be attractive in the context of functional nectar ecology. In this study, two species of *Mucuna* (Leguminosae, Faboideae) having different pollinators (Sazima and Sazima 1978; Agostini et al. 2006; Agostini 2008) were chosen to examine their nectar production dynamics and nectar sugar components to evaluate the results in the context of plant–pollinator interactions and plant reproduction.

Both species of *Mucuna* present explosive flower opening, which is a feature of most *Mucuna* species (Baker 1970; Agostini et al. 2006). *Mucuna japira* is pollinated by the large (43 g) passerine bird *Cacicus haemorrhous* (Agostini et al. 2006), and the abortion of fruits and seeds in different stages of development is common for this species

(Agostini 2008; Agostini et al. 2009). *Mucuna urens* is pollinated by the tiny (9 g) bat *Glossophaga soricina* (Sazima and Sazima 1978), and the abortion of fruits and seeds is uncommon (Agostini 2008; Agostini et al. 2009).

The knowledge of the floral ecology of this genus is scanty especially concerning floral nectar physiology and composition. This present study was undertaken to compare nectar features in *M. japira* and *M. urens*, which are visited by different types of pollinators and address the following questions: (1) What are the dynamics of nectar production throughout the lifetime of the flower and the inflorescence? (2) What is the floral response to nectar removal considering the explosive flower opening and the large differences between species in flower lifetime? (3) What is the pattern of nectar resorption in both *Mucuna* species? (4) What is the chemical composition of the nectar?

## Material and methods

### Study sites and plant species

The study site was in the coastal lowlands covered by subhumid evergreen broadleaf forest at Picinguaba (about 23°20'S, 44°52'W) in Ubatuba, São Paulo, southeastern Brazil. Average annual rainfall at the study site is 2,500 mm, and average annual temperature is 22°C (Sazima et al. 2003). These *Mucuna* species occur on river ridges or in temporarily flooded or very humid areas, and they were studied during the flowering peak of each species: in May 2002–2003 for *M. japira* and December 2004 and January 2005 for *M. urens*.

Each *M. japira* inflorescence bears 12–21 showy, yellow, odorless, and large flag-flowers (more than 6.5 cm in length), which are in anthesis simultaneously and remain fresh and active for 7 days (Tozzi et al. 2005; Agostini et al. 2006). Each *M. urens* inflorescence bears 36–54 greenish, rotten scented, and medium-sized flag-flowers (less than 5 cm length), but only 1–3 flowers are in anthesis simultaneously that last one night (Tozzi et al. 2005). The nectar is enclosed in a chamber and has no contact with the environment. Floral visitors to *Mucuna* reach the hidden nectar legitimately only after pushing the base of the keel, thus triggering the explosive opening of the flower (Agostini et al. 2006). Nectar production stops right after the explosive opening of the flower, a feature probably related to the fact that pollination occurs only once in a single event, a characteristic of all *Mucuna* species studied so far (e.g., Sazima and Sazima 1978; Endress 1994; von Helversen and von Helversen 2003; Agostini et al. 2006). Both species are mainly out-crossers (Agostini 2008).

## Nectar features and secretion pattern

The pattern of nectar production was obtained measuring 78 flowers of seven different individuals of *M. japira*, varying from 10 to 13 flowers per day of anthesis and 69 flowers of seven different individuals in *M. urens*, varying from 9 to 12 flowers every 2 h of anthesis. The flowers were marked using blue water proof pen, and each flower was sampled only once. Two variables were measured: volume of nectar using a graduated microliter syringe (Hamilton, NV, USA), and sugar concentration (percentage sucrose, *w/w*) measured with a pocket refractometer (Atago®, Tokyo, Japan 0–32%). The amount of sugar produced was expressed in milligrams and was calculated following Galetto and Bernardello (2005). It was not necessary to bag the flowers of these species of *Mucuna* because visited and non-visited flowers can be easily identified as the keel of the flower is normally closed, and to reach the nectar, the pollinator must trigger the explosive opening flower mechanism (Agostini et al. 2006). Thus, we used those flowers with the keel closed, i.e., unvisited.

Total nectar volume, concentration, and total amount of sugar were compared by one-way analysis of variance (ANOVA) test and with the Bonferroni's post hoc test for multiple comparisons among pairs of means. For statistical tests, the SPSS statistical program package (SPSS release 10.0, 1999) was used.

In order to study the effect of nectar removal on its production, nectar was repeatedly removed in another group of flowers until corolla abscission at 12-h intervals for *M. japira*, starting at 0600 h ( $n=10$ ) on the first day of anthesis and 2-h intervals for *M. urens*, starting at 1800 h ( $n=10$ ).

## Nectar composition

Nectar sugar composition was verified in 20 flowers in five different plants (four flowers/plant) in *M. urens* and in 16 flowers in five different plants (four flowers in one plant and three flowers in four plants) in *M. japira* (Table 2). Nectar sugar composition was compared in nectar samples taken from unvisited randomly chosen flowers in different periods of anthesis. Nectar drops were placed on Whatman (Maidstone, England) #1 chromatography paper and quickly dried; in the laboratory, nectar was re-dissolved and sugar separation was accomplished by gas chromatography. Nectar was lyophilized and silylated according to Sweeley et al. (1963). The derivatives were then injected into a Konik KNK 3000-HRGS gas chromatograph equipped with a Spectra-Physics SP 4290 data integrator, a flame ionization detector, and a SE 30 capillary column (30 m long, 0.25 mm diameter, and 0.25  $\mu\text{m}$  thickness of the inner pellicle). Nitrogen was the carrier gas (2 ml/min), and the

following temperature program was followed: 200°C/1 min, 1°C/min until 208°C, 10°C/min until 280°C for 2 min. Carbohydrate standards (Sigma Chem.) were prepared using the same method. We used multivariate principal component analysis (PCA; Digby and Kempton 1996) to analyze emerging nectar sugar composition patterns in both co-occurring *Mucuna* species by considering three levels of variability: species, plant, and flowers. In addition, nectar sugar composition (individual sugars and sugar ratios) was analyzed through nested ANOVAs (“species” as a fixed factor; “plant” as random factor nested within “species”; SPSS release 10.0, 1999).

## Results

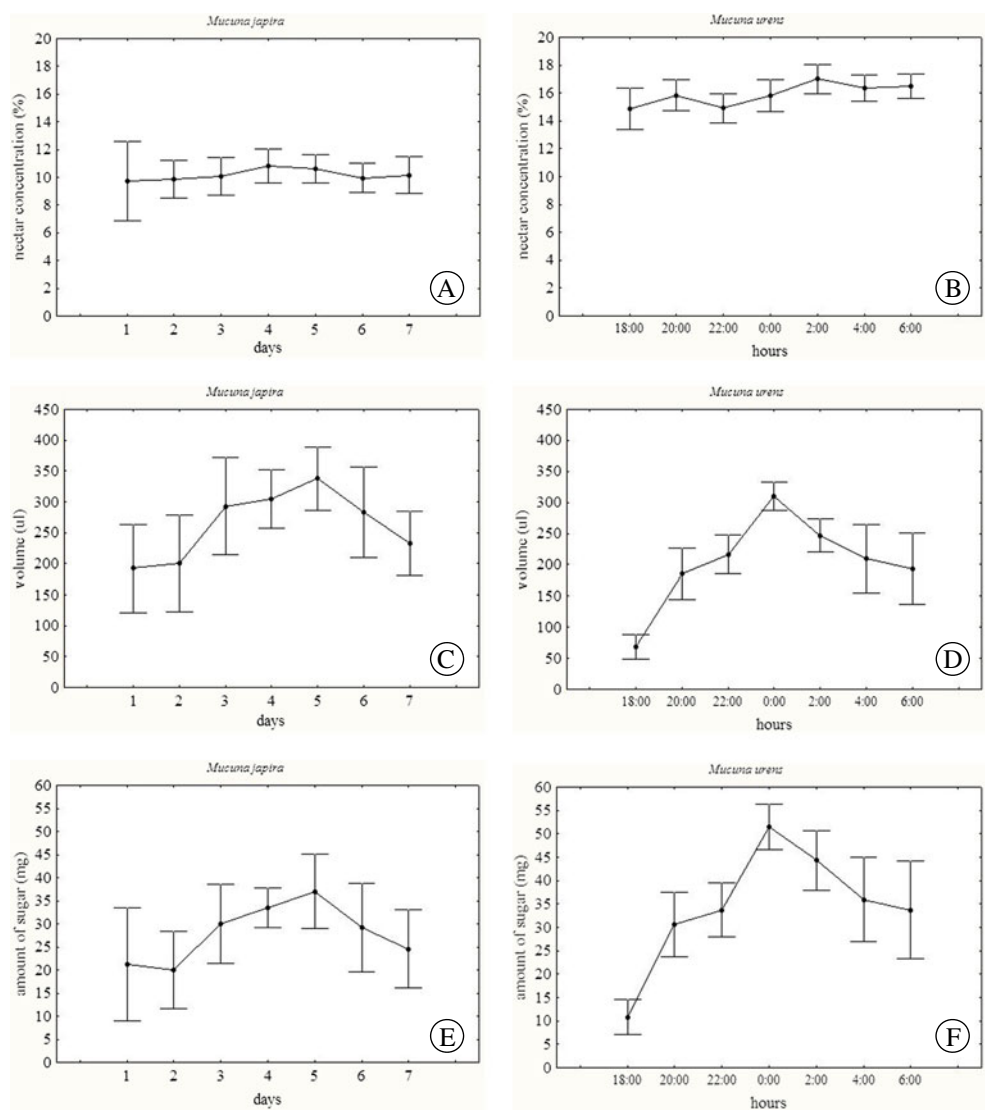
### Nectar features and secretion pattern

Mean nectar sugar concentration was relatively constant throughout the flower lifetime. Nectar sugar concentration was ca. 10% ( $F_{[4,50]}=0.71$ ,  $p=0.59$ ) for *M. japira* and ca. 16% ( $F_{[3,36]}=1.7$ ,  $p=0.19$ ) for *M. urens* (Fig. 1a, b).

Both species secreted a high volume of nectar. We assumed that nectar secretion began at the bud stage since nectar amount at the beginning of the anthesis was ca. 55% and ca. 22% of the maximum volume of nectar secreted by a flower for *M. japira* and *M. urens*, respectively (Fig. 1c, d and Table 1). The nectar secretion period lasted 5 days for *M. japira* and 6 h for *M. urens*. *M. japira* reached the maximum nectar accumulation on the fifth day of anthesis (ca. 340  $\mu\text{l}$  per flower, 5,100  $\mu\text{l}$  per inflorescence), while *M. urens* reached it at ca. 6 h after anthesis (ca. 310  $\mu\text{l}$  per flower, 310–930  $\mu\text{l}$  per inflorescence) (Fig. 1c, d). For both species, nectar secretion varied throughout the period of anthesis. For *M. japira*, two different stages of nectar secretion can be identified: the first and the second day of anthesis were comparable, ca. 200  $\mu\text{l}$ , but differed on the third, fourth, and fifth days of anthesis, when nectar accumulation reached ca. 340  $\mu\text{l}$ , ( $F_{[4,50]}=9.18$ ,  $p<0.0001$ ; Fig. 1c). For *M. urens*, three different stages of nectar secretion can be identified: at the beginning of the anthesis, ca. 70  $\mu\text{l}$ ; 2–4 h of anthesis, ca. 200  $\mu\text{l}$ ; and 6 h of anthesis, ca. 310  $\mu\text{l}$  ( $F_{[3,36]}=108.7$ ,  $p<0.0001$ ; Fig. 1d).

The total amount of sugar secreted by *M. japira* and by *M. urens* varied during the anthesis, being  $F_{[4,50]}=7.4$ ,  $p<0.0001$ ; (Fig. 1e) and  $F_{[3,36]}=91.0$ ,  $p<0.0001$ ; (Fig. 1f), respectively. The mean sugar secretion rate during the entire nectar secretion period for *M. japira* was 0.13 mg/h per flower and ca. 2 mg/h per inflorescence (Table 1) and for *M. urens* 5.07 mg/h per flower and ca. 5–15 mg/h per inflorescence. In addition, some variability in the nectar secretion pattern among flowers of both species can be inferred from standard deviations of Fig. 1. Independent of

**Fig. 1** Nectar concentration (a, b), volume (c, d), and total sugar (e, f) during anthesis in *Mucuna japura* (left) and *M. urens* (right). Anthesis period in *M. japura* is 7 days and in *M. urens* one night. For *M. japura*, 78 flowers were used of seven different individuals varying from 10 to 13 flowers per day of anthesis. For *M. urens*, 69 flowers were used of seven different individuals, varying from 9 to 12 flowers every 2 h of anthesis



the moment of anthesis that the flower receives a visit of pollinator, both species interrupt secretion after flower opening. These flowers were checked throughout their lifetime, and they did not resume nectar secretion.

Nectar resorption occurred in both species: in *M. japura*, the resorption period lasted ca. 2 days, while in *M. urens*, it lasted ca. 6 h (Fig. 1e, f). The nectar resorption rate in *M. japura* was ca. 0.25 mg/h per flower and ca. 4 mg/h per inflorescence, while in *M. urens*, it was ca. 3 mg/h per flower and 3–9 mg/h per inflorescence (Table 1).

#### Nectar composition

Nectar sugars were glucose, fructose, and sucrose for both *Mucuna* species (Table 2). Principal component analysis showed that the principal two axes provide separate ordinations of the 35 samples for nectar sugar composition of both species accounting for 98.6% of the total variation (Fig. 2). The variable “fructose” is the most

important to separate samples of both species on axis 2; samples of *M. urens* are located at the left side while those samples of *M. japura* at the right side (Fig. 2). The other variables separate samples of different “plants” and “flowers” within each species on axis 1 (Fig. 2). Some “plants” showed different nectar sugar composition among flowers (scattered pattern: for example “plant 1” for *M. urens* or “plant 6” for *M. japura*; Fig. 2), but other “plants” showed a comparable nectar sugar composition among flowers (congregate pattern: for example “plants 3 and 5” for *M. urens* or “plant 7, 8, and 9” for *M. japura*; Fig. 2). Nectar composition varied between species, among plants and flowers of the same individual (Table 2; Fig. 2). Nevertheless, statistical comparisons showed differences between species but not among plants (Table 2). Percentage of sucrose for *M. urens* and for *M. japura* was variable among individuals but did not differ between species (Table 2). On the contrary, percentages of glucose and fructose were lower in *M. urens* than in *M. japura*

**Table 1** Comparison of nectar traits between two species of *Mucuna*

Nectar traits	<i>M. japira</i>	<i>M. urens</i>
Amount at the bud stage	>55% of the maximum nectar secreted by a flower	<22% of the maximum nectar secreted by a flower
Secretion period	>5 days	8 h
Secretion rate/flower	0.13 mg/h	5.07 mg/h
Secretion rate/inflorescence	2 mg/h	5–15 mg/h
Time of maximum nectar accumulation/flower	During the fifth day (ca. 340 µl)	At midnight (ca. 310 µl)
Concentration	Constant (ca. 10%)	Constant (ca. 16%)
Interruption after nectar removal	Yes	Yes
Resorption period	2 days	6 h
Resorption rate/flower	0.25 mg/h	3 mg/h
Resorption rate/inflorescence	4 mg/h	3–9 mg/h
Sugar composition	“Unbalanced” (fructose predominant)	“Balanced” (comparable proportions of the three sugars)
Hexose dominant	Fructose	Glucose

*Mucuna japira* has 12–21 flowers/inflorescence, which are in anthesis simultaneously and last for 7 days. *M. urens* has 36–54 flowers/inflorescence, number of flowers in anthesis/inflorescence is 1–3 and anthesis period is one night

(Table 2). Hexose ratios indicated that the individuals of *M. japira* had more fructose than glucose and those of *M. urens* showed comparable proportions (Table 2; Fig. 2).

**Discussion**

Nectar features and secretion pattern

These *Mucuna* species showed similar nectar traits that can be related to the different pollinators of each one. Large

amounts of nectar seem to be a feature of several *Mucuna* species (von Helversen and von Helversen 2003), mainly those in which pollination by vertebrates occurs, because these pollinators have a higher body mass than other pollinators and they are endotherms (Stiles 1978; Faegri and van der Pijl 1980; Cruden et al. 1983; Ladley et al. 1997).

The large amount of nectar secretion (ca. 340 µl) and the very low sugar concentration (ca. 10%) of *M. japira* are well-suited features for its passerine pollinator *C. haemorrhous* (see details in Agostini et al. 2006) since such attributes are known for several plant species pollinated by passerine birds

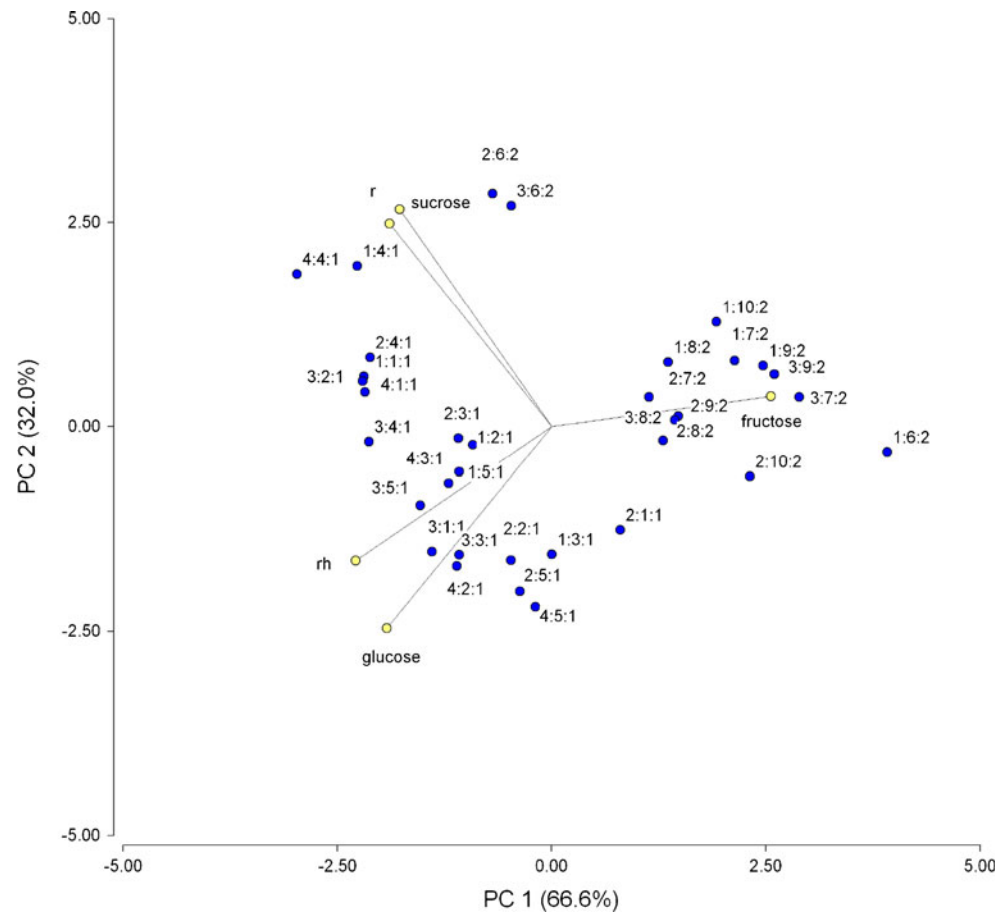
**Table 2** Percentages of nectar sugar composition of two *Mucuna* species

Plant	Fructose (F)	Glucose (G)	Sucrose (S)	$r = S/G + F$	$rh = G/F$
1 (n=4)	37.4±6.2 (0.33)	36.8±2.8 (0.15)	25.8±6.2 (0.48)	0.38±0.11 (0.60)	1.05±0.15 (0.29)
2 (n=4)	37.1±2.7 (0.15)	39.2±2.9 (0.15)	23.8±4.8 (0.41)	0.33±0.09 (0.55)	1.06±0.08 (0.15)
3 (n=4)	40.7±2.3 (0.11)	39.4±2.1 (0.11)	22.6±4.3 (0.38)	0.24±0.05 (0.41)	1.02±0.08 (0.16)
4 (n=4)	28.9±1.2 (0.13)	31.2±2.7 (0.17)	39.8±3.2 (0.16)	0.67±0.08 (0.25)	1.08±0.09 (0.17)
5 (n=4)	39.9±2.6 (0.20)	42.5±2.0 (0.09)	17.6±4.2 (0.48)	0.22±0.06 (0.55)	1.07±0.05 (0.10)
Mean <i>Mucuna urens</i> (n=20)	36.8±1.7 (0.20)	37.8±1.3 (0.16)	25.9±2.5 (0.44)	0.37±0.05 (0.60)	1.06±0.04 (0.17)
1 (n=3)	63.3±6.2 (0.44)	8.5±2.7 (0.55)	28.5±6.6 (0.82)	0.48±0.24 (0.84)	0.17±0.07 (0.71)
2 (n=3)	72.9±6.3 (0.15)	9.9±4.1 (0.71)	17.2±2.6 (0.26)	0.21±0.04 (0.33)	0.15±0.07 (0.85)
3 (n=3)	63.9±0.7 (0.02)	17.5±2.5 (0.24)	18.6±1.9 (0.18)	0.23±0.03 (0.23)	0.27±0.04 (0.25)
4 (n=3)	74.4±4.7 (0.11)	9.0±4.2 (0.82)	16.5±0.6 (0.06)	0.20±0.01 (0.08)	0.13±0.07 (0.93)
5 (n=3)	72.4±1.1 (0.03)	11.9±4.0 (0.58)	15.7±4.4 (0.48)	0.19±0.06 (0.57)	0.16±0.05 (0.57)
Mean <i>M. japira</i> (n=15)	69.3±3.3 (0.18)	11.37±1.6 (0.55)	19.3±2.77 (0.56)	0.26±0.05 (0.76)	0.18±0.03 (0.59)

Nectar composition is analyzed showing individual sugars (fructose, glucose, and sucrose) and sugar ratios ( $r$  and  $rh$ ) between species and among plants. Data are mean±S.E. (coefficient of variation). Statistical differences were found between species for hexoses (fructose,  $F_{1, 34}=82.5, P=0.0001$ ; glucose,  $F_{1, 34}=186.9, P=0.0001$ ;  $rh, F_{1, 34}=229.3, P=0.0001$ ) but not for sucrose ( $F_{1, 34}=2.5, P=0.08$ ) and sugar ratio ( $r, F_{1, 34}=1.34, P=0.10$ ). Comparisons among plants within species were not statistically significant for all variables (results not showed)



**Fig. 2** Plot of PCA scores for 35 samples of two co-occurring *Mucuna* species, showing first two principal component axes from the analysis of nectar sugar composition considering species, plant, and flower identity. Vectors correspond to the variables included in the PCA (fructose, glucose, and fructose percentages, hexose and sugar ratios = rh and r, respectively). Numbers for each sample correspond to flower:plant:species, respectively (see Table 2). Species #1=*Mucuna urens*; species #2=*M. japira*



(Johnson and Nicolson 2008). Passerine birds need a large amount of nectar, which is an expensive investment for the plant which also needs to produce floral structures and accumulate nectar (Stiles 1978). There are several congruent hypotheses to explain the low nectar concentration in the flowers pollinated by passerine birds: (1) nectar extraction by birds is more efficient (Baker 1975); (2) it does not encourage bees (Bolten and Feinsinger 1978); (3) it offers a sufficient amount of water to the birds (Baker 1975; Calder 1979; Sazima et al. 2009), and (4) it can encourage visits by birds, since the amount of sugar is low and the energy intake from few visits is not sufficient (Martínez del Rio et al. 2001; Nicolson 2007; Koehler et al. 2010).

The large nectar volume (ca. 310  $\mu$ l) and low sugar concentration (ca. 17%) recorded for *M. urens*, among other features may be related to chiropterophily (Faegri and van der Pijl 1980; von Helversen 1993), and its pollination by the bat *G. soricina* was recorded some times (Sazima and Sazima 1978; Agostini 2008). The volume and the nectar concentration of *M. urens* are similar to several bat flowers as recorded for 33 bat-pollinated species of the Neotropics (von Helversen 1993). Nectarivorous bats usually need large amounts of nectar and those bats, such as *G. soricina*, mostly use exogenous sugar generally obtained from floral nectar to fuel their metabolically expensive

activities at night (Voigt and Speakman 2007). The lower nectar concentration of *M. japira* and *M. urens* can also be related to the chemical effect on sugar concentration. Nectars with predominance in hexoses can draw more water in the nectar due to increased osmolarity than sucrose-rich nectar (Nicolson 2007).

On the other hand, some characteristics of nectar production were different between these *Mucuna* species and can be related to flower longevity and flower opening strategy. First, total amount of sugar production is higher in *M. urens* than in *M. japira*. And second, in terms of patterns of nectar sugar production, since both *Mucuna* species produce a large amount of nectar but in different periods (5 days for *M. japira* and 6 h for *M. urens*), *M. japira* is a slow-producer (secretes 5% to 10% of its maximum per hour) and *M. urens* is a fast-producer (secretes 20% to 70% of its maximum per hour), following the definition of Cruden et al. (1983).

Further, *M. japira* 12–21 flowers/inflorescence which are in anthesis simultaneously and remain fresh and active for 7 days provide a large amount of nectar, favoring geitonogamous pollination since *C. haemorrhous* visits all the flowers in the same inflorescence (Agostini et al. 2006). Nevertheless, the observed among-flowers nectar variability (i.e., nectar production and nectar sugar composition) can

be counterbalancing geitonogamous pollinations if it promotes changes in the behavior of the pollinators. In the case of *M. urens*, despite of the high number (36–54) flowers/inflorescence, only 1–3 flowers are in anthesis simultaneously and last just one night, the amount of nectar offered by an inflorescence to pollinators is not enough to fulfill the requirements of the bats. Similarly, nectar variations (i.e., nectar production and nectar sugar composition) between flowers and plants could also affect bat foraging behavior. Therefore, bats like *G. soricina* which have a diet mostly based on nectar (von Helversen 1993; Winter and von Helversen 2001), need to search for resources in other *M. urens* plants, favoring cross pollination.

Surcease of nectar production in both species after the explosive flower opening mechanism is triggered can be related to the opportunity of just one pollination since after the explosive opening, the staminal column moves towards the standard petal, and in other visits (for intake of the remaining nectar), the pollinator does not contact the reproductive organs (Baker 1970; von Helversen and von Helversen 2003; Agostini et al. 2006). Some species of Loranthaceae with explosive flower opening do not stop producing nectar after the explosive mechanism is triggered but produce negligible nectar amounts, a feature not related by Ladley et al. (1997) to pollination possibilities.

The decrease in nectar in both species of *Mucuna* can be related to nectar resorption. Evaporation is very likely excluded because the nectar is enclosed in a chamber without contact with the environment. Furthermore, the nectar volume would decrease and nectar concentration would increase if evaporation occurred. Nectar resorption has been reported more frequently in recent literature, which suggests that nectar resorption is not an unusual floral feature (Nepi and Stpicyńska 2008). Cruden et al. (1983) pointed out that resorption occurs when a maximum nectar production is reached and pollinators are inactive. Even though resorption may be energetically an expensive alternative, a flower that reabsorbs nectar can reclaim at least a part of the energy allocated for nectar production (Búrquez and Corbet 1991). This strategy of resource recovery has recently been demonstrated or hypothesized (Búrquez and Corbet 1991; Pyke 1991; Stpicyńska 2003a, b; Nepi and Stpicyńska 2007). For *M. urens* which produces more fruits and seeds than *M. japira* (Agostini 2008), nectar resorption may be an important resource for the development of the seeds since nectar resorption rate per flower for *M. urens* is higher than for *M. japira*. Besides, based on preliminary results on pollination treatments (Agostini 2008), we suggest that nectar resorption plays an important role for fruit and seed set of *M. urens*. Ecological consequences of nectar resorption have rarely been considered (Búrquez and Corbet 1991; Luyt and Johnson 2002; Ordano and Ornelas 2004). Thus,

resorption of nectar may have evident advantages since this process can increase the resources available to developing seeds (Búrquez and Corbet 1991; Pyke 1991). However, other factors can increase fruit and seed set, if we consider that successful reproduction can be strongly affected by the origin and amount of pollen deposited on stigmas (Morgensen 1975).

#### Nectar composition

Nectar sugar composition of these *Mucuna* species showed some similarities that are discussed in relation to the different pollinators. Most of samplings of *M. japira* flowers present nectars with a predominance of fructose but with 15–28% of sucrose. However, according to Lotz and Schondube (2006), oriole species (Icteridae) prefer nectar with predominance of hexoses over sucrose when the nectar has a low (15%) concentration. The higher proportion of fructose in the nectar of *M. japira* can be appropriate to the diet of *C. haemorrhous* because sugar proportions would be similar to sugars that occur in fruit pulps (Baker et al. 1998). *C. haemorrhous* is an insectivorous/frugivorous bird, including a high proportion of insects and fruits in its diet (Sick 1985; Pizo 1996; Ragusa-Netto 2002) in addition of nectar. Usually, flowers pollinated by New World passerine birds have hexose predominance in their nectar (Baker and Baker 1983; Baker et al. 1998). According to Martínez del Rio et al. (1992), New World nectar-feeding passerines have poor sucrose-digesting abilities and prefer hexoses (like fructose).

The majority of *M. urens* flowers, pollinated by *G. soricina* (Sazima and Sazima 1978), had balanced nectar (comparable proportions of fructose, glucose, and saccharose), while Baker and Baker (1983) and Baker et al. (1998) suggested that chiropterophilous flowers have nectars with hexose predominance. Although some flowers of the sampling of *M. urens* had nectar with a considerable amount of sucrose, according to Voigt and Speakman (2007), this nectar could be consumed by *G. soricina* since sucrase, the enzyme that hydrolyses the disaccharide into its monosaccharides, is abundantly present in the digestive tract of this bat species.

Nectar chemistry, including sugar proportion, may differ among individuals, populations, cultivars, or subspecies of the same species (e.g., Rivera et al. 1996; Torres and Galetto 1998; Biemaskie and Cartar 2004; Galetto and Bernardello 2005; Herrera et al. 2006; Canto et al. 2007), independently of the main pollinators of the species. The broad intra-specific variation in nectar sugar composition exhibited by the two *Mucuna* species could have important effects on the foraging patterns of pollinators and, thus, presumably, on the selective pressures exerted by them on that floral trait (Herrera et al. 2006; Canto et al. 2007). One

hypothesis suggested by Biernaskie and Cartar (2004) and Herrera et al. (2006) is that within-plant variation in nectar sugar composition, acting alone or in concert with variation in nectar volume, may likewise be advantageous to plants by decreasing the number of flowers visited per plant by variance-sensitive, risk-averse pollinator foragers, thus representing one further adaptive mechanism reducing geitonogamy. Petanidou et al. (1996) suggested that between-plant variability of nectar sugar composition can be due to a casual selection of flowers of different ages, because in some cases, sucrose breakdown in nectar can be related to flower age. The flowers of both *Mucuna* species were randomly chosen, but since the nectar of unvisited flowers of these species is not directly exposed to the environment due to constraints related to floral morphology (Agostini et al. 2006), microorganism activity to alter sugar composition may not occur.

Faboideae flowers with explosive opening mechanism secrete large amounts of nectar. It is likely that these flowers have their origin from those in which the major source was nectar (Arroyo 1981). In general, sucrose, glucose, and fructose are the sugars in the nectar, and no other type was detected, but most species have a high proportion of sucrose and relative low proportions of fructose and glucose (van Wyk 1993). Generally in this subfamily, the two hexoses occur in more or less equal proportions but often with slightly more fructose than glucose (van Wyk 1993), as is the case in *M. japira*, but not in *M. urens*, in which the proportion of glucose is higher than fructose.

Further, little is known about nectar resorption in Faboideae: some species from Argentina do not reabsorb nectar (Cocucci et al. 1992; Galetto et al. 2000), whereas *Cologania broussonetii* actively reabsorbs it (Musicante and Galetto 2008), as in both *Mucuna* species. Although more data for different species are needed, it is possible that nectar concentration, total amount of nectar produced per flower, nectar secretion pattern, and the possibility of nectar resorption seem to be conservative within the *Mucuna*.

*M. japira* and *M. urens* are dependent on their pollinators to produce fruits and seeds, and they display different characteristics in their flowers that can be interpreted as divergent strategies to promote the necessary interaction with birds or bats, respectively. Nevertheless, some floral features of these *Mucuna* species like color, odor, and flower longevity showed clear differences and seem to change easily during speciation. In contrast, other functional features such as the explosive flower opening mechanism or the pattern of nectar secretion seem to be less altered during the process of diversification.

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## References

- Agostini K (2008) Ecologia da reprodução de duas espécies de *Mucuna* (Leguminosae, Faboideae, Phaseoleae)—embriologia, citogenética e variabilidade genética—do litoral norte de São Paulo. Thesis. Universidade Estadual de Campinas
- Agostini K, Sazima M, Sazima I (2006) Bird pollination of explosive flowers while foraging for nectar and caterpillars. *Biotropica* 38:674–678
- Agostini K, Sazima M, Tozzi AMGA, Forni-Martins ER (2009) Microsporogenesis and pollen morphology of *Mucuna japira* Azevedo, Agostini & Sazima and *M. urens* (L.) Medikus. *Phytomorphology* 59:61–69
- Arroyo MTK (1981) Breeding systems and pollination biology in Leguminosae. In: Polhill RM, Raven PH (eds) *Advances in legume systematics*, part 2. Royal Botanic Garden, Kew, pp 723–769
- Baker HG (1970) Bat pollination in Central America. *Rev Biol Trop* 17:187–197
- Baker HG (1975) Sugar concentration in nectars from hummingbird flowers. *Biotropica* 7:37–41
- Baker HG, Baker I (1983) Floral nectar sugar constituents in relation to pollinator type. In: Jones CE, Little RJ (eds) *Handbook of experimental pollination biology*. Van Nostrand Reinhold Co., New York, pp 117–141
- Baker HG, Baker I, Hodges SA (1998) Sugar composition of nectars and fruits consumed by birds and bats in the tropics and subtropics. *Biotropica* 30:559–586
- Bernardello LM, Galetto L, Jaramillo J, Grijalba E (1994) Floral nectar and chemical composition of some species from Reserva Río Guajalito, Ecuador. *Biotropica* 26:113–116
- Biernaskie JM, Cartar RV (2004) Variation in rate of nectar production depends on floral display size: a pollinator manipulation hypothesis. *Funct Ecol* 18:125–129
- Bolten AB, Feinsinger P (1978) Why do hummingbird flowers secrete dilute nectar? *Biotropica* 10:307–309
- Búrquez A, Corbet SA (1991) Do flowers reabsorb nectar? *Funct Ecol* 5:369–379
- Calder WA (1979) On the temperature-dependency of optimal nectar concentrations for birds. *J Theor Biol* 78:185–196
- Canto A, Pérez R, Medrano M, Castellanos MC, Herrera CM (2007) Intra-plant variation in nectar sugar composition in two *Agulegia* species (Ranunculaceae): contrasting patterns under field and glasshouse conditions. *Ann Bot* 99:653–660
- Castellanos MC, Wilson P, Thomson JD (2002) Dynamic nectar replenishment in flowers of *Penstemon* (Scrophulariaceae). *Am J Bot* 89:111–118
- Cocucci AA, Galetto L, Sérsic A (1992) El síndrome floral de *Caesalpinia gilliesii* (Fabaceae—Caesalpinioideae). *Darwiniana* 31:111–135



- Cruden RW, Hermann SM, Peterson S (1983) Patterns of nectar production and plant–pollinator coevolution. In: Bentley B, Elias TS (eds) *The biology of nectaries*. Columbia University Press, New York, pp 80–125
- Digby PGN, Kempton RA (1996) *Multivariate analysis of ecological communities*. Chapman and Hall, London
- Endress PK (1994) *Diversity and evolutionary biology of tropical flowers*. Cambridge University Press, Cambridge
- Faegri K, van der Pijl L (1980) *The principles of pollination ecology*. Pergamon Press, New York
- Freeman CE, Worthington RD, Jackson MS (1991) Floral nectar sugar compositions of some South and Southeast Asian species. *Biotropica* 23:568–574
- Galetto L, Bernardello L (1992) Nectar secretion pattern and removal effects in six Argentinean Pitcairnioideae (Bromeliaceae). *Bot Acta* 105:292–299
- Galetto L, Bernardello G (1993) Nectar secretion pattern and removal effects in three species of Solanaceae. *Can J Bot* 71:1394–1398
- Galetto L, Bernardello G (1995) Characteristics of nectar secretion by *Lycium cestroides*, *L. ciliatum* (Solanaceae) and their hybrid. *Plant Species Biol* 11:157–163
- Galetto L, Bernardello G (2003) Nectar sugar composition in angiosperms from Chaco and Patagonia (Argentina): an animal visitor's matter? *Plant Syst Evol* 238:69–86
- Galetto L, Bernardello G (2004) Floral nectaries, nectar production dynamics and chemical composition in six *Ipomoea* species (Convolvulaceae) in relation to pollinators. *Ann Bot* 94:269–280
- Galetto L, Bernardello G (2005) Nectar. In: Dafni A, Kevan P, Husband BC (eds) *Practical pollination biology*. Enviroquest, Ontario, pp 261–313
- Galetto L, Bernardello G, Rivera G (1997) Nectar, nectaries, flower visitors, and breeding system in some Argentinean Orchidaceae. *J Plant Res* 110:393–403
- Galetto L, Bernardello G, Isele IC, Vesprini J, Speroni G, Berdue A (2000) Reproductive biology of *Erythrina crista-galli* (Fabaceae). *Ann Mo Bot Gard* 87:127–145
- Herrera CM, Pérez R, Alonso C (2006) Extreme intra-plant variation in nectar sugar composition in an insect-pollinated perennial herb. *Am J Bot* 93:575–581
- Johnson SD, Nicolson SW (2008) Evolutionary associations between nectar properties and specificity in bird pollination systems. *Biol Lett* 4:49–52
- Koehler A, Verburgt L, McWhorter TJ, Nicolson SW (2010) Energy management on a nectar diet: can sunbirds meet the challenges of low temperature and dilute food? *Funct Ecol*. doi:10.1111/j.1365-2435.2010.01728.x
- Ladley JJ, Kelly D, Robertson AW (1997) Explosive flowering, nectar production, breeding systems, and pollinators of New Zealand mistletoes (Loranthaceae). *New Zeal J Bot* 35:345–360
- Lotz CN, Schondube JE (2006) Sugar preferences in nectar- and fruit-eating birds: behavioral patterns and physiological causes. *Biotropica* 38:3–15
- Luyt R, Johnson SD (2002) Postpollination nectar reabsorption and its implications for fruit quality in an epiphytic orchid. *Biotropica* 34:442–446
- Martínez del Río C, Baker HG, Baker I (1992) Ecological and evolutionary implications of digestive processes: bird preferences and the sugar constituents of floral nectar and fruit pulp. *Experientia* 48:544–551
- Martínez del Río C, Schondube JE, McWhorter TJ, Herrera LG (2001) Intake responses in nectar feeding birds: digestive and metabolic causes, osmoregulatory consequences, and coevolutionary effects. *Am Zool* 41:902–915
- Morgensen HL (1975) Ovule abortion in *Quercus* (Fagaceae). *Am J Bot* 62:160–165
- Musicante ML, Galetto L (2008) Características del néctar de *Cologania broussonetti* (Balb.) DC. (Fabaceae) y su relación con los visitantes florales. *Ecol Austral* 18:195–204
- Nepi M, Stpicyńska M (2007) Nectar resorption and translocation in *Cucurbita pepo* L. and *Platanthera chlorantha* Custer (Rchb.). *Plant Biology* 9:93–100
- Nepi M, Stpicyńska M (2008) The complexity of nectar: secretion and resorption dynamically regulate features. *Naturwissenschaften* 95:177–184
- Nicolson SW (2007) Nectar consumers. In: Nicolson SW, Pacini E, Nepi M (eds) *Nectaries and nectar*. Springer, The Netherlands, pp 289–342
- Ordano M, Ornelas JF (2004) Generous-like flowers: nectar production in two epiphytic bromeliads and a meta-analysis of removal effects. *Oecologia* 140:495–505
- Petanidou T, van Laere AJ, Smets E (1996) Change in floral nectar components from fresh to senescent flowers of *Capparis spinosa* (Capparidaceae), a nocturnally flowering Mediterranean shrub. *Pl Syst Evol* 199:79–92
- Pizo MA (1996) Feeding ecology of two *Cacicus* species (Emberizidae, Icterinae). *Ararajuba* 4:87–92
- Pyke GH (1991) What does it cost a plant to produce floral nectar? *Nature* 350:58–59
- Ragusa-Netto J (2002) Exploitation of *Erythrina dominguezii* Hassl. (Fabaceae) nectar by perching birds in a dry forest in western Brazil. *Braz J Biol* 62:877–883
- Rivera GL, Galetto L, Bernardello G (1996) Nectar secretion pattern, removal effects, and breeding system of *Ligaria cuneifolia* (Loranthaceae). *Can J Bot* 74:1996–2001
- Sazima I, Sazima M (1978) Polinização por morcegos em *Mucuna urens* (Leguminosae). In: Resumos da XXX Reunião da Sociedade Brasileira para o Progresso da Ciência: p. 419
- Sazima M, Buzato S, Sazima I (2003) *Dyssochroma viridiflorum* (Solanaceae): a reproductively bat-dependent epiphyte from the Atlantic rainforest in Brazil. *Ann Bot* 92: 725–730
- Sazima I, Sazima C, Sazima M (2009) A catch-all leguminous tree: *Erythrina velutina* visited and pollinated by vertebrates at an oceanic island. *Aust J Bot* 57:26–30
- Sick H (1985) *Omitologia brasileira, uma introdução*. Editora da Universidade de Brasília, Brasília
- Stiles FG (1978) Ecological and evolutionary implications of bird pollination. *Am Zool* 18:715–727
- Stiles FG, Freeman CE (1993) Patterns in floral nectar characteristics in some bird–plant species from Costa Rica. *Biotropica* 25:191–205
- Stpicyńska M (2003a) Nectar resorption in the spur of *Platanthera chlorantha* Custer (Rchb.) Orchidaceae—structural and microautoradiographic study. *Plant Syst Evol* 238:119–126
- Stpicyńska M (2003b) Incorporation of [3H] sucrose after the resorption of nectar from the spur of *Platanthera chlorantha* (Custer) Rchb. *Can J Bot* 81:927–932
- Sweeley EC, Bentley R, Makita M, Wells WW (1963) Gas liquid chromatography of trimethylsilyl derivatives of sugars and related substances. *J Am Chem Soc* 85:2497–2507
- Torres C, Galetto L (1998) Patterns and implications of floral nectar secretion, chemical composition, removal effects and standing crop in *Mandevilla pentlandiana* (Apocynaceae). *Bot J Linn Soc* 127:207–223
- Tozzi AMGA, Agostini K, Sazima M (2005) A new species of *Mucuna* Adans. (Leguminosae, Papilionoideae, Phaseoleae) from southeastern Brazil, with a key to Brazilian species. *Taxon* 54:451–455

- Van Wyk BE (1993) Nectar sugar composition in southern African Papilionoideae (Fabaceae). *Biochem Syst Ecol* 21:271–277
- Voigt CC, Speakman JR (2007) Nectar-feeding bats fuel their high metabolism directly with exogenous carbohydrates. *Funct Ecol* 21:913–921
- Von Helversen O (1993) Adaptations of flowers to the pollination by Glossophagine bats. In: Barthlott W (ed) *Plant–animal interactions in tropical environments*. Museum Alexander Koenig, Bonn, pp 41–59
- Von Helversen D, von Helversen O (2003) Object recognition by echolocation: a nectar-feeding bat exploiting the flowers of a rain forest vine. *J Com Phys A* 189:327–336
- Willson MF (1983) *Plant reproductive ecology*. John Wiley and Sons, New York
- Winter Y, von Helversen O (2001) Bats as pollinators: foraging energetics and floral adaptations. In: Chittka L, Thomson JD (eds) *Cognitive ecology of pollination*. Cambridge University Press, Cambridge, pp 148–170