



Seed dispersal for the unusual inflated berries of *Burmeistera* (Campanulaceae)

Diana Gamba^{a*} , N. Rossana Maguiña^a, Camilo A. Calderón-Acevedo^a , Karina Torres^b and Nathan C. Muchhala^a

^aDepartment of Biology, University of Missouri – St. Louis, St. Louis, MO, USA; ^bDepartamento de Biología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador

(Received 22 June 2016; accepted 6 November 2016)

Examining dispersal is critical for understanding the diversity of Andean-centered plant lineages, like *Burmeistera* (Campanulaceae). One-third of its species present an unusual inflated berry. Unlike the bright colors of non-inflated fruits in the genus, these fruits are typically dull-green; however, the fact that the seeds are loosely held in the placenta and easily removed when touched seems to suggest adaptation to animal dispersal. We studied two inflated-baccate species, *Burmeistera glabrata* and *B. borjensis*, with the aim of testing the non-exclusive hypotheses that their seeds are dispersed by (1) small mammals, (2) slugs, or (3) adult flies that develop inside the fruits. In two sites in the Ecuadorian Andes, we performed observations at dusk and dawn to examine the fate of fruits and seeds; recording fruit fall, formation of holes in the fruits, and seed loss from the placenta. We documented fruit visitors with cameras, and surveyed unopened fruits for the presence of insect larvae and seed condition. Finally, we performed an experiment to examine the effect of holes and rain in germination, in order to evaluate if holes are required for seeds to leave the fruits and subsequently germinate. For both species, most fruits fell and decomposed beneath the mother plant. However, we found limited support for small mammal dispersal; videos and observations revealed that mice and squirrels are potential, but rare, seed dispersers. We found no evidence for slug or fly dispersal; fly larvae were common inside fruits, but acted exclusively as seed predators. Crickets often chewed holes in fruits on plants and on the ground. Holes did not have an effect on germination, which was induced only by rain. Hence, the majority of seeds end up under the mother plant, with rare but potentially important events of primary or secondary dispersal by small mammals. The combination of limited dispersal due to gravity and rare events of mammal dispersal may have played a critical role in the rapid diversification of *Burmeistera*.

Keywords: Andes; *Burmeistera*; cloud forest; inflated berries; limited dispersal

Estudiar la dispersión de semillas es importante para entender la gran diversidad de plantas andinas, como *Burmeistera* (Campanulaceae). Un tercio de sus especies se caracteriza por tener bayas infladas muy inusuales. A diferencia de las bayas coloridas no infladas de las otras especies en este género, estos frutos son verde opaco. Sin embargo, sus semillas están débilmente adheridas a la placenta, y son fácilmente removibles al tacto, sugiriendo una adaptación a dispersión biótica. Estudiamos dos especies con bayas infladas, *Burmeistera glabrata* y *B. borjensis*, con el fin de poner a prueba tres hipótesis no exclusivas de dispersión de semillas por: (1) mamíferos pequeños, (2) babosas, (3) moscas adultas que se desarrollan dentro del fruto. En dos sitios de los Andes ecuatorianos realizamos observaciones al amanecer y al anochecer para examinar el destino de frutos y semillas; registramos la caída de frutos, la formación de huecos en los frutos, y la pérdida de semillas. Documentamos visitantes de frutos por medio de cámaras, e inspeccionamos frutos aún sin abrir para evaluar la presencia de larvas y la condición de las semillas. Finalmente realizamos un experimento para evaluar el efecto de huecos y de lluvia en la germinación de semillas, con el fin de verificar si la presencia de huecos es necesaria para que las semillas salgan del fruto y subsecuentemente germinen. Para ambas especies, la mayoría de frutos se cayeron y descompusieron debajo de la planta madre. Sin embargo, dispersión por mamíferos pequeños también ocurrió, aunque fue muy escasa; videos y observaciones revelaron ratones y ardillas alimentándose de frutos en raras ocasiones. No encontramos evidencia de dispersión por babosas o moscas; larvas de moscas fueron comunes dentro de frutos, pero actuaron exclusivamente como predadores de semillas. Documentamos grillos haciendo huecos frecuentemente en los frutos. Los huecos no influyeron en la germinación de semillas, la cual fue inducida sólo por la lluvia. Aunque la mayoría de semillas simplemente caen bajo la planta madre, los eventos escasos de dispersión por mamíferos pequeños pueden ser de gran importancia. La combinación de dispersión limitada y eventos raros de dispersión por mamíferos que documentamos en este estudio pudo haber jugado un rol importante en la rápida diversificación de *Burmeistera*.

Palabras clave: Andes; *Burmeistera*; bosque nublado; bayas infladas; dispersión limitada

*Corresponding author. Email: dlgtk5@mail.umsl.edu

Introduction

Seed dispersal is a critical stage in the life history of plants [1,2], and different species can vary widely in how far they are able to disperse their seeds [3–5]. This dispersal ability largely determines the spatial distribution of individuals [6,7] and the connectivity of populations through gene flow [1,8–10]. Thus identifying dispersal mechanisms is critical for understanding processes of diversification, particularly in species-rich regions such as tropical rainforests; however, in these same regions such information is often lacking.

Here, we study the dispersal of a highly unusual fruit type found in the genus *Burmeistera* H. Karst. & Triana. The thin walls of these berries surround an air-filled expansion, with hundreds of tiny seeds loosely held in a central placenta (Figure 1). Given this structure and their dull coloration (green with occasional magenta splotches), it is not readily obvious to what dispersal agent these fruits may be adapted. Approximately one-third of the ~120 species of *Burmeistera* have these fruits [11], while the other

two-thirds have brightly colored fleshy fruits (red, pink, purple, yellow, orange, or blue) suggestive of bird dispersal syndrome [12,13]. Preliminary observations support bird dispersal for these fleshy fruits, as they often disappear from plants with the subtending pedicel left behind, while inflated fruits typically simply fall to the ground along with the pedicel when ripe [11]. Interestingly, dipteran larvae have been found inside these inflated fruits (as well as a slug on one occasion), and holes of various sizes (from pinpoints to most of the fruit) regularly appear in the outer wall on fruits both on the mother plant and on the ground [Muchhala, pers. obs.]. *Burmeistera* fruits are indehiscent, unlike the dehiscent capsules of the closely related genus *Siphocampylus* [14], thus holes may aid in allowing seeds to be washed out by rain. Additionally, the mature seeds are somewhat sticky to the touch and readily dislodge from the placenta, suggestive of adaptation to adhere to the bodies of dispersers.

In this study, we aim to test three non-exclusive hypotheses as to the principle mode of dispersal of these

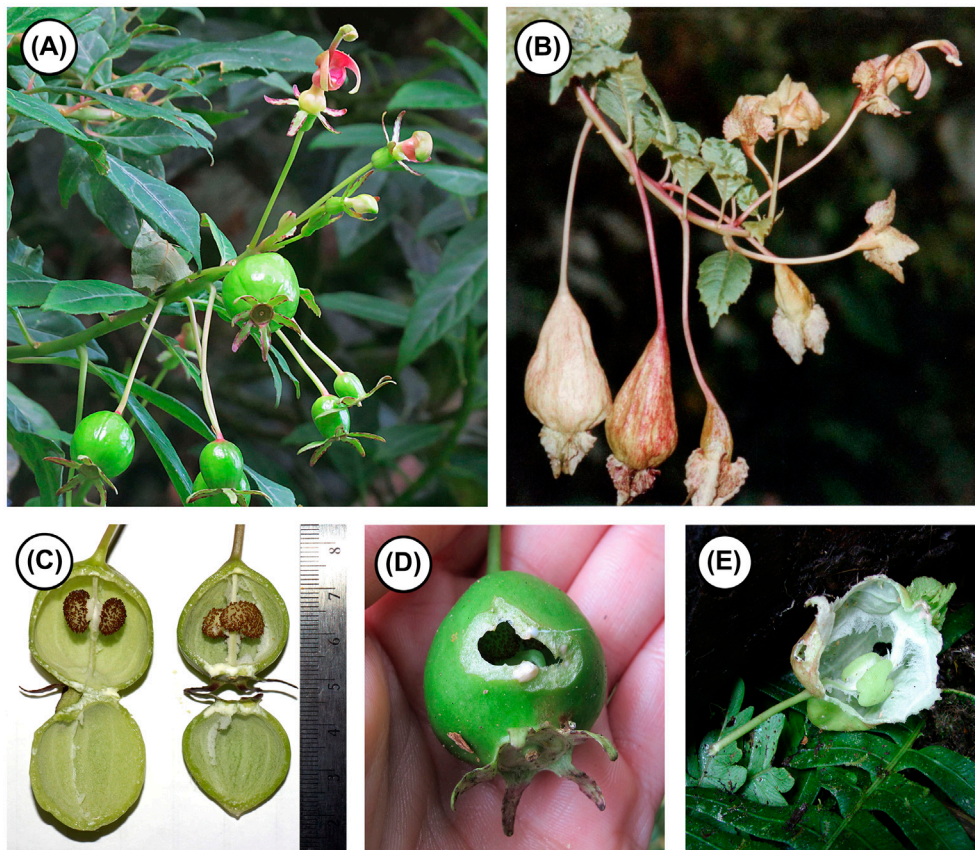


Figure 1. The unusual inflated fruits of species of *Burmeistera*. (A) Fruits and flowers of *B. glabrata* (photo by Dick Culbert). (B) Fruits and flowers of *B. borjensis*. (C) Bisectioned fruits of *B. glabrata*, showing central placenta with attached seeds, with ruler for scale (in cm). (D) Fruit of *B. glabrata* with a hole made by a cricket; note milky latex and undisturbed seeds inside. (E) Fruit of *B. borjensis* on the ground, the day after a nocturnal videotaping of a mouse chewing on outer fruit wall; note the central placenta with the majority of seeds missing.

fruits: (1) Rodents or other small mammals consume the outer wall and/or placenta; seeds attach to their fur; (2) Slugs chew holes in fruit walls; seeds attach to their bodies; (3) Dipteran larvae develop inside the fruits, molt, and the adult flies carry off seeds. We focused on two inflated-baccate species of *Burmeistera* in different cloud-forest sites in Ecuador, *B. glabrata* (Kunth) Benth. & Hook. f. ex B.D. Jacks. and *B. borjensis* Jeppesen, documenting visitation using video cameras and camera traps, and tracking seed and fruit fates via twice-daily observations (at dusk and dawn) for six weeks, noting presence of holes, seed content, and whether fruits are on the plant or on the ground. We also surveyed a number of unopened fruits for the presence of larvae. Finally, to explore the role of holes in seed dispersal, we performed an experimental manipulation to evaluate germination of seeds in fruits with/without holes and with/without exposure to rain. Our study represents the first effort to clarify seed dispersal of these unusual inflated fruits.

Materials and methods

Study sites and study species

Burmeistera contains approximately 120 species distributed from Guatemala to Peru, with a center of diversity in the Andean cloud forests of Colombia and Ecuador [14,15]. Extensive pollination studies show that species are primarily bat-pollinated, with secondary pollination by hummingbirds [16–18], yet dispersal modes remain unknown. Here, we focus on two species with inflated fruits: *B. glabrata* in Wildsumaco Biological Station (0°40'S, 77°35'W) and *B. borjensis* in Yanayacu Biological Station (0°36'S, 77°53'W). Both sites are located in the province of Napo, on the eastern slopes of the Ecuadorian Andes. Wildsumaco sits near the buffer zone of the Sumaco National Park and International Biosphere Reserve, close to the small villages of Pacto Sumaco and Guagua Sumaco. The elevation is about 1400 m and the habitats include primary lower montane rain forest, as well as secondary forest and pasture land. Yanayacu is located on the slope of the Antisana Volcano, five kilometers west of the town of Cosanga. The research station is at an elevation of 2100 m and the habitat around it comprised primary cloud forest, and connects directly to the Antisana Ecological Reserve. The remaining land is abandoned cattle pasture, interspersed with naturally occurring *Chusquea* bamboo and secondary cloud forest. Precipitation seasonality in both places corresponds to that of the Ecuadorian Amazon Basin, specifically to the high intra-Andean basins, where there is a marked bimodal rainfall regime, with peaks in March–April and in September–October, and a drier season from June to August. Nevertheless, rainfall is common throughout the year in both sites with total annual rainfall ranging from 2300 to 3500 mm [19,20].

The *Burmeistera* species studied here are freestanding herbs to subshrubs that climb nearby vegetation [21]. These species commonly grow in forest gaps and forest edges, frequently among disturbed vegetation or in secondary forests. Berries of both species are green and greatly inflated when mature; those of *B. glabrata* are typically globose and ca. $2\text{--}3.5 \times 1.5\text{--}2$ cm, and those of *B. borjensis* are ovoid and slightly larger, ca. $3\text{--}3.5 \times 2.5$ cm [16,21]. Although both species occur in the Napo province, they do not seem to co-exist in any locality from current known collections. *Burmeistera glabrata* is fairly widespread in Colombia (in the departments of Antioquia, Cundinamarca, and Putumayo) and Ecuador (in the provinces of Carchi, Napo, Morona-Santiago, Pastaza, Pichincha, Sucumbios, and Tungurahua), while *B. borjensis* is endemic to the Napo province [21].

Quantitative estimations and twice-daily observations

From 15 June to 26 July 2014, we marked 11 individual plants and followed a total of 118 fruits in *B. glabrata*, and 20 plants and 46 fruits in *B. borjensis*. Plants were marked with flagging tape and chosen along hiking trails within sites and at least 3 m apart from each other. Fruits were marked with white masking tape and a unique number written with black waterproof ink. Marked fruits on the same plant were distributed from the bottom to the top of plants. Eight of these marked fruits were found on the ground below plants at the beginning of observations in *B. glabrata*, and three in *B. borjensis*. We measured each fruit every three days to estimate average growth rate per day. We also calculated the average size at which fruits fell beneath the plant, and how long it took for fruits to rot or disappear from the ground. In order to cover the time frame of fruit development, the marked fruits ranged from immature (ca. 2×2 cm, for both species) to mature (ca. $3\text{--}3.5 \times 2.5$ cm, in *B. glabrata*, and ca. 3.5×3.5 cm, in *B. borjensis*) for both species.

In order to document when fruit drop, hole formation, and seed loss occurred, we performed observations twice a day on each of these fruits, at 0600 and 1800 h, recording the position of the fruit (on ground, on plant, or gone), the number of holes (if present), and the percent of seeds still attached to the placenta (when visible through holes; 0, 25, 50, 75, or 100%). We assumed 100% seed presence for fruits without holes.

Video and photographic records

We followed fruits with video cameras and camera traps to register possible fruit visitors, whether seed predators or seed dispersers. For *B. glabrata*, we videotaped five fruits on four *B. glabrata* plants, one of which we continued recording after it fell to the ground. For *B. borjensis*, we videotaped three fruits on different plants, and five

fruits on the ground. We recorded during the day and night using a SONY camcorder with a Nightshot function (SONY Corporation of America, USA). Additionally, we used RECONYX camera traps with infrared mode (RECONYX, Inc., Holmen, Wisconsin, USA) to follow fruits (2–7) of five *B. glabrata* plants and two *B. borjensis* plants. We set the camera in the RC55 Rapidfire mode to take photographs every minute (day and night; the infrared automatically activates at night), and to take five instant photographs per second when movement was detected.

Insect larval survey

In order to evaluate the frequency of insect larvae inside *B. glabrata* fruits, we chose randomly five mother plants and collected a total of 240 mature fruits from the ground and from plants at the end of our study (on 2 August 2014). Fruits were either intact (200 fruits), or presented a pinpoint hole on the pericarp (40 fruits). Fruits attached to plants were chosen along the height of each plant. We opened all fruits, recording larvae presence and the condition of the placenta and seeds (if intact, damaged, or gone).

Effect of holes and rain on B. glabrata germination

In June–August 2015, we set up an experiment at Wildsumaco Biological Station in order to assess whether holes in *B. glabrata* fruits promote seed germination by allowing rain to wash the seeds out of the fruit. On 7 June 2015, we picked 40 intact mature fruits with similar dimensions (ca. 3.5×3 cm) from 20 different plants. We artificially made one hole (around 5 mm diameter) in 20 fruits and left the other 20 intact. We placed each fruit in separate plastic containers (10 cm diameter, 6 cm tall) with soil collected from the area surrounding the station. We positioned 20 of these under a roof sheltered from the rain and 20 under open sky exposed to the rain, placing equidistantly 10 fruits with holes and 10 with no holes under each condition. We monitored the fruits through 7 July 2015. On 3 August 2015 we counted the number of resulting seedlings for each pot. We analyzed results with a two-way ANOVA in R v.3.1.1 [R Core Team 2013], with the presence/absence of rain as one factor, the presence/absence of holes as a second factor, and the number of seedlings as the response variable.

Results

Quantitative estimations and twice-daily observations

Of the 110 fruits of *B. glabrata* that we began to follow while still on the plant, 58 (53%) remained attached to the plant after 42 days of observations, 49 (45%) fell to

the ground beneath the plant (with the pedicel attached to the fruit), and only three (2%) disappeared from the plant (with the pedicel still attached to the plant; Table 1). Fruits increased in size slowly while on the plant at an average rate of 0.2 mm (± 0.2 SD) in length per day and 0.2 mm (± 0.2 SD) in width per day ($N = 105$). The mean size just before fruits fell off the plant was 3 cm (± 0.6 SD) \times 2.5 cm (± 0.5 SD; $N = 49$). Of the fruits that fell to the ground, 11 remained there at the end of our recording period, and the other 38 fully decomposed below the plant; i.e., the seeds were liberated on the ground, thus none of these fruits were secondarily dispersed from the ground. We recorded holes formed in 50 fruits (42%); these appeared while fruits were attached to the plant (20 fruits) or after they had fallen to the ground (30 fruits). For half of the fruits with holes formed while on the plant (10 of 20), and all fruits with holes formed on the ground (30), seeds began to disappear from the central placenta one to three days after hole formation.

In *B. borjensis*, of the 43 fruits that we started to track while still on the plant, 21 (49%) remained attached to the plant after the 42 days of our observations, 20 (46%) fell to the ground below the plant, and only 2 (5%) disappeared from the plant (Table 1). These fruits increased in size while on the plant at an average rate of 0.3 mm (± 0.2 SD) in length per day and 0.4 mm (± 0.3 SD) in width per day ($N = 37$). The mean size just before fruits fell off beneath the plant was 3.8 cm (± 1.3 SD) \times 3.3 cm (± 1.1 SD) ($N = 19$). Of the fruits that fell to the ground, 2 remained there at the end of our recording period, 15 fully decomposed below the plant, and 3 disappeared around two weeks after falling to the ground (Table 1). We documented holes formed in 23 fruits (50%); these appeared while the fruits were attached to the plant (13 fruits) or after they had fallen to the ground (10 fruits). All fruits which presented holes while on the plant and on the ground began to lose seeds around two days after holes were formed.

Most dispersal-related activity occurred at night for both species (Figures 2 and 3). From the total fruits that fell to the ground, 71 and 60% fell during the night in *B. glabrata* and *B. borjensis*, respectively (Figure 2). Of the three fruits that disappeared from a plant in *B. glabrata*, two did so at night and one during the day.

Table 1. Dispersal-related fates of fruits tracked over one month for two *Burmeistera* species, numbers in parentheses correspond to fruits that disappeared from the ground.

Fruit	<i>B. glabrata</i>	<i>B. borjensis</i>
Remained on plant until end	58	21
Fell beneath plant	49 (0)	20 (3)
Disappeared from plant	3	2
Total followed	118	46

In *B. borjensis*, the two fruits that disappeared from a plant did so at night, as did the three fruits that disappeared from the ground. We documented a total of 96 holes formed in 50 fruits of *B. glabrata*, and a total of 60 holes in 23 fruits of *B. borjensis*. For *B. glabrata*, the great majority were formed during the night (88%) and in fruits on the ground (77%; Figure 3(A)). For *B. borjensis*, 68% of holes were formed during the night and 52% in fruits on the ground (Figure 3(A)). We recorded a total of 86 events of seed loss for *B. glabrata* and 75 for *B. borjensis*. In the former species, 81% of these events happened in fruits on the ground, and 78% during the night (Figure 3(B)). In the latter species, 75% of seed loss occurred in fruits on the ground, and 69% of seed loss was recorded at night (Figure 3(B)).

Video-photographic records

With the SONY camcorders we recorded a total of 51.28 h in *B. glabrata*, of which 13.95 were videotaped during the day, and 37.33 during the night. We recorded occasional activity throughout the day and night corresponding to unidentified insects landing on the surface of fruits. On two occasions, crickets were recorded eating the outer shell of the fruits for more than five minutes each time. They landed on the fruit surface and ate the pericarp, while leaving the placenta and seeds intact and produced holes similar to those we documented from our observations in *B. glabrata*, around 0.5–2 cm in diameter. In *B. borjensis*, we recorded a total of 17.12 h, of which 5.12 were videotaped during the day, and 12.00 during the night. On four occasions, mice ate the outer shell of fruits on the ground, making holes in the pericarp. They forced their head inside the fruits on three occasions, thus potentially picking up seeds on their fur. A squirrel was also observed (but not videotaped)

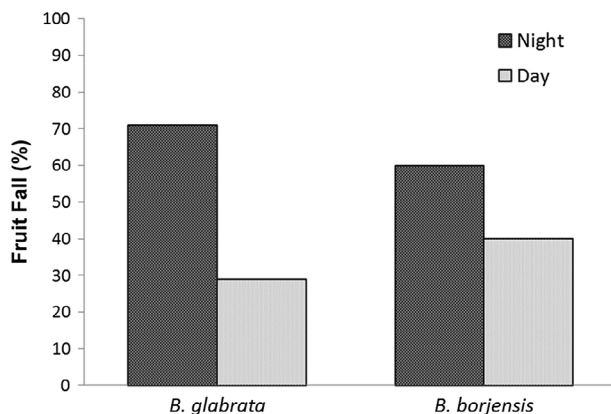


Figure 2. Percent of fruits that fell during the night versus the day of the fruits that fell under the plant, for *B. glabrata* and *B. borjensis*.

coming down a branch and taking a fruit during the day. For approximately 10 minutes it sat and ate the outer shell with the fruit between its paws, again potentially picking up seeds on its fur.

The camera traps captured a total of 46,661 photographs of *B. glabrata* fruits, and 5,151 photographs of *B. borjensis* fruits. None of these showed dispersal-related activity, in terms of the movement of seeds or fruits by animals. Insects contacted the fruits in 531 of the *B. glabrata* photos, but only landed on the outer surface and did not contact the seeds. No activity was documented from pictures taken of *B. borjensis* fruits; insects flew around the fruits but did not contact them.

Insect larval survey

From the 240 fruits of *B. glabrata* collected and opened, 33 (14%) contained insect larvae inside. From these 33 fruits, 30 (91%) had the seeds partially or completely eaten, sometimes crushed along with the placenta. An additional 15 (6%) of the 240 fruits had similarly crushed seeds, yet no insect larvae were found inside.

Effect of rain and holes in *B. glabrata* germination

For our germination experiments, seeds only germinated in the presence of rain for both fruits with holes (mean seedlings = 7.4 ± 7.8 SD, Figure 4) and without holes (mean seedlings = 4.5 ± 5.5 SD, Figure 4). We found that rain is the most important factor affecting seed germination, and it is statistically significant ($F_{1,36} = 15.49$, $p < 0.001$, Table 2). The presence of holes was not an important factor influencing seed germination ($F_{1,36} = 0.92$, $p = 0.34$, Table 2). Nor was there an interaction between holes and rain ($F_{1,36} = 0.92$, $p = 0.34$, Table 2).

Discussion

We hypothesized that the unusual inflated fruits found in many species of *Burmeistera* may be adapted to dispersal by (1) small mammals, (2) slugs, or (3) dipteran larvae metamorphosing to adults in the developing fruits. Of these three hypotheses, our study of seed dispersal of *B. glabrata* and *B. borjensis* only found limited support for the first. Observations and video recordings suggest squirrels and mice will consume the fruits, but apparently only play a minor role in dispersal since only 5 of 153 fruits were found to be removed from the plants in our twice-daily observations (the other 148 fell to the ground under the mother plant). Slugs were never observed inside fruits in 68.64 h of videotaping. Similarly, dipteran adults were not observed to emerge from the fruits. In fact, the fly larvae found in fruits acted primarily as seed predators (a frequently documented result;

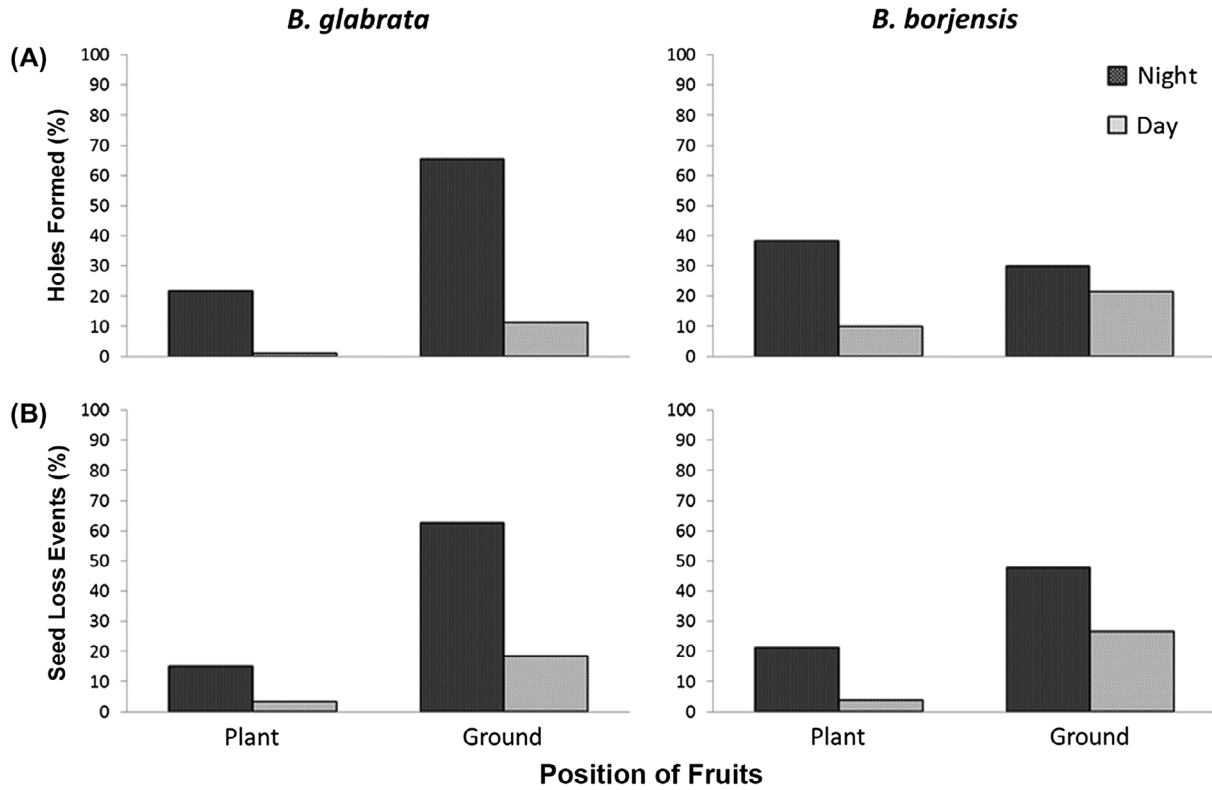


Figure 3. Timing of dispersal activity for fruits of *B. glabrata* and *B. borjensis*. Shows timing for fruits on the ground and on the plant; dark gray bars correspond to events documented at night, light gray to events during the day. (A) Of the total number of holes formed in fruit walls, gives percent formed in day versus night and on ground versus on plant. (B) Of the total seed loss events for each species, gives percent lost in day versus night and on ground versus on plant.

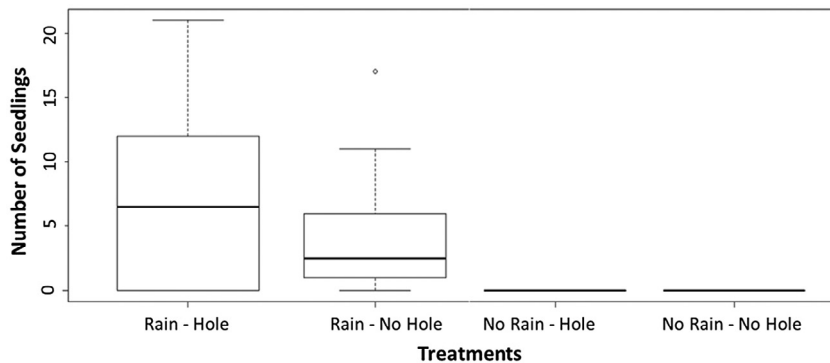


Figure 4. Number of *B. glabrata* seedlings (with mean and SD) that germinated from fruits placed in pots either exposed to rain or sheltered from it, and either with holes in the fruits or without holes.

e.g. [22,23]). In a survey of 240 fruits obtained from plants, 33 were found to contain larvae, and for 30 of these the larvae had consumed and destroyed some (and often all) of the fruit's seeds. Furthermore, pupae shells were never found in any fruit, suggesting the larvae metamorphose into adults only after exiting the fruits. Thus, no support was found for the hypothesis that adult

flies disperse *Burmeistera* when they exit fruits with seeds adhering to their bodies.

Our data instead suggest that the inflated fruits of *Burmeistera* are mainly gravity-dispersed, with only occasional primary or secondary dispersal by small mammals. For both species, most dispersal-related activity was nocturnal, i.e. events of fruit fall, fruit

Table 2. Two-way ANOVA table for the effects of rain and holes on the germination of *B. glabrata* seeds.

Factors	df	MS	F	P
Rain	1	354	15.49	<0.001
Holes	1	21	0.92	0.34
Rain*Holes	1	21	0.92	0.34
Error	36	22.9		

disappearance, formation of holes, and seed loss were more frequent at night (Figures 2 and 3). The rare events of frugivory by small mammals that we documented can be a mechanism for dispersal in addition to gravity. Such rare long-distance dispersal events can have a disproportionate importance to plant biology compared to gravity [4,8,10,13,24,25], since they can allow the seeds to escape from specialized pathogens and diseases associated with the mother plant [26], in line with the Janzen–Connell model of density and distance-dependent survival [27].

In *B. glabrata*, the only organisms we observed making holes in the fruits were crickets, and mice and squirrels in *B. borjensis*. In our twice-daily rounds, we frequently observed crickets on the fruits, thus they appear to be primarily responsible for the formation of holes. These crickets did not enter the fruits, hence there is no evidence that they were acting as dispersal agents by picking up seeds on their bodies. In fact no other animals were observed to act as secondary dispersal agents for those fruits that we videotaped on the ground, with the exception of the mice mentioned previously for *B. borjensis*. What role do these holes themselves play in seed dispersal? Because *Burmeistera* fruits are indehiscent, we suspected holes might be required in order for seeds to exit. However, our experimental manipulation demonstrates that the presence of holes does not affect germination (our proxy for seed release); only rainfall increased germination rates (Table 2, Figure 4). In fact, we observed that rain alone weakens the pericarp wall, promoting its decomposition and subsequent seed release from the placenta.

From a macroevolutionary perspective, *Burmeistera* is monophyletic and part of the centropogonid clade, sensu [14], along with *Centropogon* and *Siphocampylus* [11,28]. Initial phylogenetic results suggest that species with inflated berries form a clade derived from a fleshy berry ancestor [14]. Phylogenetic comparative studies of various Andean angiosperm lineages show that *Burmeistera* has the highest speciation rate of all groups surveyed [29]. In particular, the diversification rate in *Burmeistera* is higher relative to the closely related capsular taxa, which are presumably dispersed by wind [14]. The limited dispersal reported here may promote diversification by disrupting gene flow among populations, increasing population differentiation, and the potential for parapatric/

allopatric speciation [30]. A study examining the effects of seed dispersal mode on the geographic distribution and population genetic structure across species of the centropogonid clade would help clarify the importance of dispersal in the high diversification rate reported in *Burmeistera*.

We conclude that gravity is an important dispersal mode in *B. glabrata* and *B. borjensis*, and small mammals are occasional biotic dispersal agents. Although the majority of seeds end up under the mother plant, rare dispersal events by small mammals are potentially important as they may transport seeds away from predators and diseases associated with the mother plant [26,27]. Dispersal occurs mainly at night, and precipitation facilitates germination with and without fruit holes. The limited seed dispersal of these inflated fruits could influence the high rate of speciation documented in *Burmeistera* [14].

Geolocation Information

This research was performed in two Biological Stations located in the Napo province, in the Amazonian Andes of Ecuador: Wildsumaco Biological Station (0°40'S, 77°35'W) and Yanayacu Biological Station (0°36'S, 77°53'W).

Author contributions

D. Gamba designed the study, collected data, and wrote the manuscript. N.R. Maguiña and C.A. Calderón-Acevedo designed the study and collected data. K. Torres collected data. N.C. Muchhala proposed the hypotheses, designed the study, and wrote the manuscript.

Acknowledgments

We thank the Ecuadorian Ministry of the Environment for permission to conduct this research (permit No. 13-IC-FLO-DPAN/MA), the staff at Wildsumaco and Yanayacu Biological Stations for lodging and logistical aid, and Andrea Vallejo, Ricardo Zambrano, Verónica Leiva, and Mayra Ninazunta for assistance with the fieldwork. Harold Greeney and Lou Jost helped to brainstorm possible dispersal agents. Brian Arbogast, Travis Knowles, and Mitch Lysinger kindly loaned us camera traps, and Patricia Horack helped with examining and summarizing the camera trap photographs.

Associate Editor: Veerle Vanacker

Disclosure statement

No financial interest or benefit was derived from this research.

Funding

This work was supported by the Department of Biology at the University of Missouri – St. Louis, St. Louis, MO, USA.

ORCID

Diana Gamba  <http://orcid.org/0000-0002-0421-6437>

Camilo A. Calderón-Acevedo  <http://orcid.org/0000-0002-1468-3565>

References

- [1] Levin SA, Muller-Landau HC, Nathan R, et al. The ecology and evolution of seed dispersal: a theoretical perspective. *Annu Rev Ecol Evol Syst.* 2003;34:575–604.
- [2] Beckman NG, Rogers HS. Consequences of seed dispersal for plant recruitment in tropical forests: interactions within the seedscape. *Biotropica.* 2013;45:666–681.
- [3] Higgins SI, Nathan R, Cain ML. Are long-distance dispersal events in plants usually caused by nonstandard means of dispersal? *Ecology.* 2003;84:1945–1956.
- [4] Nathan R. Long-distance dispersal of plants. *Science.* 2006;313:786–788.
- [5] Gillespie RG, Baldwin BG, Waters JM, et al. Long-distance dispersal: a framework for hypothesis testing. *Trends Ecol Evol.* 2012;27:47–56.
- [6] Loiselle BA, Blake JG. Spatial distribution of understory fruit-eating birds and fruiting plants in a neotropical lowland wet forest. *Vegetatio.* 1993;107:177–189.
- [7] Nathan R, Muller-Landau HC. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends Ecol Evol.* 2000;15:278–285.
- [8] Hamrick JL, Murawski DA, Nason JD. The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. *Vegetatio.* 1993;107:281–297.
- [9] Seidler TG, Plotkin JB. Seed dispersal and spatial pattern in tropical trees. *PLoS Biol.* 2006;4:e344.
- [10] Hamrick JL. The influence of seed dispersal mechanisms on the genetic structure of plant populations. In: Estrada A, Flemming TH, editors. *Frugivores and seed dispersal.* New York (NY): Springer; 1986. p. 211–223.
- [11] Knox EB, Muasya AM, Muchhala N. The predominantly South American clade of Lobeliaceae. *Syst Bot.* 2008;33:462–468.
- [12] Howe HF, Smallwood J. Ecology of seed dispersal. *Annu Rev Ecol Syst.* 1982;13:201–228.
- [13] Van der Pijl L. Principles of dispersal. New York (NY): Springer; 1982. 218p.
- [14] Lagomarsino LP, Antonelli A, Muchhala N, et al. Phylogeny, classification, and fruit evolution of the species-rich Neotropical bellflowers (Campanulaceae: Lobelioideae). *Am J Bot.* 2014;101:2097–2112.
- [15] Lammers TG. World checklist and bibliography of Campanulaceae. London: Royal Botanical Gardens, Kew; 2007. 685 p.
- [16] Muchhala N. The pollination biology of *Burmeistera* (Campanulaceae): specialization and syndromes. *Am J Bot.* 2006;93:1081–1089.
- [17] Muchhala N. Adaptive trade-off in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds. *Am Nat.* 2007;169:494–504.
- [18] Muchhala N. functional significance of interspecific variation in *Burmeistera* flower morphology: evidence from nectar bat captures in Ecuador. *Biotropica.* 2008;40:332–337.
- [19] Laraque A, Ronchail J, Cochonneau G, et al. Heterogeneous distribution of rainfall and discharge regimes in the Ecuadorian Amazon basin. *J Hydrometeorol.* 2007;8:1364–1381.
- [20] Greeney HF. Bimodal breeding seasonality of an understory bird, *Premnoplex brunnescens*, in an Ecuadorian cloud forest. *J Trop Ecol.* 2010;26:547–549.
- [21] Jeppesen S. Lobeliaceae. In: Gunnar H, Sparre B, editors. *Flora of Ecuador.* 14. Stockholm: Swedish Natural Science Research Council; 1981. p. 9–170.
- [22] Janzen D. Seed predation by animals. *Annu Rev Ecol Syst.* 1971;2:465–492.
- [23] Herrera CM. Selective pressures on fruit seediness: differential predation of fly larvae on the fruits of *Berberis hispanica*. *Oikos.* 1984;42:166–170.
- [24] Fragoso JMV, Silvius KM, Correa JA. Long-distance seed dispersal by tapirs increases seed survival and aggregates tropical trees. *Ecology.* 2003;84:1998–2006.
- [25] Ogales M, Heleno R, Traveset A, et al. Evidence for overlooked mechanisms of long-distance seed dispersal to and between oceanic islands. *New Phytol.* 2012;194:313–317.
- [26] Mordecai EA. Pathogen impacts on plant communities: unifying theory, concepts, and empirical work. *Ecological Monographs.* 2011;81:429–441.
- [27] Comita LS, Queenborough SA, Murphy SJ, et al. Testing predictions of the Janzen-Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. *J Ecol.* 2014;102:845–856.
- [28] Antonelli A. Higher level phylogeny and evolutionary trends in Campanulaceae subfam. Lobelioideae: molecular signal overshadows morphology. *Mol Phylogenet Evol.* 2008;46:1–18.
- [29] Pennington RT, Lavin M, Särkinen T, et al. Contrasting plant diversification histories within the Andean biodiversity hotspot. *Proc Nat Acad Sci.* 2010;107:13783–13787.
- [30] Givnish TJ. Ecology of plant speciation. *Taxon.* 2010;59:1326–1366.