

Pollination by hummingbirds and bees in eight syntopic species and a putative hybrid of Ericaceae in Southeastern Brazil

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Abstract. We report on flowering phenology, floral morphology, pollinators, and nectar for eight species and a putative natural hybrid belonging to *Agarista*, *Gaultheria* and *Gaylussacia* that occur syntopically in a montane area. The campanulate to tubular flowers of eight out of nine Ericaceae taxa are primarily pollinated by either hummingbirds or bees. Flowering overlaps in all species but slight differences of floral shape, colour, and nectar characterize pollination by each pollinator group. Differences in floral traits are not large enough to exclude secondary pollinators. Thus, either the main pollinators of a species belonging to its syndrome, or secondary pollinators of a species belonging to different syndromes, may allow for inter-specific crosses.

Key words: *Agarista*, bee, *Gaultheria*, *Gaylussacia*, hummingbird, hybrid, nectar, pollination syndromes.

In the Neotropics, the Ericaceae are an Andean-centered family represented by 46 genera and about 800 species (94% endemic) mostly adapted to moist, open, cool, montane environments (Luteyn 2002). Southeastern Brazil is not usually thought of as being

particularly significant in the biogeography of the Ericaceae, but it seems to be important for the genera *Agarista*, *Gaultheria* and *Gaylussacia* of the subfamily Vaccinioideae (Luteyn 1989, Stevens 1995, Floyd 2002). Only two of the 66 Vaccinioideae species from southeastern Brazil occur in other regions (i.e. 97% of the species are endemic), suggesting species evolution in this region (Luteyn 2002).

The diversification of Ericaceae in the Neotropics seems to have several causes. For example, the mosaic arrangement of certain montane landscapes by physical and chemical irregularities provides a host of discrete habitats (Luteyn 2002). Interactions with pollinators also appear to be a key feature. However, there are few studies on pollination biology of *Agarista*, *Gaultheria* and *Gaylussacia* (e.g. Reader 1977, Mirick and Quinn 1981), especially among the Brazilian species (see Luteyn 1995). Furthermore, these genera present several disjunct geographic distributions, hybrids and problems to circumscribe species (Middleton 1991, Luteyn 1995, Stevens 1995), for which field data on floral biology and pollinators may be useful.

Particular combinations of floral traits have long been considered as a reflection of pollinator types (see Fenster et al. 2004 and references therein). Studies on pollination biology of congeneric species are important to indicate floral traits that are conserved among taxa and those that diverge under the influence of different pollinator types. Interaction between plants and pollinators is postulated as a central mechanism in the diversification of many angiosperm lineages, because evolutionary specialization by plants on their pollinators may drive adaptive divergence in floral traits and may contribute to the origin and maintenance of reproductive isolation (Grant 1981; Stebbins 1970, 1977; but see Waser 1998). In this view, describing the relationship between floral traits and pollinator types in sympatric species is a step in the study of the reproductive isolation of related species (e.g. Grant 1952, Brantjes 1982, Johnson and Steiner 1997, Hodges et al. 2002, Kay and Schemske 2003).

In this paper we report on flowering phenology, floral characteristics, pollinators, and production and sugar composition of nectar for eight species belonging to *Agarista*, *Gaultheria* and *Gaylussacia* and a putative

natural hybrid. The study concentrates on finding suites of floral traits related to pollination by hummingbirds versus bees.

Material and methods

Study species and site. Observations were made in high-altitude areas covered by grasslands, *Araucaria* forests and cloud forests at Serra da Bocaina National Park, Serra do Mar range, southeastern Brazil (ca. 22°44'S, 44°36'W, 1450–1800 m a.s.l.). Vegetation features of this region are summarized by Eiten (1970, 1992) and Safford (1999). These montane areas are classified as Cfb, after Köppen's climatic regions, i.e. mesothermic, with mean annual temperature between 12 and 20° C, moderate winters, and mild wet summers.

We studied eight species and a putative hybrid (Table 1, Appendix). *Agarista hispidula*, *Gaylussacia chamissonis* and *G. jordanensis* occur in well-drained grassland sites, whereas *Gaultheria eriophylla*, *G. serrata* and *Gaylussacia densa* occur at the edge of *Araucaria* and cloud forests. *Agarista oleifolia* is found at the edge of *Araucaria* forests along rivers and streams, whereas *Gaultheria sleumeriana* occurs almost exclusively in grassy areas up to 10 m from the banks of the Rio Mambucaba at Serra da Bocaina. In general, individuals of these species were common at the study sites, with the exception of

Table 1. Floral attributes and pollinator groups of nine Ericaceae taxa. Values are means \pm s.d. Abbreviations: CAM = campanulate, INF = infundibuliform, TUB = tubular, URC = urceolate, BL = large bees, BS = small bees, HB = hummingbirds, WA = wasps. Five and ten flowers were sampled, respectively, for floral dimensions and nectar production of each species (but six for *Gaylussacia* “hybrid”)

Taxon	Corolla shape	Main corolla color	Corolla length (mm)	Corolla opening (mm)	Nectar production (mg sugar)	Main pollinator group	Secondary pollinator group
<i>Gaylussacia chamissonis</i>	CAM	White	6.8 \pm 0.57	3.5 \pm 0.50	0.2 \pm 0.09	BS ^a	WA
<i>Gaylussacia jordanensis</i>	CAM/INF	White	9.3 \pm 0.67	5.8 \pm 0.57	0.2 \pm 0.11	BS	BL, WA
“ <i>Gaylussacia</i> hybrid”	CAM	White/pink	8.7 \pm 0.27	3.8 \pm 0.27	0.3 \pm 0.10	BL	BS, HB
<i>Gaultheria sleumeriana</i>	CAM/URC	White	5.4 \pm 0.42	2.3 \pm 0.27	0.3 \pm 0.12	BL	BS, HB
<i>Gaultheria serrata</i>	URC	Red	6.8 \pm 0.27	1.7 \pm 0.27	0.4 \pm 0.19	HB	–
<i>Gaultheria eriophylla</i>	URC	Red	6.5 \pm 0.46	1.1 \pm 0.11	0.8 \pm 0.40	HB	–
<i>Gaylussacia densa</i>	URC/TUB	Red	9.8 \pm 0.27	1.6 \pm 0.21	1.0 \pm 0.65	HB	BL
<i>Agarista hispidula</i>	URC/TUB	Red	10.6 \pm 0.89	2.0 \pm 0.11	2.8 \pm 1.22	HB	–
<i>Agarista oleifolia</i>	URC/TUB	White	10.9 \pm 0.82	2.0 \pm 0.11	1.5 \pm 0.60	few data	–

^a Mainly the exotic *Apis mellifera*.

Agarista hispidula and *Gaultheria sleumeriana*, with four and ca. 50 individuals, respectively. The latter species is endemic to Serra da Bocaina (Kinoshita-Gouvêa 1981). At two sites in the locality known as Fazenda Mariana, eight individuals were recorded, with reproductive and vegetative traits (e.g. corolla shape and size; leaf pilosity and size) intermediate between *Gaylussacia densa* and *G. jordanensis*, suggesting the existence of a natural hybrid of these species, hereafter called “*Gaylussacia* hybrid”. Individuals of “*Gaylussacia* hybrid” are found growing between populations of *G. densa* and *G. jordanensis*, at the edge of the cloud forest. Voucher specimens were deposited in the Universidade Estadual de Campinas Herbarium (UEC) (see Appendix).

Flowering and floral characteristics. Ten tagged individuals of each species (six of “*Gaylussacia* hybrid”) were observed monthly from January to December 1999, and two phenology parameters were recorded: flowering time months, in which each species was in flower; and blooming peak months, in which more than half of the individuals of each species had flowers. Terminology for phenological patterns follows Newstrom et al. (1994). Floral longevity, shape, dimensions and color, and phases of anthesis were recorded in the field. Drawings were made using a camera lucida. Pollen fertility was estimated by its cytoplasmic stainability, using carmine acetate test (Radford et al. 1974). Corolla colors follow Kornerup and Wanscher (1963).

Nectar. Flowers were tagged and bagged in bud stage, and on the following day, nectar volume was measured with a graduated microliter syringe (Hamilton, Reno, USA), and nectar sugar concentration was measured with a hand refractometer (Atago, Tokyo, Japan). 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 done by gas chromatography. Nectar was lyophilized and silylated according to Sweeley et al. (1963). 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 OV 101 column (2 m long), 3% on Chromosorb G/AW-DMcS mesh 100–120. Nitrogen was the carrier gas (30 ml/min) with the following temperature regime: 200° C for 2 min, 1° C/min until 215° C was reached, 15° C/min until reaching 310° C for 5 min. Chromatographic sugar analyses were made at least

twice for each sample. Carbohydrate standards (Sigma Chem.) were prepared using the same method. The sugar ratio ($r = \text{sucrose} / \text{fructose} + \text{glucose}$) and the hexose ratio ($r_h = \text{glucose} / \text{fructose}$) were calculated (after Baker and Baker 1983).

Floral visitors. Pollinators of each species were determined through diurnal observation of flowers for 49 days (total of 266 h) from April 1998 to December 1999. Only observations carried out under good weather condition were considered. The frequency of visits of each pollinator species to each focal plant was recorded. Visitors were directly observed and photographed at the flowers to scrutinize their visiting behavior. Insect specimens were collected to detect the presence of pollen on their bodies, and insects were identified by specialists. Hummingbirds were identified in the field or through photographs. The primary pollinators of each species were determined taking into account behavior during visits, the capacity to contact anthers and stigmas, frequency of visits to the focal plant, and number of flowers visited.

Results

Phenology. The flowering pattern of Ericaceae was continuous (flowering every month or in

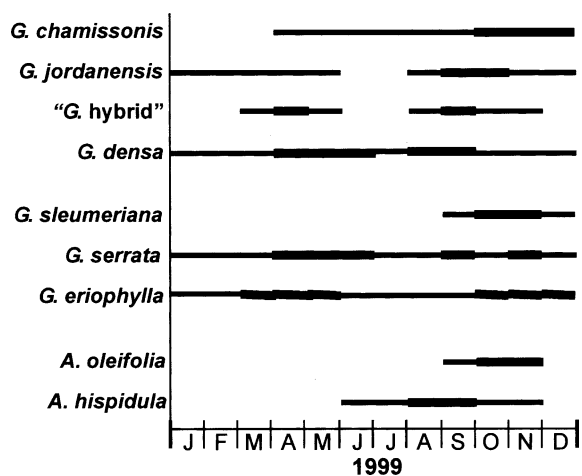


Fig. 1. Flowering patterns of *Gaylussacia*, *Gaultheria* and *Agarista* species from the Serra da Bocaina highlands. Thin and thick lines indicate, respectively, time and peak of flowering

brief sporadic breaks) or annual (only one major cycle per year), and in the case of “*Gaylussacia* hybrid” with two flowering cycles per year (Fig. 1). Extended flowering (> 5 mo) was observed in most species, while *Agarista oleifolia* and *Gaultheria sleumeriana* had intermediate flowering. Due to the extended flowering time, at least four species were in flower simultaneously every month, and all species flowered from September to November (Fig. 1).

Floral biology. The axillary racemose inflorescences (fascicle-like in *Agarista hispidula*) bear a few to dozens of sympetalous, pentamerous, pendulous flowers with either superior ovaries having hundreds of ovules in *Agarista* and *Gaultheria* or inferior ovaries with ten ovules in *Gaylussacia*. Corolla shape varies from campanulate to urceolate-tubular (Table 1, Fig. 2). Flowers are small, measuring from 5 to 12 mm (Table 1). Corolla length differs significantly among species (Kruskall Wallis, $H_{8, 45} = 40.5$, $p < 0.001$), but not among three pollination groups, namely, species pollinated by small bees, large bees, and hummingbirds (Kruskall Wallis, $H_{2, 40} = 4.9$, $p = 0.09$). Corolla opening width varies from 1 to 6.5 mm (mean = 2.6, s.d. = 1.44 mm, $n = 45$, Table 1), and is significantly different among species (Kruskall Wallis, $H_{8, 45} = 40.4$, $p < 0.001$), and also among pollination groups (Kruskall Wallis, $H_{2, 40} = 29.6$, $p < 0.001$); there is no difference in corolla opening width between species pollinated by small and large bees, but it is wider than that of hummingbird-pollinated species. Sparse to moderate pubescence (dense in *Gaultheria eriophylla*) is present within the corolla, in particular near the corolla opening, forming a ring of hairs, except

for *Agarista oleifolia*, *Gaylussacia chamissonis* and *G. jordanensis* where the petals are glabrous (Fig. 2). Corollas are monochromatic, white to red, except for those of “*Gaylussacia* hybrid”, which are pale pink with darker pink or red spots, and individuals of *Agarista oleifolia* bear pinkish- or greenish-white flowers (Table 1, Fig. 3). The stamens are always included in the corolla tube and are arranged in a ring. Filaments are apically slender but broaden near the corolla base in all species except *Gaultheria sleumeriana*. The filaments form a chamber between the stamens and pistil, where nectar accumulates (Fig. 2). Anthers in *Gaylussacia* narrow distally. In *Gaultheria eriophylla* and *G. serrata* anthers are short and oblong with two terminal awns on each anther sac. In contrast, in *G. sleumeriana* anthers are long, slightly elongated into short tubules and without awns. In *Agarista* species, anthers are bellows-shaped and filaments are distally curved (geniculate), surrounding the base of the anther (Fig. 2 h–j). Anthers dehisce introrsely by two apical pores and the smallish pollen is presented in tetrads. Pollen fertility was high in all pure species (range 84.5% to 94.0%, $n = 3$ flowers for each species), but for “*Gaylussacia* hybrid” it ranged from 66.5% to 79.0% ($n = 5$ flowers). In *Agarista hispidula*, *Gaultheria eriophylla* and *G. serrata* the style is cylindrical and inserted within the corolla and in the remaining species is slightly exerted or positioned level with the throat of the corolla (Fig. 2). The stigma is truncate to obtuse in all species and a viscid droplet reveals its receptivity. Flowers are slightly herkogamous, with stigma and anther pores placed at different positions for each species (Fig. 2). Floral longevity ranges from

Fig. 2 A–I. Long-sections of flowers. **A.** *Gaylussacia jordanensis*. **B.** *Gaylussacia chamissonis*. **C.** “*Gaylussacia* hybrid”. **D.** *Gaylussacia densa*. **E.** *Gaultheria sleumeriana*. **F.** *Gaultheria serrata*. **G.** *Gaultheria eriophylla*. **H.** *Agarista oleifolia*. **I.** *Agarista hispidula*. **Fig. 2 J–L.** Mechanism of pollen release of *A. hispidula* flowers. **J.** Detail of an anther in an unvisited flower. **K.** *Chlorostilbon aureoventris* visiting a flower. **L.** Detail of the anther during the visit. Note that the bill squeezes the anther base and a pollen cloud is expelled through the pores. Abbreviations: *c* corolla, *hb* hummingbird bill, *n* nectary, *s* style

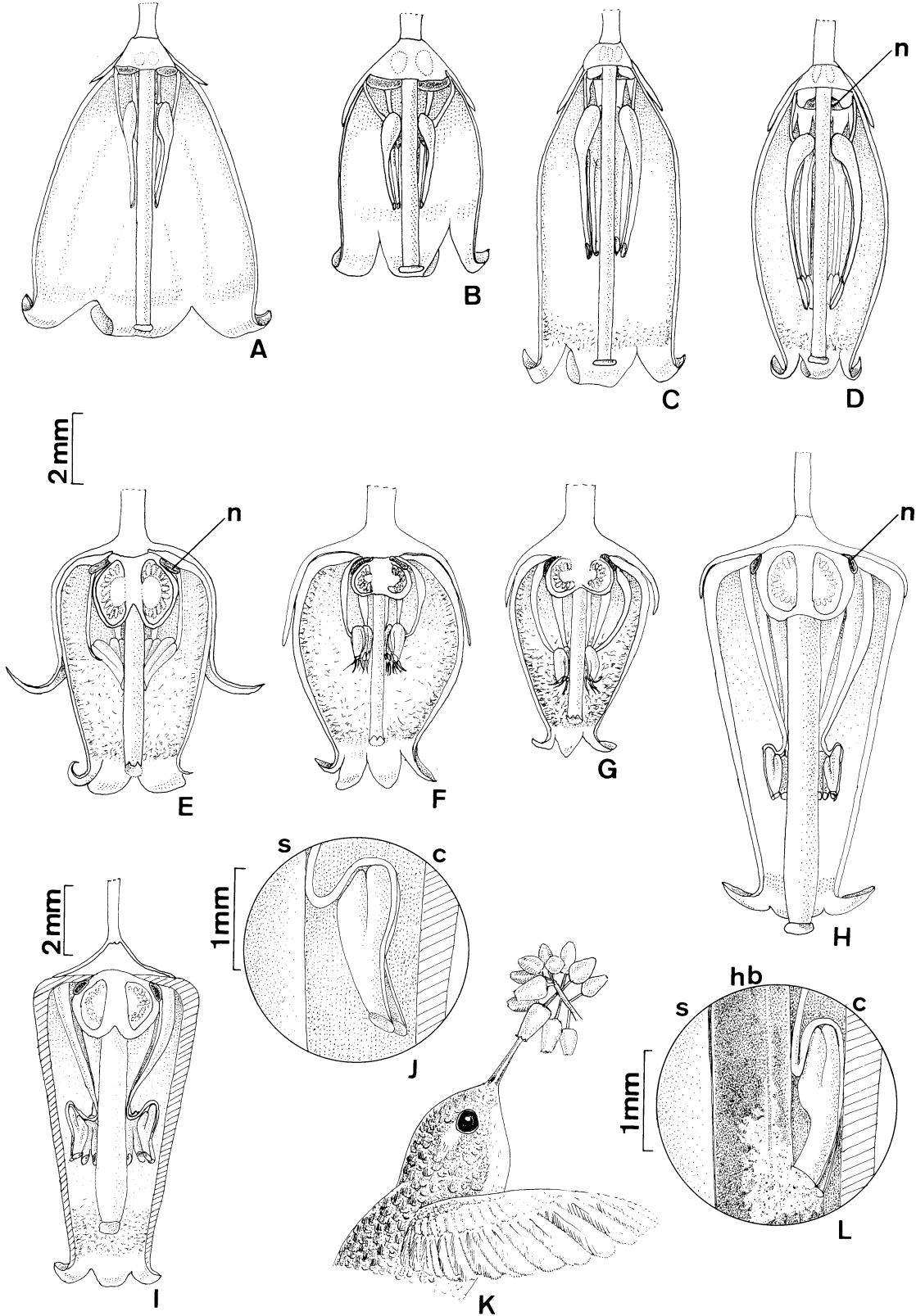




Fig. 3. Flowers and pollinators of Ericaceae species. **Fig. 3 A–B.** Flowers pollinated mainly by small bees. **A.** A female of *Ceratalictus* sp. (Halictidae) visiting a flower of *Gaylussacia jordanensis*. Note that the bee enters the flower to take nectar. **B.** A female of *Polybia* sp. (Vespidae) visiting a flower of *Gaylussacia chamissonis*. **Fig. 3 C–E.** Flowers pollinated mainly by large bees. Queens of *Bombus atratus* visiting, respectively, the flowers of “*Gaylussacia* hybrid” (**C**) and *Gaultheria sleumeriana* (**D**). Note that only a small part of the bee’s head penetrates the corolla. **E.** A male of *Stephanoxis lalandi* leaving a flower of “*Gaylussacia* hybrid” after the visit. **Fig. 3 F–H.** Flowers pollinated mainly or exclusively by hummingbirds. Males of *Stephanoxis lalandi* visiting, respectively, the flowers of *Gaultheria serrata* (**F**) and *Gaylussacia densa* (**G**). **H.** A female of *Chlorostilbon aureoventris* visiting a flower of *Agarista hispidula*. Note the pollen mass on the bill (**E**), (**F**) and (**H**). **Fig. 3 I.** Flowers of *Agarista oleifolia*, species with an uncertain pollination system

five to eleven days. Pollen is already mature in bud, and in species with urceolate-tubular hairy corollas, some pollen is shed prior to anthesis on the hair ring at the corolla opening. During anthesis, pollen is shed consecutively on the corolla hair ring. In species with this secondary pollen presentation, pollen may be rubbed off on the pollinator in two ways: from the corolla hair ring or directly from anther pores. The stigma becomes receptive some hours after anthesis; separation of male and female functions is thus limited to a small period of the flower's lifetime, characterizing a partial protandrous mechanism (sensu Mallick 2001). Since sexual phases overlap for several days, spontaneous self-pollination is possible in species with secondary pollen presentation, because the stigma and the corolla hair-ring are positioned at the same level. No detailed studies were carried out to establish the status of compatibility, but partial fruit production from bagged flowers of *Agarista hispidula*, *Gaultheria eriophylla*, *G. serrata* and *Gaylussacia densa* was recorded. Most of the species have odorless flowers. A sweet odor was detected in flowers of *Gaylussacia chamissonis*

and a fainter one in *G. jordanensis*, “*G. hybrid*”, and *Agarista oleifolia*.

Nectar and nectaries. Floral nectaries are continuous with inter-staminal projections around the ovary base in *Gaultheria*, ten-segmented around the ovary base in *Agarista*, and continuous around the style over the ovary in *Gaylussacia* (Fig. 2). Nectar sugar production (24 h interval, Table 1) was significantly heterogeneous among species (Kruskall Wallis, $H_{8, 86} = 68.8$, $p < 0.001$), and among the three pollination groups (Kruskall Wallis, $H_{2, 76} = 42.2$, $p < 0.001$). There were no differences in sugar production between species pollinated by small and large bees, which secrete smaller nectar sugar amounts than hummingbird-pollinated species. Nectar volume was low to moderate (range 1.2 to 11.7 μ l per flower) and nectar concentration was dilute ($< 15\%$), except for *Agarista hispidula* and *Gaultheria sleumeriana* (Table 2). Nectar sugar composition was comparable among species, with little or no sucrose (i.e. hexose dominant) and similar amounts of fructose and glucose (Table 2). Bee-pollinated species had no sucrose and hummingbird-pollinated species

Table 2. Nectar volume, concentration and sugar composition of nine Ericaceae taxa. Values are means \pm s.d. Abbreviations: N = number of sampled plants and number of flowers in parenthesis from which the nectar was obtained for chemical analysis, r = sucrose ratio, r_h = hexose ratio

Taxa	Vol, μ l	[], %	N	Sugar composition			r	r_h
				Sucrose	Fructose	Glucose		
<i>Gaylussacia chamissonis</i>	1.5 \pm 0,62	10.0 \pm 2,36	3 (7)	0	49.91	50.08	0	1.00
<i>Gaylussacia jordanensis</i>	2.0 \pm 0,78	11.6 \pm 1,49	2 (3)	0	39.45	60.54	0	1.53
<i>Gaylussacia</i> “hybrid”	3.5 \pm 0,89	8.3 \pm 2,36	2 (5)	0	53.94	46.05	0	0.85
<i>Gaylussacia densa</i>	6.9 \pm 2,84	13.4 \pm 4,25	1 (7)	1.27	46.51	52.21	0.01	1.12
			1 (6)	7.60	41.48	50.91	0.08	1.23
<i>Gaultheria sleumeriana</i>	1.2 \pm 0,54	27.3 \pm 5,46	2 (9)	0	47.65	52.34	0	1.09
			3 (10)	0	47.28	52.71	0	1.11
<i>Gaultheria serrata</i>	4.4 \pm 2,84	9.5 \pm 2,27	2 (11)	0	48.18	51.81	0	1.07
			1 (5)	0	53.25	46.74	0	0.88
<i>Gaultheria eriophylla</i>	5.5 \pm 1,76	14.0 \pm 3,56	2 (12)	6.12	42.99	50.88	0.07	1.18
			1 (7)	7.95	42.61	49.42	0.09	1.16
<i>Agarista hispidula</i>	11.7 \pm 4,22	22.6 \pm 4,60	1 (4)	18.92	40.72	40.35	0.23	0.99
<i>Agarista oleifolia</i>	11.6 \pm 3,38	12.4 \pm 3,32	2 (6)	64.44	18.86	16.68	1.81	0.88
			2 (8)	1.06	47.31	51.62	0.01	1.09

had a small amount, except for *Gaultheria serrata*. *Agarista oleifolia* samples had sucrose-dominated nectar and the other had hexose-dominated nectar (Table 2).

Pollinators and pollination mechanisms. Small bees, large bees or hummingbirds were the primary pollinators of these plant species (Table 1). Small bees in search of nectar (Halictidae and Meliponini-Apidae) were the main native pollinators of *Gaylussacia jordanensis* and *G. chamissonis*, respectively. However, the European *Apis mellifera* was by far the most common visitor, and consequently the main pollinator of *G. chamissonis* (Appendix). Pollination mechanisms are similar for these two species with their small white campanulate flowers. The bees land directly on the corolla, so that the ventral parts of the bee's body touch the stigma. Early in the bee's visit, contact with the stigma could favour deposition of out-crossed pollen, since the stamens are shorter than the stigma; a similar situation occurs in the other species. Very small bees, such as *Ceratalictus* sp. visiting *G. jordanensis* (Fig. 3a), fully enter the corolla directing their head to the inter-filament space in order to reach the nectar. At this time, the bee's head presses the anther base and, as a result, a fine mist of pollen is released over its ventral thorax and abdomen. Somewhat larger bees, such as *Melipona bicolor* and *Apis mellifera*, as well as wasps, introduce only the head and thorax while visiting *G. chamissonis* and *G. jordanensis* flowers (Fig. 3b). They press the anthers like the small bees, the pollen dusting mainly to the ventral thorax.

Large bees, in particular *Bombus atratus* queens, were the main pollinators of *Gaultheria sleumeriana* and "*Gaylussacia* hybrid". During visits, the bees grasp nearby flowers and introduce the extended proboscis and part of the head into a flower (Fig. 3c–d). During nectar intake, the pollen on the bee's head is rubbed off on the stigma; as the stigma is touched, movement is transferred to the anthers that release a new pollen load to the head.

The remaining species (except for *Agarista oleifolia*), with reddish, urceolate or tubular

corollas, were pollinated mainly or exclusively by hummingbirds. The black-breasted plover-crest, *Stephanoxis lalandi*, was by far the most frequent pollinator of *Gaultheria eriophylla*, *G. serrata* and *Gaylussacia densa*, which grow at forest edges, usually in clumps. The hummingbirds approach a flower from below, and while introducing the bill, they touch the stigma with the pollen-covered bill tip (Fig. 3e–g). As the bill touches the anthers, pollen is released on the hummingbird bill and also on the corolla hair-ring. *Stephanoxis lalandi* visited these three species indiscriminately, since they grow close together with overlapping flowering. The similar flower morphology of the three determines a similar location of pollen deposition on the hummingbirds, with the consequent mixture of inter-specific pollen. *Agarista hispidula* was visited mainly by the glittering-bellied emerald, *Chlorostilbon aureoventris* (Fig. 3h), and only early in the morning (before 8:30 h). This hummingbird introduces its bill between the style and the anther-ring in order to access the nectar. Thus causing pressure at the anther's base, squeezing it between the corolla and the bill, where the pollen is deposited (Fig. 2j–l).

Only a single event of floral visitation to *Agarista oleifolia* in 34 observation hours was recorded (Appendix). On that occasion, a *Bombus atratus* queen visited several flowers of *Gaultheria sleumeriana*, and then visited eight flowers of *Agarista oleifolia* nearby. Since the bumblebee visits *A. oleifolia* similarly to how it visits *G. sleumeriana* and "*Gaylussacia* hybrid" flowers, it may also bring about the pollination of the former species. On several occasions, individuals of four hummingbird species visited flowers of different ornithophilous species growing near *A. oleifolia* plants, but the latter were never visited.

In addition to the main pollinators, animals of other groups occasionally visited flowers of most species (Table 1, Appendix). Moreover, in several cases, one animal species pollinated more than one Ericaceae species. For example, the small bee *Dialictus* sp. 1 visited flowers of *Gaylussacia jordanensis* and

“*G. hybrid*”, and *Bombus atratus* visited *Agarista oleifolia*, *Gaultheria sleumeriana*, *Gaylussacia densa* and “*G. hybrid*” (Appendix). Similar events involved hummingbirds. *Chlorostilbon aureoventris* visited flowers of *Agarista hispida*, *Gaultheria eriophylla* and *G. sleumeriana*, and *Stephanoxis lalandi* visited flowers of *Gaultheria eriophylla*, *G. serrata*, *Gaylussacia densa* and “*G. hybrid*”.

Discussion

Taxonomists have noted that flower shape, size and color are only of slight use taxonomically in *Agarista*, *Gaultheria* and *Gaylussacia*, because they are relatively uniform within these genera (Judd 1995, Luteyn 1995, Floyd 2002). This suggests uniformity in the pollination system of the species. However, either hummingbirds or bees are the primary pollinators of the species we studied, except possibly *Agarista oleifolia*. We were able to show suites of characters (corolla opening width, nectar amount per flower, and flower color) by which bee- and hummingbird-pollinated species differed. This pattern may reflect a convergence of phenotypic traits for plant species adapted to the characteristics of a certain pollinator group (i.e. pollination syndrome after Faegri and van der Pijl 1971). Campanulate, white, relatively low-rewarding flowers are melittophilous, while tubular, red, relatively high-rewarding flowers are ornithophilous. In fact, these characteristics have been pointed out as typical of melittophily and ornithophily for other plant groups (e.g. Grant and Grant 1965, Vogel 1990, Wilson et al. 2004).

No previous information on pollination biology of Brazilian Ericaceae is available, and the floral traits presented above as differing by pollinator type need to be checked in other species of Vaccinioideae, Ericaceae. Data from *Gaultheria* and *Gaylussacia* species occurring outside Brazil give some support to the relationship between corolla color plus morphology and bees versus hummingbirds: bees were recorded visiting the white or pale-colored, campanulate flowers of *Gaultheria buxifolia* in

Bolivia and *Gaultheria reticulata* in Peru (Luteyn 1995), and *Gaultheria procumbens* (Mirick and Quinn 1981) and *Gaylussacia frondosa* (Rathcke 1988) in the USA; hummingbirds were recorded visiting the tubular red flowers of *Gaultheria bracteata* in Bolivia, *Gaultheria strigosa* in Ecuador, and *Gaultheria erecta* and *Gaultheria schultesii* in Mexico (Luteyn 1995). For *Agarista oleifolia* a single pollination event was recorded at Serra da Bocaina, and in a study of hummingbird-pollinated floras at two high altitude areas in São Paulo State (Cunha and Campos do Jordão), Buzato et al. (2000) recorded no visitors. The scarcity of floral visits in different areas precludes an evaluation of the pollination system of this species.

In some plant groups, a positive correlation between pollinator type and nectar sugar components has been reported (e.g. Baker and Baker 1983, Dafni et al. 1988), but in other groups the available data indicate that sugar composition is conservative (e.g. van Wyk et al. 1993, Galetto et al. 1998, Perret et al. 2001, Galetto and Bernardello 2003). Three species of *Vaccinium* pollinated mainly by large bees in Belgium showed large differences in their sugar ratios (Jacquemart 1992). In a study of 37 ornithophilous (passerine birds) and 13 entomophilous species of *Erica* from the South African Cape Region, sugar composition showed a marked dichotomy (sucrose- or hexose-dominant), but both nectar types were found in ornithophilous and entomophilous categories (Barnes et al. 1995). Analogously, similarities in the nectar of hummingbird- and bee-flowers contrast with specific morphological traits associated with these two syndromes in 45 species of Sinningieae (Gesneriaceae) (Perret et al. 2001). This trend reported for *Erica* and Sinningieae seems to be the same for the Ericaceae studied here. Nectar composition seems to be a more conservative trait than floral morphology for this group of Vaccinioideae from Brazil, suggesting that plant-visitor interactions may cause more rapid change in floral structure than in nectar composition.

The Serra da Bocaina highlands harbor at least three other Ericaceae species (*Agarista eucalyptoides*, *Agarista pulchella*, and *Gaylussacia amoena* - Appendix) in addition to the eight species studied here. Considering these 11 taxa, 55 inter-specific pairs occur in this area. In spite of numerous possibilities for hybridization, hybrids of Ericaceae seem to be infrequent at Serra da Bocaina. Mechanisms of reproductive isolation in plants include prezygotic (e.g. habitat and flowering segregation, specialization on a certain pollinator, and incompatible pollen-pistil interactions), and postzygotic, which affect hybrid viability, survivorship or fertility (Arnold 1997, Kay and Schemske 2003). Our results show that differences of habitat (grassland or forest) and phenology (time of flowering peak) of plants, and also, specialization on the pollinator group may contribute to prezygotic mechanisms of reproductive isolation of these Ericaceae at Serra da Bocaina.

Nevertheless, possible isolation mechanisms suggested above are probably not enough for complete reproductive isolation. Contact between forest and grassland is frequently abrupt, and flowering overlap occurs on several occasions due to the long flowering time of most species. Due to occasional pollinators, specialization on a certain pollinator group probably also fails in promoting complete isolation between species of different syndromes. For example, both *Gaylussacia densa* (mainly pollinated by hummingbirds) and *G. jordanensis* (mainly pollinated by small bees) were visited by large bees, which are the most probable vector accountable for the origin of “*Gaylussacia* hybrid”. Hybridization and introgression seem to be common in *Gaylussacia* history and are the possible causes of significant incongruencies found between nuclear and chloroplast data sets of the genus analyzed cladistically (Floyd 2002). Similarly, Sleumer (1952) proposed the idea that hybridization has played an important evolutionary role in *Gaultheria*. Moreover, putative intrageneric hybrids of *Agarista* and

Gaultheria have been reported (e.g. Franklin 1964, Middleton 1991, Judd 1995), including specimens of *G. eriophylla* x *G. serrata* (Luteyn 1995). Considering these reports, it is clear that prezygotic and postzygotic mechanisms of reproductive isolation are in need of greater study to evaluate the role of hybridization in the evolution of subfamily Vaccinioideae from Brazil.

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Appendix

Ericaceae from Serra da Bocaina and the pollinators captured or recorded on their flowers are listed. Plant habit, collector number (L. Freitas) of the plant specimens deposited at UEC, and total time of observation are given in brackets. The number of visits (nv) to the focal plant follows each pollinator name. Abbreviations are BS - small bees, BL - large bees, HB - hummingbirds, WA - wasps, uncoll - uncollected. Non-pollinating visitors were excluded.

Agarista hispidula (DC.) Hook. f. ex Nied. (shrub) (23, 431, 462) (38.5h): HB – *Chlorostilbon aureoventris berlepschi* Pinto (nv 6), *Leucochloris albicollis* (Vieillot) (nv 1).

Agarista oleifolia (Cham.) G. Don. var. *oleifolia* (treelet) (462) (34h): BL – *Bombus atratus* Franklin (Bombini) (nv 1).

Gaultheria eriophylla (Pers.) Sleumer ex Burt var. *eriophylla* (shrub) (860) (40.5h): HB – *Chlorostilbon aureoventris* (nv 1), *Stephanoxis lalandi lalandi* (Vieillot) (nv 12).

Gaultheria serrata var. *organensis* (Meisn.) Luteyn (shrub) (176, 179, 355, 360, 859)

(21.5h): HB - *Leucochloris albicollis* (nv 1), *Stephanoxis lalandi* (nv 18).

Gaultheria sleumeriana Kin.-Gouv. (shrub) (444, 717) (23h): BS - *Melipona bicolor bicolor* Lepeletier (Meliponini) (nv 4); BL - *Bombus atratus* (nv 4); HB - *Chlorostilbon aureoventris* (nv 2).

Gaylussacia chamissonis Meisn. (sub-shrub) (469) (16.5h): BS - *Apis mellifera* L. (Apini) (nv 38), *Melipona bicolor* (nv 5), *Augochloropsis* sp. 1 (Augochlorini) (nv 1), uncoll Meliponini (1 sp., nv 1); WA - uncoll *Polybia* (Vespidae) (2 spp., nv 3).

Gaylussacia densa var. *bocainae* Sleumer (shrub) (858) (51.5h): BL - *Bombus atratus* (nv 1); HB - *Leucochloris albicollis* (nv 1), *Stephanoxis lalandi* (nv 36).

Gaylussacia jordanensis Sleumer (sub-shrub) (167, 370, 432, 572, 712) (31.5h): BS - *Apis mellifera* (nv 3), *Plebeia saiqui* (Friese) (Meliponini) (nv 2), *Augochloropsis cyanea* (Schrottky) (nv 2), *Augochloropsis* sp. 1 (nv 4), *Ceratalictus* sp. (Augochlorini) (nv 15), *Dialictus* sp. 1 (Halictini) (nv 1), uncoll Meliponini (1 sp., nv 3); BL - *Megachile iheringi* Schrottky (Megachilini) (nv 1); WA - uncoll Vespidae (1 sp., nv 1).

“*Gaylussacia hybrid*” (shrub) (620, 711) (9.5h): BS - *Apis mellifera* (nv 1), *Dialictus* sp. 1 (nv 3); BL - *Bombus atratus* (nv 3), *Xylocopa brasiliatorum* (L.) (Xylocopini) (nv 1), uncoll *Megachile* (nv 1); HB - *Stephanoxis lalandi* (nv 2).

Ericaceae species collected at Serra da Bocaina, in addition to the species here studied: *Agarista eucalyptoides* (Cham. & Schltdl.) G. Don (716), *Agarista pulchella* var. *cordifolia* (Meisn.) Judd (426), *Gaylussacia amoena* Cham. (168).

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