Notes on the Phenology, Morphometry and Floral Biology of *Anaphyllopsis americana* (Araceae)

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ABSTRACT

Anaphyllopsis americana (Engler) A. Hay was studied in French Guiana. It possesses one of the longest floral cycles in Araceae, about 40 d, with each sexual phase lasting about 3 wk. In association with such a flowering cycle, the thermogenic pattern is similarly long, and consists of one temperature peak of low intensity (1.5–6°C) occurring every day between 1000 and 1500 hours. The presence of a daily thermogenic pattern over a long period of time (14–30 d) has not been observed to date in other Aroids.

The phenology and reproductive success of the studied population appear to vary seasonally. In fact, the proportion of flowering individuals and reproductive success are higher after the dry season when rains resume (November). Regardless of the season, reproductive success remains low, with an overall mean of 17% maturing infructescences. We have shown that the species cannot self-pollinate and thus relies entirely on external vectors for pollination. Despite extensive observation, we were unable to identify day-time pollinators. Due to the low frequency and abundance of insects present within the floral chambers, the fact that these insects (ants, crickets, cockroaches,...) are not very efficient pollinators, and the low fruit set, we suggest that the pollination system in *A. americana* is apparently inefficient. Finally, a long flowering cycle plus flowering periods all year long may be a flowering strategy in *A. americana* or the result of an inefficient pollination system achieved by inefficient pollinating insects in order to increase the probability of cross-pollination.

INTRODUCTION

The genus *Anaphyllopsis*, which now comprises three species, was first created to accommodate *Cyrtosperma ameri-*

canum Engler, the only Neotropical species of the Asian genus *Cyrtosperma* (Hay, 1988; Gonçalves, 2005). *Anaphyllopsis* belongs to the Lasioideae subfamily (Engler) which is composed of 10 genera: *Dracontium* L., *Dracontioides* Engler, *Anaphyllopsis* A. Hay, *Pycnospatha* Gagnepain, *Anaphyllum* Schott, *Cyrtosperma* Griffith, *Lasimorpha* Schott, *Podolasia* N.E. Brown, *Lasia* Loureiro, *Urospatha* Schott (Hay, 1992; Mayo *et al.*, 1997).

Some floral characters of *Anaphyllopsis americana* (Engler) A. Hay have been studied in different comparative works on the Araceae family (Chouteau *et al.*, 2006, 2008; Barabé & Lacroix, 2008; Barabé *et al.*, 2008; Gibernau *et al.*, 2010). In summary, *Anaphyllopsis americana* appears to have a relatively small pollen grain which possesses a long viability since 50% of the pollen is viable after 70 hr (3 d), and 210 hr (9 d) are necessary for a total loss of viability (Barabé *et al.*, 2008). Its Pollen/Ovule (PO) ratio is very high (about 100,000:1) suggesting a non-efficient pollination system which might be linked with a long flowering cycle, for an Araceae, of about 3–4 wk. No pollinators have been documented in this species or even for the genus (Gibernau, 2003; Chouteau *et al.*, 2006, 2008). Multivariate analyzes performed on Araceae in order to study the relationships between the type of pollinators (bee, fly and beetles) and floral traits have shown that *Anaphyllopsis americana* is distinct from the three main pollination "syndromes" (Chouteau *et al.*, 2008; Gibernau *et al.*, 2010). The floral characters of *A. americana* appear to cluster with those of two other Lasiodeae species (*Dracontium polyphyllum* L. and *Dracontioides desciscens* (Schott) Engl.) and those of two species from the Orontioideae subfamily (*Lysichiton camtschatcensis* Schott and *Symplocarpus renifolius* Schott) to form a putative group of species with a generalist pollination system. Generalist pollination systems are rare in Araceae and only documented in two species, *Lysichiton camtschatcensis* (Tanaka, 2004) and *Symplocarpus renifolius* (Uemera *et al.*, 1993). In these cases, various insects, a few of them known as efficient pollinators, can be found in low frequencies in the inflorescences.

Although some floral characters of *A. americana* are well known, phenology and thermogenesis need to be documented. In the present paper we describe the reproductive and flowering cycles, and analyze the thermogenic pattern in one population growing in French Guiana.

MATERIAL AND METHODS

Anaphyllopsis americana (Engler) A. Hay was studied in French Guiana during five different periods from May–September 2003, as well as in July 2005, May 2006, July 2008 and November 2009. One population was sampled and observed at Kilometer Point 19 along the road leading to Petit Saut dam (Kourou region). *Anaphyllopsis* individuals were growing in seasonally inundated forest gaps near a marsh. A voucher specimen: *Barabé et al. 258* was deposited at the Marie-Victorin Herbarium (MT).

A phenological survey in terms of number of leaves and inflorescences per individual was performed on 88 individuals.

Vegetative morphometric measures (leaf size, spathe height and spadix length) were performed on 30 inflorescences. Reproductive morphometric characters (height and width of the spathe, spadix length, flower number, number of ovules and seeds, aborted ovules and flowers) were measured on 16 inflorescences and 17 infructescences.

Experiments on spontaneous self-pollination, consisting of bagging inflorescences from before opening until complete drying or maturation, were performed on 7 inflorescences from different individuals.

Inflorescence thermogenesis was recorded on 11 inflorescences in 2003, 2 inflorescences in 2005 and 4 inflorescences in 2006. Temperatures of the spadix and ambient air were recorded every 10 min using two Digi-Sense® DualLogR® thermocouple thermometers. One of the thermometer's probes was inserted approximately 5 mm into the middle of the spadix and the second probe was used to record ambient air temperature. The temperatures of the leaf base and the peduncle were also recorded on 6 and 4 specimens respectively. For the leaf, the probe was inserted in the petiole 20 cm above ground level, and for the peduncle, 5 cm below the base of the spathe.

RESULTS

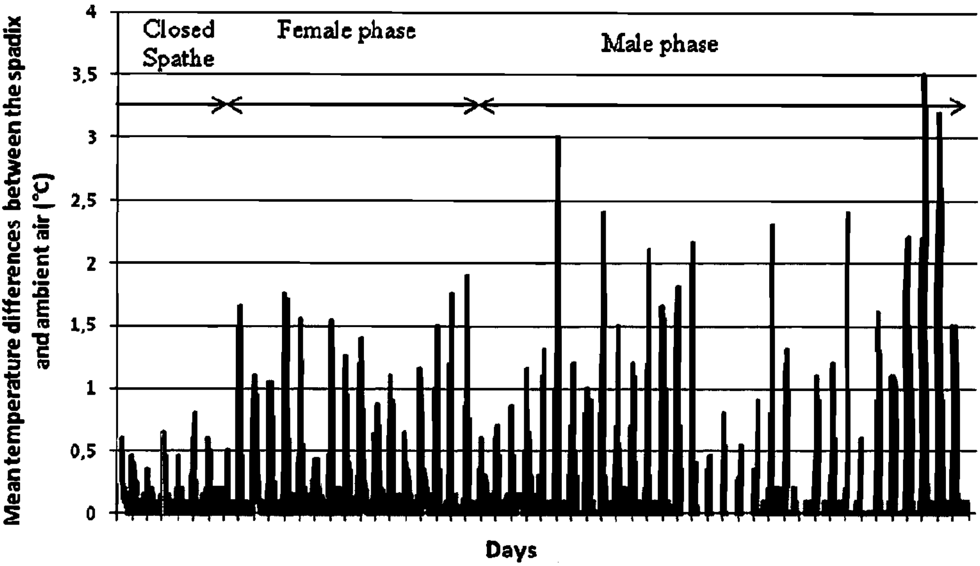
Floral cycle—The floral cycle started with the opening of the spathe and stigmas appeared to be receptive at this time. Interestingly, the spathe started to open from the apex and formed a funnel entrance but also separated at the base leaving a direct access to the spadix (about 0.5–1 cm wide). The floral cycle lasted about 40 d (range: 34–48 d), starting with the female phase (stigmas receptive) which lasted between 14 and 22 d followed by the male phase which lasted between 17 and 24 d. The anthesis occurred from the top to the base of the inflorescence. Our observations did not document the existence of a bisexual intermediate stage.

Thermogenesis—The long thermogenic pattern (Fig. 1) seemed to be linked with the long flowering cycle and consisted of a single daily temperature increase of low intensity occurring between 1000 and 1500 hours. The temperature difference between the spadix and ambient air varied between 1.5 and 6°C, but more commonly between 2 and 3°C (Fig. 1). The spadix heating was confirmed when looking at the difference between the spadix and the leaf petiole, a non-thermogenic tissue (Fig. 2). The spadix temperature was between 3 and 5°C warmer than the petiole during the day.

The temperature increase was very feeble before the spathe opened, less than 1.5°C, certainly due to solar radiation (Fig. 1). During the female phase, the amplitude of the pattern was very irregular. The values of the temperature difference peaks varied between 1 and 6.5°C among and within an individual. During the male phase, the temperature differences between the air and the spadix were not constant. In some individuals, they were equivalent to the temperature differences occurring during the female phase (Fig. 1), in others, they were lower and corresponded to a "non-heating" spadix (Fig. 1). However, in still other individuals, the temperature increase during the male phase was higher than in the female phase (Fig. 1). After that, the temperature increase was weak, varying between 1 and 1.5°C (Fig. 1). In 2005 and 2006, the temperature records between the spadix and the leaf petiole suggest that the temperature difference is high during the female and very low, virtually insignificant, during the male phase (Fig. 2).

Insect visitors—In July 2006, the floral chambers of 16 open inflorescences were observed between 0900–1100 hours. Only 5 inflorescences had visitors, respectively: two and five ants (Hymenoptera), one cockroach (Blattodea), one woodlouse (Isopoda) and two crickets (Orthoptera). In July 2008, between 1600–1800, with 8 open inflorescences, only one cricket (Orthoptera) was observed. In November 2009, between 0830–1030, with 11 open inflorescences at female stage, only one had insects: one black stingless bee (Hymenoptera) and one black grasshopper (Orthoptera).

Phenological Frequencies—The studied population contained more than one hundred individuals (Table 1). In July 2006, 114 individuals were counted, and half (51%) produced at least one inflorescence. Among reproductive individuals, a quarter (26%) produced 2 or 3 inflorescences. The 58 reproductive individuals produced 76 inflorescences, 23 of them during the present flowering period. Hence, 53 inflorescences were issued from the previous blooming period, 46 were dried and 7 maturing as infructescences. In July 2008, 50 individuals were marked, and half (54%) produced one inflorescence. Of the 28 inflorescences observed, 12 were from the present flowering period and 16 from the



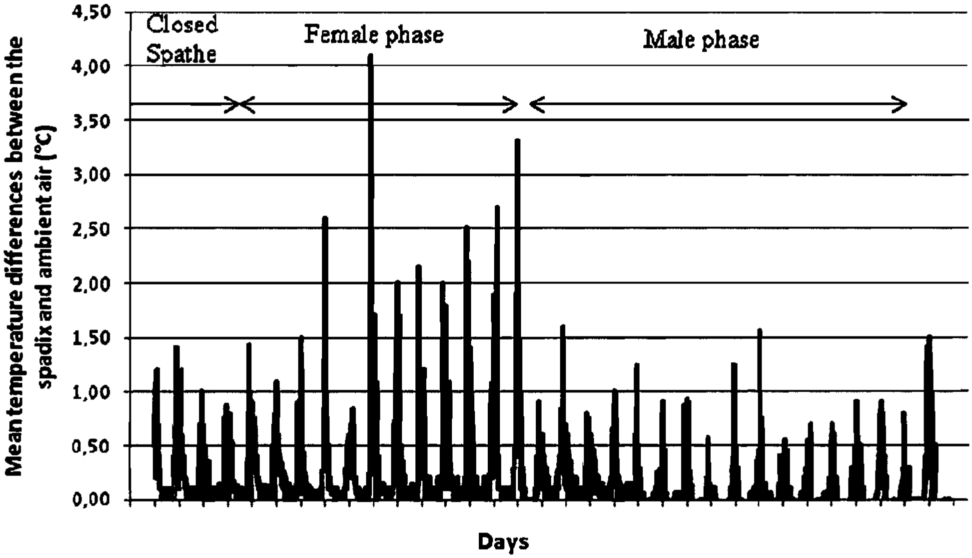


Fig. 1. Mean temperature differences between the spadix and ambient air recorded during about 60 d on 4 individuals (upper graph) and during 30 d on 3 individuals (lower graph). Each peak of temperature difference represents a different day (around midday).

previous one, of which 14 were dried and 2 maturing as infructescences. In November 2009, we observed 71 individuals. Among them only 28% of the individuals (N = 14) marked the previous year had any aboveground organ. Marking plants in July 2008 allowed us to estimate the class "0 leaf – 0 inflorescence" in 2009. It is not known whether the non-recovered individuals were dormant or dead. Finally, 75% of the

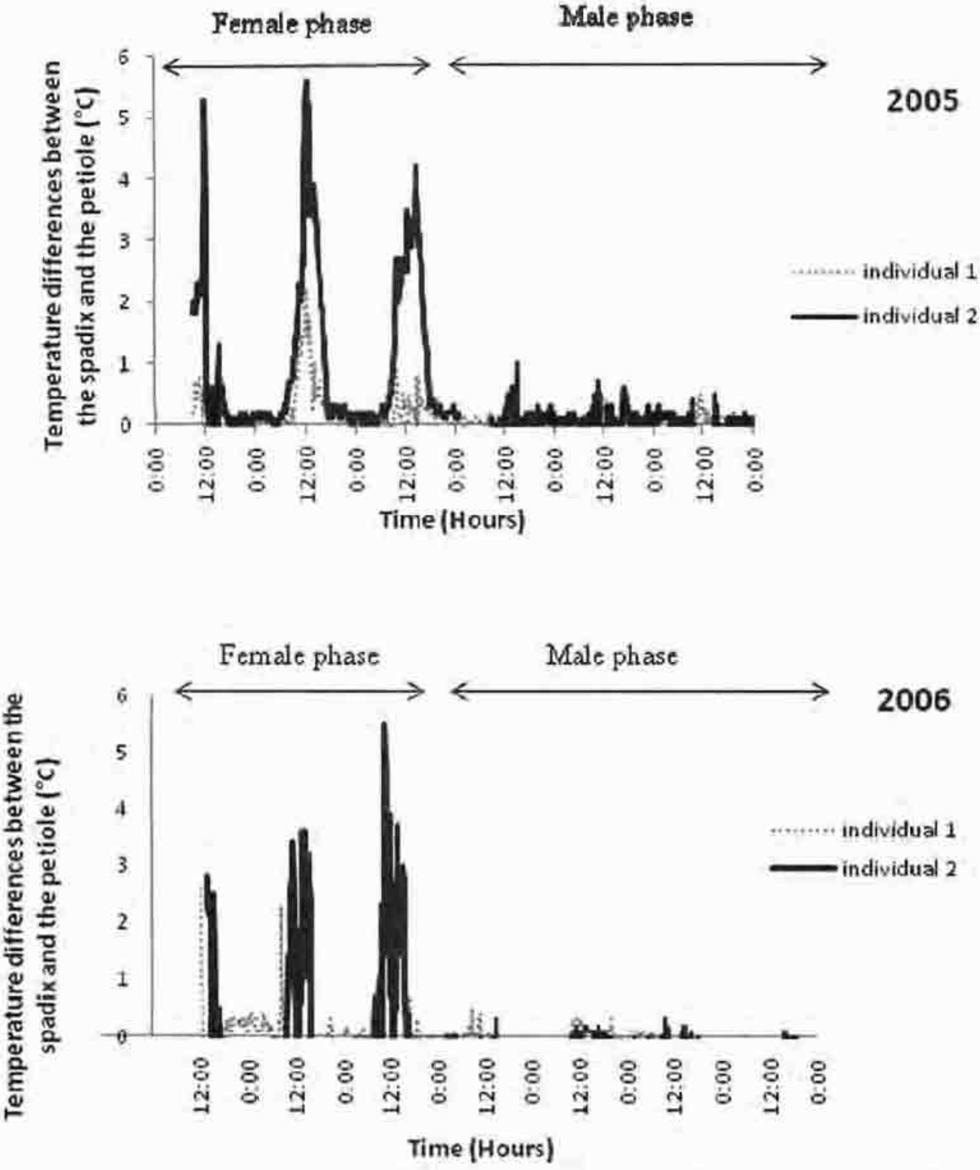


Fig. 2. Mean temperature differences between the spadix and the petiole recorded during 7 days on two individuals in 2005 (upper graph) and in 2006 (lower graph) during female and male phases.

plants (N = 43) produced at least one inflorescence and only 12% of the reproductive individuals had two or three inflorescences. The 43 reproductive individuals produced 49 inflorescences, including 36 during the present flowering period. Only 13 inflorescences were issued from the previous blooming period, 8 were dried and 5 maturing as infructescences. In conclusion, in November 2009, the population flowering pattern was different from July 2006 or 2008 ($Chi^2_2 = 9.96$, $P = .007$). In July (2006 and 08), half of the population was composed of reproductive individuals (no difference between 2006 and 2008: $Chi^2_1 = .07$, $P = .79$) whereas in November

Table 1. Phenological stage of the population "PK19" in three different years and at two different seasons. The plain line in the table separates the non reproductive individual from the reproductive ones.

Number of leaves	Number of inflorescence	Frequency (observations) July 2006 (N = 114)	July 2008 (N = 50)	November 09 (N = 71)
0	0	N.A.	N.A.	14
1	0	32	19	12
2	0	21	4	2
3	0	3	-	-
0	1	-	3	14
1	1	29	18	18
2	1	12	4	6
3	1	2	2	-
1	2	9	-	4
2	2	3	-	-
2–3	3	3	-	1

*N.A. Not Available

2009, 75% of the population was reproductive. Considering only the previous flowering period, the percentages of developing infructescences in the population were the same (13%) in July 2006 and 2008 (Chi^2_1 = .05, P = .94) but higher (38%) in November 2009 (Chi^2_1 = 4.47, P = .034).

Morphometry—The leaf (N = 30) was 58.9 ± 14.6 cm long (range: 28–90) and 42.9 ± 10.7 cm wide (range: 16–65). There was a significant positive linear relationship between leaf length and spadix height ($Y = .285 * X + 7.725$) ($R^2 = .71$; $F_{1,32} = 79.1$; $P < 10^{-3}$). The spathe was on average 24.4 ± 4.3 cm long (N = 58, range: 12–34) and the spadix 4.12 ± .88 cm high (N = 42, range: 2.5–6.7). The positive relationship between spathe and spadix heights ($Y = .188 * X - .606$) was significant ($R^2 = .61$; $F_{1,32} = 50.40$; $P < 10^{-3}$).

The inflorescence bears bisexual flowers; each composed of 4 stamens surrounding the unilocular ovary containing one ovule. The average number of flowers per inflorescence was 149 ± 33 (N = 32, range: 79–244). However, we found a positive ($Y = 5.636 * X + 5.721$) and significant ($R^2 = .47$; $F_{1,14} = 12.63$; $P = .003$) linear relationship between spathe height and the number of flowers . The same held true between spadix height and the number of flowers ($Y = 26.306 * X + 32.409$; $R^2 = .76$; $F_{1,14} = 43.97$; $P < 10^{-3}$).

Reproductive success—Reproductive success was low. The rate of non-maturing inflorescences was high, estimated at between 60 and 90% (N = 82); the number of maturing seeds per infructescence was low, since only 52.3 ± 31.7 berries (N = 25, range: 8–103) matured per infructescence, representing only one third of the total number of flowers per inflorescence. Thus, on average, two thirds of flowers were not pollinated or did not develop fruit. Moreover, there was no linear relationship between the size of the inflorescence (in term of flower number) and the number of maturing seeds ($R^2 = .007$). Finally, none of the 7 bagged inflorescences set fruits, indicating that spontaneous self-pollination might be rare.

DISCUSSION

The lasioid species, *Anaphyllopsis americana* (Engler) A. Hay, possesses one of the longest floral cycles in Araceae, about 40 d. With each sexual phase lasting about 3 wk, such results are comparable to some long-flowering *Anthurium* species (Croat, 1980). Contrary to many *Anthurium* species (Croat, 1980), there is no efficient bisexual intermediate stage in *A. americana* since none of the bagged inflorescences fructified.

The thermogenic pattern is also long (Fig. 1) and seems to be linked with the unusually long flowering cycle. It consists of one temperature peak of low intensity occurring every day between 1000 and 1500 hours. Although the thermogenic pattern appears to be periodic, the amplitude of the daily thermogenic peak varies considerably between and within an individual. Hence the pattern of thermogenesis is not well defined as is the case in other Aroids, as for example in *Monstera* (Chouteau *et al.*, 2009), *Syngonium* (Chouteau *et al.*, 2007) or *Philodendron* (Barabé *et al.*, 2002). This might be due to extrinsic (weather) or intrinsic (physiology, morphology) factors or both. Even if the spadix is protected at the bottom of an above-open floral chamber, its small size (about 4 cm high and less than 1 cm width) may prevent it from being highly thermogenic, because of the high surface – volume ratio. Applying heat transfer models (Gibernau *et al.*, 2005), temperature differences in the small spadix of *A. americana* should range between 1.8 and 6°C, which is in accordance with our measures. However, we do not possess enough data to clarify this question. The presence of a daily thermogenic pattern over a long period of time (14–30 d) has not been observed to date in other Aroids (Gibernau *et al.*, 2005). However, we do not know whether this particular thermogenic pattern plays a role in the emission of odors or in pollination mechanisms.

The phenology and reproductive success of the studied population appeared to vary seasonally. One can expect a weather effect, particularly with regard to the impact of rain on pollination success. In fact, the proportion of flowering individuals and reproductive success were higher after the dry season when rains resumed. In July, at the beginning of the dry season, half of the population was reproductive, versus 75% in November, at the beginning of the rainy season. Similarly, reproductive success was three times higher in November than in July. However, further studies are needed to address this question in more detail.

Whatever the season, reproductive success remained low with an overall mean of 17% maturing infructescences. Since the species cannot self-pollinate, it relies entirely on external vectors for pollination, and apparently on insects. Based on herbarium specimens, the species appears to flower all year long (Hoff *et al.*, 2007). This may be a flowering strategy associated with a non-efficient pollination system. Multiplying flowering events at different periods throughout the year may increase the probability of cross-pollination by inefficient pollinating insects.

Although we have made extensive observations, we have no precise data regarding pollinators. From the low frequency and abundance of insects within the floral chambers, the fact that these insects (ants, crickets, cockroaches,...) are not very efficient pollinators, and the low fruit set, we suggest that the pollination system in *A. americana* appears to be rather inefficient. One could simply hypothesize that the long flowering cycle increases the probability of visits to an inflorescence by inefficient pollinating insects. Such pollination systems have been already studied in other plants, and cockroaches in particular have been reported as pollinators in the Asian Annonaceae, *Uvaria elmeri* (Nagamitsu & Inoue, 1997) and in one French-Guianan *Clusia* (Vlasakova *et al.*, 2008). On one hand, observations made in these previous studies can explain some of our observations on *A. americana*. The flower visits occurred at night. Flowers were visited by a variety of insects (ants, crickets, bugs). The cockroaches fed on stigmatic exudates and pollen, and the visit frequency was low (less than 2 per hour). On the other hand, these previous studies present some differences with regard to our observations. In *Clusia*, cockroaches were efficient pollinators leading to a fruit production of 42%

(Vlasakova *et al.*, 2008), whereas in *Uvaria*, cockroaches had a low pollen load and poor pollen efficiency leading to a fruit set of 2%, (Nagamitsu & Inoue, 1997). By contrast with *A. americana*, the flowering cycle of *Uvaria* is short since a flower bloomed for 50 hr (Nagamitsu & Inoue, 1997). Finally, the flowers of *Clusia* appeared to be highly specialized to cockroach visits with secretory organs producing an attractive non-sugary liquid secretion and a buttery floral odor rich in acetoin (Vlasakova *et al.*, 2008). Hence night pollination should be studied more carefully, since some of our observations are concordant with such a hypothetical pollinator.

The flowering biology of the other lasioid genera is not known (Gibernau, 2003). Only a few mentions of visiting insects are available. In *Dracontium* and *Dracontioides*, the inflorescences are visited by flies (Boos, 1997; Zhu & Croat, 2004; Gonçalves, 2005; Grayum, pers. observ.). Nitidulidae (Coleoptera) are reported to visit inflorescences of the African *Cyrtosperma* or the Neotropical *Urospatha* (Knecht, 1983; Boos, pers. observ.). Interestingly, *Urospatha* has a habit and a floral morphology very close to *Anaphyllopsis*, but unfortunately no more precise information is available.

ACKNOWLEDGMENTS

We are grateful to the Staff of *Laboratoire Environnemental de Petit Saut* (Hydreco), Kourou, French Guiana, for logistical help. This research was supported in part by an individual operating grant from the Natural Sciences and Engineering Research Council of Canada to D.B and by the "*Project Amazonia*" from the CNRS-Guyane.

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