Dynastine scarab Beetle Pollination in *Dieffenbachia longispatha* (Araceae) on Barro Colorado Island (Panama) Compared with La Selva Biological Station (Costa Rica)

Dr. Danny N. Beath
Corston
Cardington
Nr. Church Stretton
Shropshire SY6 7JZ
England, UK

ABSTRACT

The aim of this study was to evaluate the differences between two flowering populations of *Dieffenbachia longispatha*; one on Barro Colorado Island, Panama and the other at La Selva Biological Station, Costa Rica. The Barro Colorado population exhibited green spathes and 10–15 loosely packed bright orange female florets in the lower spadix zone, compared to the La Selva populations, which had slightly larger yellow-green spathes and 40–50 densely packed yellow female florets in the lower spadix zone. Other differences included floral odor, which was sweet and spicy in the Barro Colorado population and rancid smelling on La Selva plants, together with numbers, species and timings of beetle visitors. On Barro Colorado the dominant beetle visitors were *Cyclocephala gravis* and *Cyclocephala sexpunctata* compared to *Cyclocephala amblyopsis* and *Cyclocephala gravis* at La Selva. In conclusion, the effects of selection by the local beetle populations in each locality appears to have led to the evolution of different sub-species of *D. longispatha* on Barro Colorado Island in Panama and at La Selva Biological Station, Costa Rica.

KEY WORDS

Beetles, *Dieffenbachia longispatha*, evolution, sub-species.

INTRODUCTION

Summary of Previous Findings

Studies of Dynastine scarab beetle pollination in the Araceae in the neotropics have been done in Brazil by Gottsberger & Amaral (1984), Gottsberger (1990) and Gottsberger et al. (1991) and in Costa Rica by Grayum (1996), Schatz (1990) and Valerio (1986).

Gottsberger & Amaral (1984) and Gottsberger (1990) reviewed beetle pollination in several *Philodendron* species in Brazil, which detailed nocturnal anthesis and thermogenesis in *P. bipinnatifidum* Schott, together with *P. selloum* Koch and pollination by Dynastine scarab beetles. Gottsberger also participated in a detailed study of *Erioscelis emarginata* Mannh visiting inflorescences of *Philodendron selloum,* where the role of floral scents was implicated in long range attraction of beetles, coupled with short range visual cues from open inflorescences (Gottsberger & Silberbauer-Gottsberger, 1991).

At La Selva in Costa Rica Grayum (1996) has studied beetle pollination on several species of *Philodendron* subgenus *Pteromischum* at La Selva, with detailed observations on the seasonality and vertical stratification of different species of *Philodendron,* together with the evolution of subtle differences in floral odors in the speciation of *Philodendron* subgenus *Pteromischum* at La Selva.

Studies on pollination by Dynastine scarab beetles (*Cyclocephala* and *Erioscelis* sp.) on *Dieffenbachia* in Costa Rica have been done on *D. oerstedii* Schott (Valerio, 1984) and *D. longispatha* Engler & Krause (Young, 1986 & 1988).

Young undertook a detailed study of *D. longispatha* over three years in La Selva and identified 9 species of *Cyclocephala* and one *Erioscelis* species as well as a number of nitidulid and staphylid beetles, Mirid bugs, fruit flies, thrips and earwig species visiting open inflorescences. Young established two species of *Cyclocephala* as the main pollinators of *D. longispatha* which attracted pollinating beetles during nocturnal anthesis with strong floral odors. Young (1986 & 1988) marked beetles visiting open blooms and tracked their movements between different inflorescences on successive nights and found that beetles were capable of flying up to 1000 m in locating newly opened inflorescences. A similar study involving marked beetles and odor driven nocturnal pollination was carried out by Beath (1993) in the old world tropics in Ghana, on *Amorphophallus johnsonii* N. E. Brown which was pollinated by carrion beetles (*Phaeochrous amplus* Arrow).

Floral odors in Araceae at La Selva have been reviewed by Schatz (1990) where the fragrances of a number of aroid genera were screened and essential compounds were identified using gas chromatography. Schatz paired up specific odors with 21 species of aroids studied at La Selva using a Kruskall Multidimensional Scaling (MDS) analysis to produce a graph of odor profiles. These were matched up to reveal odor specificity in the beetle pollinators.

Schatz discovered very specific beetle-aroid relationship at La Selva involving *Philodendron radiatum* Schott and an unidentified *Cyclocephala* species (since identified as *C. ampliota* Bates). *Philodendron radiatum* exhibited a unique odor profile and was the only species to be visited by *C. ampliota* at La Selva. Grayum even suggested that some of these aroid odors may be acting as sex pheromones for the visiting beetles.

The Dieffenbachia Inflorescence

The flowering organ of Araceae is characterised in *Dieffenbachia* by a compound monoecious inflorescence, comprising separate zones of female and male florets on a rod-like organ or spadix. The female florets are set in a proximal position with a short sterile zone separating them from the male florets on the upper spadix (Fig. 1b and 1d). The spadix is enveloped by a leafy bract called a spathe. (Fig. 1a and 1c). In *D. longispatha* the female florets are single ovaries with sticky pad-like stigmas and these are surrounded by numerous rod like sterile staminodia. The male florets consist of closely packed stamens, which produce pollen via apical pores. Anthesis in *Dieffenbachia* is protogynous and lasts two days, with the female florets being receptive on the first night, followed by pollen production on the second night.

METHODS AND MATERIALS

Observations

Observations on *D. longispatha* were made at two lowland rain forest habitats, one on Barro Colorado Island (BCI) in Panama during July and the other at La Selva Biological Station in north east Costa Rica during September. In both cases, continuous observations were made between dusk (1730 at La Selva and 1830 on BCI) and 2100 on both nights of anthesis (female and male phases). Nine inflorescences were observed on BCI, compared to 13 inflorescences at La Selva. Each observed bloom was assigned a number. Beetle arrivals and departures were recorded, together with observations of beetle behavior inside the inflorescence. Beetle numbers were counted between dusk and 2100 hours on the first night of anthesis.

Beetle Marking and Insect Identifications

At La Selva, beetle movements between inflorescences were recorded in a small colony of flowering *Dieffenbachia* plants. The beetles were marked by spraying UV sensitive fluorescent dye over the beetles

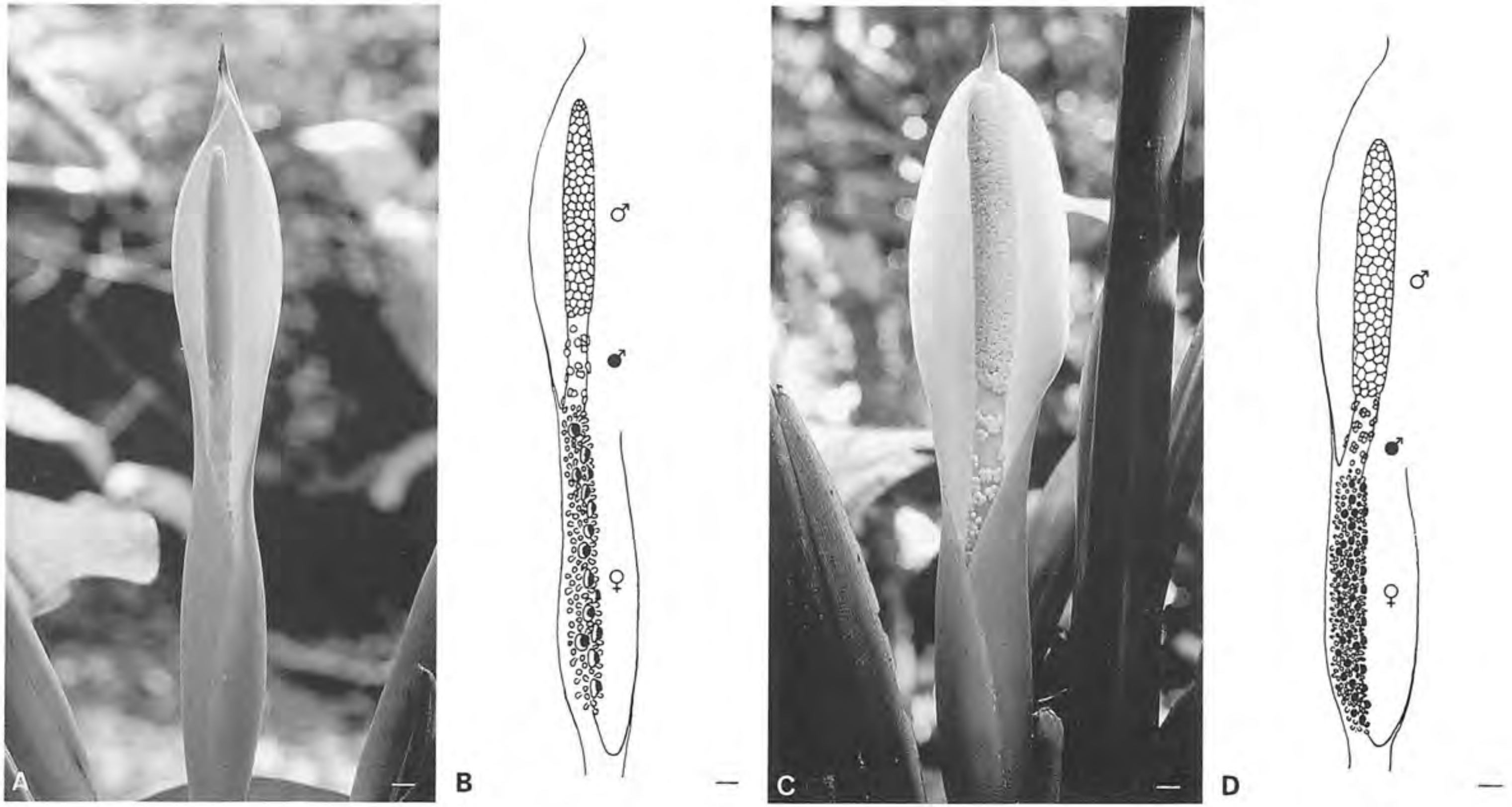

Fig. 1. Inflorescences of *Dieffenbachia longispatha:* from Barro Colorado Island, Panama, showing (A) inflorescence at anthesis and (B) cut away section of spathe and spadix showing details of female florets and sterile staminodes, ♀, sterile male zone, ♂, and fertile male zone, ♂,: and from La Selva Biological Station, Costa Rica, showing (C) inflorescence at anthesis and (D) cut away section of spathe and spadix showing details of female florets and sterile staminodes ♀, sterile male zone ♂, and fertile male zone ♂. (— = 1 cm).

inside inflorescences on day two of anthesis. The movement of individual beetles after dusk was traced by using a portable UV fluorescent lamp to show up sprayed beetles as they arrived at newly opened inflorescences. Variously colored fluorescent dyes (FIESTA "Astral Pink," "Blaze A5" and "Arc Chrome A6") were used to distinguish between beetles coming from different inflorescences.

Beetles on BCI were identified by cross referencing specimens to the Stockwell Beetle Collection in the Smithsonian Tropical Research Institute, Panama. Insects collected at La Selva were identified at the large collection in the INBio (Instituto Nacional de Biodiversidad) headquarters in San Jose, Costa Rica, with the help of Dr. Angel Solis.

RESULTS

Floral Morphology

Flowering individuals of *D. longispatha* on Barro Colorado Island exhibited dark green spathes which opened to reveal all of the upper male spadix zone, plus the sterile spadix zone and a tiny portion of the female spadix zone (Fig. 1a). Female florets on BCI specimens were bright orange and sparsely spaced out in a single line of between 10–15 florets along the lower spadix zone (Fig. 1b). Inflorescences of *D. longispatha* at La Selva typically produced slightly larger yellow-green spathes which opened to reveal the upper male spadix zone, plus the sterile spadix zone and a small portion of the female spadix zone (Fig. 1c). Female florets on La Selva plants were pale yellow and numerous with between 40–50 florets (Fig. 1d). In both BCI and La Selva plants the female florets were interspersed by closely packed sterile staminodes.

Anthesis

Inflorescences began opening the day before anthesis and by morning on day one the upper spathe was wide open.

Anthesis began just after dusk on day one and was accompanied by floral odor. On BCI the odor was strongest between 1915 and 1945 and was emitted as a strong spicy perfume, while at La Selva the odor peaked between 1800 and 1845 and was characterised by a strong, sweetly rancid, odor. Dynastine scarab beetles started arriving between 1810–1815 hours (La Selva) and 1920–1930 hours (BCI) and continued to arrive until around 2030 in both cases. Upon arrival, the beetles crawled down from the upper spathe limb into the lower spathe tube and began to feed on starch-rich sterile staminodia surrounding the fertile female florets. The beetles remained active and fed until 2100 hours after which they became quiescent and rested in the bottom of the spathe chamber. Beetles remained inside the spathe overnight and throughout the next day (day two).

On BCI, typically 2–4 beetles visited inflorescences and consisted of three species of *Cyclocephala, C. carbonaria* Lewis, *C. gravis* Bates and *C. sexpunctata* Cast (Table 1a). At La Selva up to 12 beetles were found in single blooms and consisted mainly of two *Cyclocephala* species; *C. amblyopsis* Bates and *C. gravis*, together with more occasional visits by *Cyclocephala sexpunctata* and *Erioscelis columbica* Endrodi (Table 1b). Inflorescences at La Selva were also visited by small numbers of nitidulid (Nitidulidae) and staphylinid (Staphylinidae) beetles, earwigs (Dermaptera), thrips (Thysanoptera), mirid bugs (Miridae) and occasional fruit flies (*Drosophila*). These genera were absent on BCI except for the mirid bugs, earwigs and fruit flies.

Just after dusk on day two (1745 La Selva, 1845 BCI) the dynastine scarab beetles became active and climbed up the spadix onto the upper male zone. Pollen emerged between 1755–1805 hours (La Selva) and 1855–1905 hours (BCI) and the waiting beetles fed voraciously on this pollen as it was produced. Beetles started to leave male phase inflorescences by 1820 (La Selva) and 1920 (BCI), but some remained on the male spadix zone until after 2000 in both cases. The lower spathe tube became constricted and the upper spathe limb started to close soon after pollen production. On day three the spathe remained partly open all day with no odor and closed completely after dusk.

Beetle Movements

Experiments with marked *Cyclocephala* and *Erioscelis* beetles on a colony of *D. longispatha* at La Selva revealed a proportion of beetles moving from male phase (2nd day) blooms to female phase (1st day) blooms between 1800 and 1830 hours. Distances travelled within the colony were quite short, between 2.5 and 15 meters and two beetle species were involved, with seven *C. gravis* and two *C. amblyopsis* being observed (Table 2). No individuals of *Cyclocephala sexpunctata* and only one individual of *Erioscelis columbica* were seen moving from male to female phase blooms in *D. longispatha* in La Selva.

DISCUSSION

Comparisons between beetle pollinated populations of *Dieffenbachia longispatha* in sympatric rainforest habitats in Costa Rica and Panama have revealed subtle differences in floral morphology, anthesis and pollination. In both localities, the inflorescences exhibit protogynous anthesis spread over two nights, typical of dynastine beetle pollination in the neotropics.

The vegetative appearance of *D. longispatha* in the field on BCI and at La Selva is indistinguishable and they grow in small colonies on the forest floor. However, the colonies of *D. longispatha* in La Selva are mostly found growing in low lying boggy areas while on BCI it is more often found growing on drier sites, often on sloping ground (pers. observ.). Colonies of *D. longispatha* are also larger at La Selva.

The most obvious physical difference between the two populations is apparent in the spathe color, which is dark green on BCI plants and yellow green on La Selva plants. This difference was consistent in all the individuals studied and would appear to represent a significant variant feature (Table 3). The color and density of female florets on the lower spadix constituted another difference between the two populations, with BCI specimens having large bright orange female florets well spaced out on the spadix, compared to the more densely packed smaller yellow florets on La Selva plants (Table 3, Fig. 1).

The other noticeable difference in the inflorescences was evident during anthesis, when BCI plants produced a spicy perfume, compared to a distinctly rancid odor on La Selva specimens (Table 3).

These differences appear to mark a significant ecological variation and the evolution of a separate sub-species in *D. longispatha*. Ongoing taxonomic revision of *Dieffenbachia* in Central America by Croat (pers. comm.) suggests the existence of a new sub-species of *D. longispatha* in Central America.

The driving force for this kind of variation in inflorescences of the same species between the two habitats would be supplied by selection pressure from the most efficient pollinators at each site. In theory, any differences in the numbers and type of beetles visiting the inflorescences of *D. longispatha* at BCI and La Selva might explain why inflorescences have evolved these differences. The observations on visiting beetles and recapture results from La Selva provide evidence for pollinator driven variation (Tables 1 and 2).

On Barro Colorado Island the most abundant beetle visitors were *C. gravis* and *C. sexpunctata* (Table 1a), while the most common beetle visitors to *D. longispatha* at La Selva were *C. amblyopsis* and *C. gravis* (Table 1b). Tests of significance (chi-squared tests) between the ocurrence of *C. gravis* at BCI and La Selva suggest a probability value of $p = 1.0$, which is not significant, indicating that in both localities this species is a common visitor and an important pollinator (Table 1a and 1b). The numbers of individuals of *C. gravis* recaptured at La Selva support this theory, although the number of beetles recaptured was too small to run a reliable chi-squared test on (Table 2).

However, differences in beetle visitation become more apparent when comparisons between the other two beetle species are made, where *C. amblyopsis* is an im-

Table 1. Numbers of Dynastine Scarab beetles (*Cyclocephala* spp. and *Erioscelis* sp.) visiting inflorescences of *Dieffenbachia longispatha* on Barro Colorado Island and La Selva Biological Station between July and September 1997. Beetles were counted inside the spathes on the first night of anthesis, during the female phase, between 2000 and 2100 hours (♀ female phase; ♂ male phase; — no observations; p = Chi-squared comparisons).

	Number of beetle visitors				
Anthesis dates ♀/♂ phases	***Cyclocephala* sp.**				***Erioscelis* sp.**
	C. amblyopsis	***C. carbonaria***	***C. gravis***	***C. sexpunctata***	***E. columbica***
1a. Barro Colorado Island, Panama					
9/10:7	—	—	1	2	—
11/12:7	—	1	2	—	—
14/15:7	—	—	1	1	—
15/16:7	—	1	—	1	—
17/18:7	—	1	1	2	—
21/22:7	—	—	2	2	—
23/24:7	—	—	—	1	—
26/27:7	—	1	2	2	—
27/28:7	—	—	1	3	—
Totals	0	4	10	14	0
1b. La Selva Biological Station, Costa Rica					
9/10:9	—	—	—	1	—
12/13:9	4	—	7	—	1
14/15:9	3	—	4	—	—
15/16:9	1	—	2	1	1
16/17:9	—	—	2	—	1
17/18:9	6	—	1	—	—
17/18:9	3	—	2	—	—
19/20:9	2	—	—	—	—
20/21:9	1	—	1	1	—
21/22:9	2	—	4	—	—
21/22:9	—	—	1	—	—
22/23:9	3	—	1	—	3
26/27:9	—	—	1	—	—
Totals	25	0	26	3	6
Chi-squared comparisons	p = .002	p = .017	p = 1.0	p = .008	p = .115

portant visitor at La Selva, compared *C. sexpunctata*, which is prevalent on *D. longispatha* at BCI. Chi-squared test results of p = .002 for *C. amblyopsis* and p = .008 for *C. sexpunctata* represent highly significant differences.

The absence *C. amblyopsis* on BCI would explain its absence from inflorescences of *D. longispatha* on BCI, but *C. sexpunctata* was present at both sites and was a common visitor to *D. longispatha* on BCI while being much rarer at La Selva (Table 1). The greater abundance of *C. sexpunctata* on inflorescences on BCI is

Table 2. Marked beetle movements from male phase to female phase inflorescences in a small colony of *Dieffenbachia longispatha* at La Selva during September 1997 (infl. inflorescence, — no observations or zero).

Male phase inflorescences			Female phase inflorescences			
Infl. no.	Marking date	No. of beetles marked	Infl. no.	Nos. of beetles recaptured	Date recaptured	Beetle species recaptured (nos. caught)
1	15:9	7	2	3	15:9	*C. amblyopsis* (1), *C. gravis* (2)
2	16:9	5	3	3	16:9	*C. gravis* (2), *E. columbica* (1)
3	17:9	3	4	1	17:9	*C. gravis*
4	18:9	7	—	—	—	—
5	20:9	2	6	1	20:9	*C. amblyopsis*
6	21:9	3	7	1	21:9	*C. gravis*
7	22:9	1	8	1	22:9	*C. gravis*
8	23:9	7	—	—	—	—
		35		10		

most likely to be caused by the odor of *D. longispatha* on BCI being more attractive to *C. sexpunctata* than the differently perfumed inflorescences at La Selva.

The attraction of high numbers of *C. amblyopsis* to *D. longispatha* at La Selva could be the result of the evolution of an odor that is specially attractive to *C. amblyopsis* at La Selva. The recapture results at La Selva indicate that *C. amblyopsis* is an important pollinator alongside *C. gravis* (Table 2). Recapture experiments done by Young (1986) at La Selva support these results by indicating *C. gravis* and *C. amblyopsis* as the principle pollinators.

Occasional visits by smaller numbers of other Dynastine Scarab beetle species were a feature of *D. longispatha* at both

Table 3. Comparisons of the differences between floral morphology, anthesis and pollination of *Dieffenbachia longispatha* at Barro Colorado Island, Panama and La Selva Biological Station, Costa Rica.

Difference	Barro Colorado Island	La Selva Biological Station
Spathe color	dark green	yellow-green
Female floret color	bright orange	pale yellow
Number of female florets	10–15	40–50
Odor at anthesis	sweet spicy scent	rancid odor
Average no. of beetles/infl.	3.1	4.6
Presence/absence of:		
Nitidulidae (beetles)	no	yes
Staphylinidae (beetles)	no	yes
Thysanoptera (thrips)	no	yes
Dermaptera (earwigs)	yes	yes
Drosophila (fruit flies)	yes	yes
Miridae (mirid bugs)	yes	yes

sites, but the numbers involved, (combined with the recapture results at La Selva) indicate that these would be ineffective or very minor pollinators.

On BCI *Cyclocephala carbonaria* was an occasional visitor, while *Erioscelis columbica* sometimes appeared in La Selva inflorescences. In both cases these beetles were absent from the other field site and the Chi-squared tests revealed non significant comparative values between the two beetle species at each field site (Table 2).

The role of the other insects (Nitidulid and Staphylinid beetles, thrips, earwigs, mirid bugs and fruit flies, Table 3) seen on *D. longispatha* at both field sites in pollination would appear to be insignificant, due to their small numbers and ineffective behavior syndromes to become effective pollinators. The evolution of the differing inflorescence morphologies and floral odors *D. longispatha* appears to be a response to the different suites of pollinating Dynastine Scarab beetles at each site. The spicy odor on BCI plants would seem to be attracting *C. sexpunctata* more effectively than the rancid odor on La Selva plants while the rancid odor present in La Selva plants may have evolved to attract *C. amblyopsis* (which is not present on BCI). The presence of *C. gravis* as a principle pollinator at BCI and La Selva indicates that the attractive odoriferous element for this beetle species is present at both field sites.

Further investigations are needed to confirm these hypothesis, with a special need to identify the exact chemical composition of the floral odors in each subspecies, which will help to quantify the differences between the BCI and La Selva populations of *D. longispatha*.

Once these odors are identified, it will be possible to undertake experiments with artificial components of the naturally occurring floral odors to see which elements were important in attracting the key pollinator species in the field. In this way it will be possible to identify the exact compounds which are responsible for attracting specific beetle species at each site. I propose to undertake these studies on BCI and at La Selva in future research.

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