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Oviposition in *Heliconius erato* (Lepidoptera, Nymphalidae): how Essential Is Drumming Behavior for Host-Plant Selection?

Sabrina C. Thiele[1] · Daniela Rodrigues[2] ·
Gilson R.P. Moreira[1]

Abstract Quantitative studies on the behavioral events involved in oviposition by phytophagous insects are scant. One of these events is drumming behavior, a rapid extension of forelegs after landing on a host, which remains largely under studied. In this study, quali- and quantitative analyses of *Heliconius erato phyllis* (Lepidoptera: Nymphalidae) oviposition events and drumming behavior in relation to both preferred (*Passiflora misera* and *P. suberosa*) and non-preferred (*P. caerulea* and *P. alata*) hosts were performed. For the first time, drumming behavior is described by images made during the butterfly oviposition process. The same set of females was assigned to both single- and multiple-choice tests under insectary conditions, and their behaviors toward each host were recorded on video. The resulting images were analyzed frame by frame. Both frequency and duration of behavioral events related to oviposition varied on preferred vs non-preferred hosts. On preferred hosts, behaviors that were associated with egg deposition (inspecting flight, drumming and abdomen touching) were pronounced; subsequently, oviposition occurred within a few seconds. On non-preferred host plants, behaviors that did not predict oviposition (flying and resting) predominated, and oviposition on these hosts was negligible. Drumming occurred on all plants and

✉ Daniela Rodrigues
drodriguesufrj@gmail.com

[1] PPG Biologia Animal, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, Porto Alegre, Rio Grande do Sul 91501-970, Brazil

[2] Departamento de Ecologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, CP 68020, Rio de Janeiro, Rio de Janeiro 21941-902, Brazil

resulted in a faster decision-making process on high-quality hosts (prompt acceptance) as well as on lethal hosts (prompt rejection) compared to the host that confers poor larval performance (delayed rejection). Thus, drumming is crucial for decision-making related in host-plant selection by *H. erato phyllis*, and influences other behaviors involved in the oviposition process.

Introduction

In phytophagous insects, host-plant selection by the parental female is determinant for success of the offspring. A recent meta-analysis has shown that oviposition preference is positively associated with larval performance, and this association is stronger in oligophagous species compared to polyphagous ones (Gripenberg et al. 2010). A positive link between oviposition preference and offspring performance is even more crucial in species with larvae possessing limited mobility (Dethier 1982; Feeny et al. 1983; Singer 1986). Thus, natural selection has shaped the behavioral steps involved in efficient host-plant recognition by ovipositing females.

Oviposition in insects, as any other behavior, is mediated by a number of both internal and external stimuli (Harris and Foster 1995). Butterflies and moths generally follow a sequence of behavioral events that includes searching and finding a potential host plant, evaluation and contact with the plant and subsequent acceptance or rejection of the plant (Ramaswamy 1988; Renwick and Chew 1994). Visual and olfactory cues may induce females to land on a potential host (Prokopy and Owens 1983; Bruce et al. 2005). After landing, perceiving chemical compounds through tactile receptors is of paramount importance for evaluating the appropriateness of the host (Feeny et al. 1983). As a consequence, the decision of whether to accept or reject a certain host plant will depend on the balance between stimulant and deterrent compounds (Roessingh et al. 1991; Nishida 2005).

Drumming behavior occurs in several insect orders and is involved in mate finding, acoustic communication, substrate recognition, and oviposition. In the context of oviposition, drumming is performed with the foretarsi, after ovipositing females land on a host. With respect to Lepidoptera, drumming has been examined in butterflies, and less is known about drumming in moths (Renwick and Chew 1994). Fox (1966) hypothesized that, when butterfly females contact the host surface with the foretarsi, plant chemical compounds are released and perceived by the corresponding gustatory papillae. In Nymphalidae and closely related lineages, these tarsi are reduced and have no cursorial function in both sexes (Wolfe et al. 2011). Male foretarsi lack these sensilla, and recently Briscoe et al. (2013) demonstrated that gene expression and evolution of these chemical receptors are in fact driven by females. Over the decades, drumming has been mentioned as an important step in the decision-making process for oviposition (e.g. Myers 1969; Ma and Schoonhoven 1973; Ichinosé and Honda 1978; Renou 1983; Ramaswamy et al. 1987). However, these studies consisted of behavioral catalogs, electrophysiological records, or experiments based on removal or inactivation of legs and/or antennae. Although drumming behavior is difficult to observe (Haribal

and Renwick 1998), studies on the subject have apparently been performed with the naked eye (see references above); as a consequence, inaccurate descriptions of drumming are probably an issue in past studies. The only mention of a record of drumming behavior in Lepidoptera using equipment dates back to the 1930s, in a movie presented at the Meeting of the Entomological Society of Germany (see second reference in Ilse 1937). Apparently, these images have not been available in the literature. Quantitative analyses of this behavior in particular, as well as other behaviors involved in oviposition in Lepidoptera, are scant.

The present study examined drumming behavior in *Heliconius erato* (Linnaeus) (Lepidoptera: Nymphalidae), a passion vine butterfly widely distributed in the Neotropical Region (Rosser et al. 2012). *Heliconius erato* is considered oligophagous sensu Bernays and Chapman (1994), and uses several species of Passifloraceae as host plants (Beccaloni et al. 2008). In southern Brazil, nine passion-vine species have been recorded as hosts of *H. erato phyllis* (Dell'Erba et al. 2005). In this region, females prefer to lay eggs on *Passiflora misera* and *P. suberosa*, and these host plants confer superior larval performance over other *Passiflora* species. *Passiflora caerulea* is a less used host on which larval performance is poor, whereas *H. erato phyllis* females avoid ovipositing on *P. alata*, since it is lethal to larvae (Menna-Barreto and Araújo 1985; Périco and Araújo 1991; Périco 1995; Rodrigues and Moreira 2002; Kerpel and Moreira 2005; Silva et al. 2014). Adults of *H. erato phyllis* have strong visual acuity (Benson et al. 1975) and females are able to select large-sized larval hosts, which present terminal buds and no conspecifics (Mugrabi-Oliveira and Moreira 1996a, b). Eggs are laid singly, on tendrils and young leaves (Benson 1978), and oviposition preference is innate and not learned (Kerpel and Moreira 2005). Females possess gustatory sensilla associated with the tarsal cuticular spines, and inactivation of these sensilla prevents oviposition (Silva 2015). Adults of *Heliconius* are long-lived probably due to consumption of both nectar and pollen from various sources; oviposition rates vary from one to four eggs per day (Gilbert 1972; Dunlap-Pianka et al. 1977; Corrêa et al. 2001). Females learn host plant location, and frequently inspect larval host plants when foraging for pollen and nectar (Gilbert 1975). These studies indicate that host-plant selection by *H. erato phyllis* occurs through assessment of host plant attributes during the oviposition process, and not through previous experience.

Although *H. erato phyllis* host use and preference have been extensively examined (Périco and Araújo 1991; Périco 1995; Rodrigues and Moreira 2002; Kerpel and Moreira 2005), the behavioral events that precede oviposition in this species were not fully investigated. Périco and Araújo (1991) pointed out that *H. erato phyllis* performs drumming on different hosts; however, the number of females tested in that study was not reported, which limits understanding of the actual frequency of this behavior on hosts that differ in quality. In this study, we examined the behavioral events involved in the oviposition behavior of *H. erato phyllis* in relation to preferred vs. non-preferred hosts, using both quali- and quantitative approaches. We predicted that the oviposition behavior of *H. erato phyllis* would differ when females encounter preferred hosts compared to non-preferred ones. Moreover, we hypothesized that drumming is an important step in decision-making processes related to egg laying in *H. erato phyllis*. When butterflies contact preferred larval host plants, oviposition would take place. Conversely, plant abandonment after drumming would occur when the host is non-preferred.

Material and Methods

Study System

Four species of host plant were used: *Passiflora misera*, *P. suberosa*, *P. caerulea* and *P. alata*. These vines have tendrils that allow them to climb over trees and other substrates (Ulmer and MacDougal 2004). As stated above, these hosts differ in terms of *H. erato phyllis* oviposition preference and larval performance. Thus, *P. misera* and *P. suberosa* were the preferred hosts used for *H. erato phyllis*, and *P. caerulea* and *P. alata* were the non-preferred ones. Seedlings of the four passion-vine species were cultivated in a greenhouse at the Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, southern Brazil (30°02′ S; 51°13′ W).

Adults of *H. erato phyllis* were obtained by rearing larvae collected from the field population existing at the above locality. Larvae were kept individually in transparent plastic pots (500 mL) and fed ad libitum with *P. misera* shoots, as larval performance is superior on this host. Larvae were reared under controlled abiotic conditions (25 ± 1 °C; 14 L: 10 D) and inspected daily until pupation; cleaning and food replacement were done whenever necessary. Newly emerged adults were numbered on the forewings and transferred to outdoor insectaries (2 x 1.5 x 1.5 m each).

Females were fed daily with a semi-natural diet consisting of a mixture of pollen, honey and distilled water (proportion 1:2:7) (Ferro 1998). The diet was offered to butterflies through artificial feeders, which were constructed from 0.5 mL Eppendorf tubes without lids, and a round piece of yellow ethylene vinyl acetate (EVA) (diameter: 4 cm) with a hole in its center. In order to mimic a real flower, the EVA was arranged around the opening of the Eppendorf tube. Males and females were kept together to allow mating. Then, during the pre-oviposition phase, females were separated from the males and kept in two insectaries (*n* = 5 females / insectary). For each group, either *P. misera* or *P. suberosa* was available to the females. This procedure was done before tests commenced, to make sure the females to be tested were able to lay eggs. These host plants were chosen because *H. erato phyllis* have innate preferences for *P. misera* and then *P. suberosa*, and oviposition preference does not change according to either larval or adult experience (Kerpel and Moreira 2005). When oviposition was observed, the host plants were removed, and the females were tested after 24 h.

Behavioral Events Involved in Oviposition

Behavioral observations made in the single choice tests (see below) were used to construct a qualitative description of the behavioral events involved in *H. erato phyllis* oviposition. Observation and record methods followed focal sampling rules, as described in Martin and Bateson (2007). In order to do so, videos were made to capture female activity in relation to the hosts, through a Sony® Cyber-shot DSC-H10 video camera. To properly observe drumming, additional images were recorded at a distance of ca. 20 cm. Checking the movements of the anterior legs was possible through sequential images taken frame by frame from the videos, which were decomposed with the software Windows Movie Maker®.

The behavioral events were based on Dinesh and Venkatesha (2013) (inspecting flight), Ilse (1937) and Renwick and Chew (1994) (drumming; Fig. 1), and Chadha and
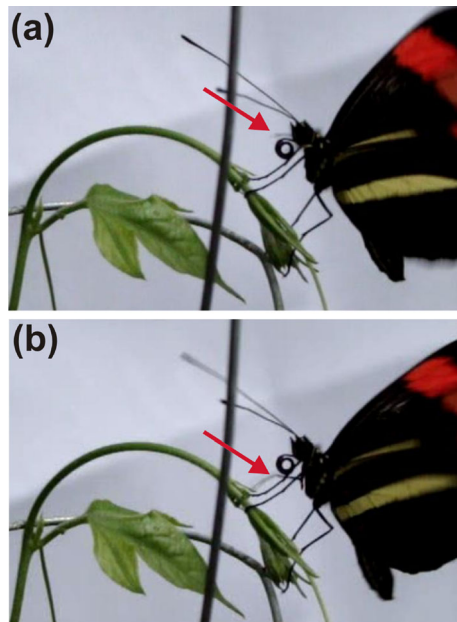
Fig. 1 Close-up of *Heliconius erato phyllis* drumming behavior, indicating variation in position of forelegs *(arrows)*: (**a**) raised, kept close to the female's body; (**b**) lowered, touching the host plant surface

Roome (1980) (abdomen touching). Flying and resting were also taken into account. These five behavioral events were divided into two categories that are associated with egg deposition or not, according to the descriptions listed in Table 1. The number of transitions between behavioral events (that is, the beginning of a certain behavioral event after the preceding one has finished) was also recorded.

When oviposition occurred, the observations were halted.

Table 1 Behavioral events performed by *Heliconius erato phyllis* ovipositing females when tested on preferred and non-preferred host plants

Behavioral events	Description
Association with egg deposition	
Inspecting flight	Flying around the host within a maximum radius of ca. 20 cm; females approaching very close to the host, but not touching it (images available in online resource 1)
Drumming	Forelegs extending rapidly and alternately after landing on the host, simultaneously with rapid wing movements (Fig. 1a, b; images available in online resource 2)
Abdomen touching	Abdomen curling, followed by touching the host surface with the ovipositor; no oviposition (images available in online resource 3)
Non-association with egg deposition	
Flying	Moving the wings and keeping the body in the air in a radius of more than 20 cm distant from the host
Resting	Wings motionless and legs settled, with cursorial legs on the substrate

Experimental Design

To examine the behavioral events of *H. erato phyllis* in relation to preferred vs non-preferred hosts, single- and multiple-choice tests were run ($n = 10$ / test). In both tests, the same butterflies were tested individually; each butterfly was tested only once a day. For the single-choice tests, only females that displayed behaviors either associated with egg deposition or non-associated with egg deposition in relation to all four hosts were included in the analysis.

Passiflora misera, *P. suberosa*, *P. caerulea* and *P. alata* shoots used in the experiments were standardized, i.e., all had intact apical bud as well as five open leaves, and had no reproductive parts. Five artificial feeders were arranged on a round styrofoam base (10 cm diameter) and placed at a distance of 1 m from the hosts. Experiments were performed from 09:00 to 14:00 hs, the period when females are most active (Mugrabi-Oliveira and Moreira 1996b).

Observations were made through the aid of a video recording with the camera, which was supported on a tripod. Both the equipment and the observer were located outside the insectary, ca. 1 m distant from the target passion-vine shoot, to avoid influencing butterfly behavior. Females were placed individually in the insectary, and all the behaviors that they performed after contacting the plant (i.e., after the first inspecting flight) were recorded. The trials lasted until oviposition occurred. If no eggs were laid, the observations continued for 30 min. For each behavioral event, the absolute frequency and duration were recorded.

Single-Choice Tests

Only one host was offered in a 500-ml plastic bottle with water, placed in the center of the insectary. To minimize eventual effects of abiotic factors and the previous experience of the females on oviposition behavior, the sequences in which hosts were offered to each butterfly were randomized. In these tests, the absolute frequency of all behavioral events was recorded in sequence.

Multiple-Choice Tests

In the multiple-choice tests, one shoot of each host was placed in the center of the insectary as described above. The shoots were placed 30 cm apart from each other and 1 m distant from the artificial feeder. Host position was randomized in order to avoid spatial conditioning of the females (see Ney-Nifle et al. 2001). In these tests, only the behaviors that are associated with deposition of an egg were recorded, as flight and resting are not related to any host in particular and thus are not informative in a multiple-choice situation.

Statistical Analysis

With respect to both single and multiple choice tests, the total testing time was calculated (that is, the time at which a female started to interact with the hosts until the end of the test). For single choice tests, the latency period until egg laying (on preferred hosts) or host abandonment (in the case of non-preferred hosts) was also quantified. To do so, we

considered the first drumming event as the onset of the latency period, and laying an egg or abandoning the host as the end of it. The percent of time spent performing each behavior was calculated from summing all the behavioral events, which were then divided by the total testing time and finally multiplied by 100. The data regarding absolute frequency and duration of each behavioral event on each host, as well as latency and percent of time performing each behavior relative to total testing time, were analyzed with respect to normality and homoscedasticity of variances through Kolmogorov-Smirnov and Bartlett tests, respectively. Because of data dependency, the data that showed normal distribution and homoscedastic variances were submitted to Repeated Measures ANOVA, followed by Tukey's multiple comparison tests. The data that did not show a normal distribution and homoscedastic variances were log $(x + 1)$ transformed, and tested again with respect to normality and variance homoscedasticity. The normalized data after transformation were compared by Repeated Measures ANOVA, as above, and those in which no transformation was successful were compared through non-parametric tests (Friedman tests, followed by Dunn's multiple comparison tests). The total testing time in both tests was compared among hosts in the same way. Analyses followed the procedures described in Zar (1999) and Conover (1999), and were run using GraphPad Prism® software (Motulsky 1999).

Results

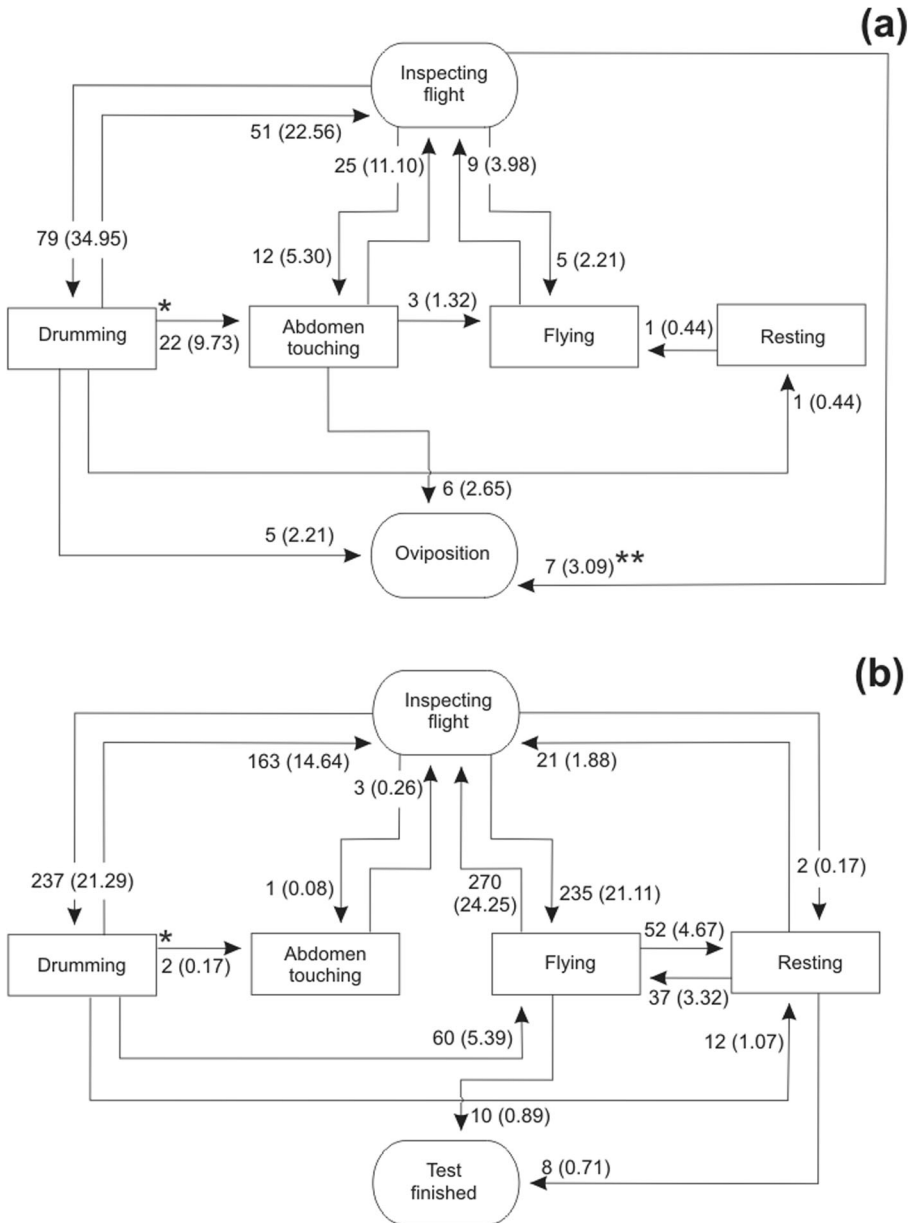### Behavioral Events Involved in Oviposition

The behavioral events involved in oviposition of *H. erato phyllis* varied with respect to sequences in relation to preferred vs non-preferred hosts, as well as their corresponding absolute frequencies. There was a total of 226 transitions between behavioral events when the hosts were preferred (*P. misera*: 119–52.65 %; *P. suberosa*: 107–47.35 %), and 1113 transitions relative to non-preferred hosts (*P. caerulea*: 657–59.03 %; *P. alata*: 456–40.97 %) (Fig. 2a, b). Drumming behavior occurred in all cases, regardless of the host.

In the presence of preferred hosts, inspecting flight was succeeded by drumming (79 cases - 34.95 %) and abdomen touching (12 cases - 5.3 %). The transition from inspecting flight to oviposition always occurred after other, intervening behavioral events, and after at least one drumming event. After drumming, in most cases there were additional flight inspections (51–22.56 %) and / or abdomen touching (22–9.73 %). For both preferred hosts, oviposition occurred in all trials (Fig. 2a).

For non-preferred hosts, the number of transitions between behavioral events increased markedly, and no eggs were laid. With these hosts, inspecting flights were pronounced, followed by drumming (237 cases - 21.29 %), flight (235 cases - 21.11 %) and resting (2 cases - 0.17 %). In contrast, abdomen touching occurred only three times on *P. caerulea* and never on *P. alata* (Fig. 2b).

Single-Choice Tests

One female did not respond to all hosts, and was therefore excluded from the analysis. *Heliconius erato phyllis* varied both in terms of number of events, as well as test

Fig. 2 Behavioral events involved in *Heliconius erato phyllis* oviposition in relation to: (**a**) preferred hosts (**b**) non-preferred hosts (*n* = 9) in a single-choice situation. Arrows indicate the direction of each event toward the subsequent one. Arabic numbers denote the absolute frequency of transition, and numbers in the parenthesis denote the corresponding relative frequency. *Drumming behavior was recorded in all tests; **Transition from inspecting flight to oviposition always occurred after other behavioral events, including previous inspecting flights, abdomen touching and at least one drumming event

duration. Females spent significantly different amounts of time in the tests, depending on the host ($F_{3,24} = 125.9$; $P < 0.0001$). The testing time was significantly shorter when females were tested on *P. misera* (minimum and maximum of 22.72 and 167.50 s,

respectively) and *P. suberosa* (18.80 and 95.20 s) than on *P. caerulea* (349.40 and 1800 s) and *P. alata* (596.20 and 1800 s) (Tukey's multiple comparison tests, $P < 0.05$). Latency differed among the hosts ($F_{3,24} = 26.48$, $P < 0.0001$). *Passiflora misera* and *P. suberosa* differed from both *P. caerulea* and *P. alata* in terms of latency from first drumming to either laying an egg (*P. misera* and *P. suberosa*) or leaving the host (*P. caerulea* and *P. alata*) (Tukey's multiple comparison tests, $P < 0.05$) (Fig. 3).

The different hosts also influenced the percent of time spent in each behavior (Fig. 4). When tested on preferred hosts, females spent more time in behaviors associated with egg deposition. The percent of time spent in inspection flight was higher on *P. misera* and *P. suberosa* than *P. caerulea* and *P. alata* ($F_{3,24} = 50.30$, $P < 0.0001$; Tukey's multiple comparison tests, $P < 0.05$; Online Resource 1); the same pattern occurred for drumming (Friedman statistic = 22.45, $P < 0.0001$; Dunn's multiple comparison tests, $P < 0.05$) (Fig. 4a, b, respectively; Online Resource 2). Females spent more time displaying abdomen touching on *P. misera* and *P. suberosa* than on *P. alata* (Friedman statistic = 16.77, $P < 0.001$; Dunn's multiple comparison tests, $P < 0.05$) (Fig. 4c; Online Resource 3). On *P. caerulea* and *P. alata*, females spent more time performing behaviors not associated with egg deposition. The percent of time spent flying was higher on *P. caerulea* and *P. alata* compared to *P. suberosa* (Friedman statistic = 16.36, $P = 0.001$; Dunn's multiple comparison tests, $P < 0.05$), and was also higher on *P. alata* than *P. misera* and *P. suberosa* (Dunn's multiple comparison tests, $P < 0.05$) (Fig. 4d). Percent of time spent resting was higher on
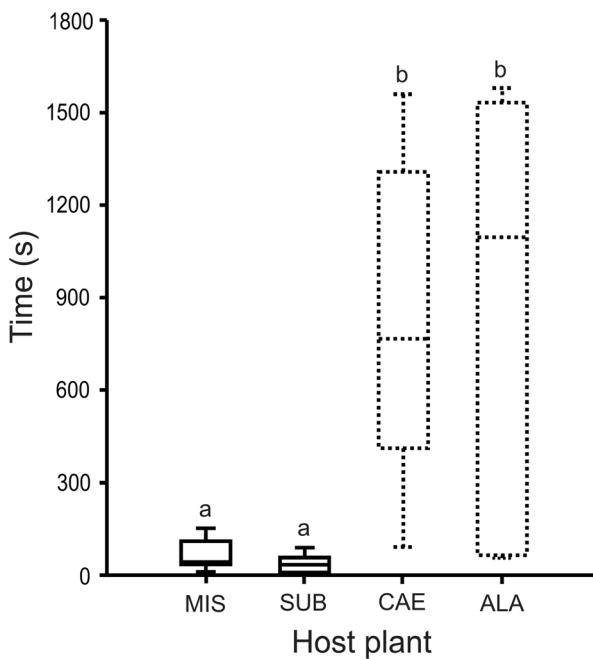
Fig. 3 Latency of *Heliconius erato phyllis* from first drumming event to either laying an egg (preferred hosts) or leaving the host (non-preferred hosts) ($n = 9$). Solid bars denote preferred hosts; dashed bars, non-preferred hosts. (MIS = *Passiflora misera*, SUB = *Passiflora suberosa*, CAE = *Passiflora caerulea* and ALA = *Passiflora alata*) (median ± interquartile range). For each panel, different letters above boxplots denote significant differences among hosts (Tukey's multiple comparison tests, $P < 0.05$)
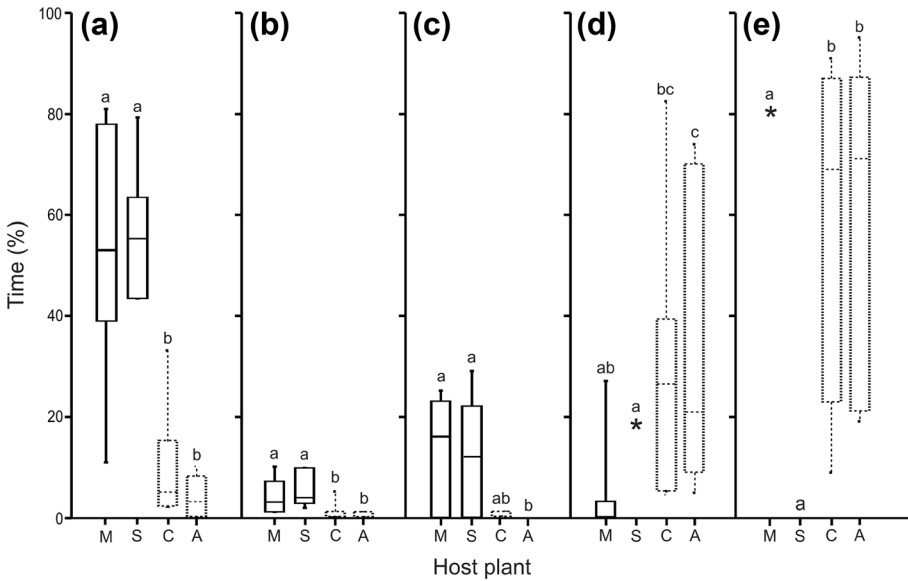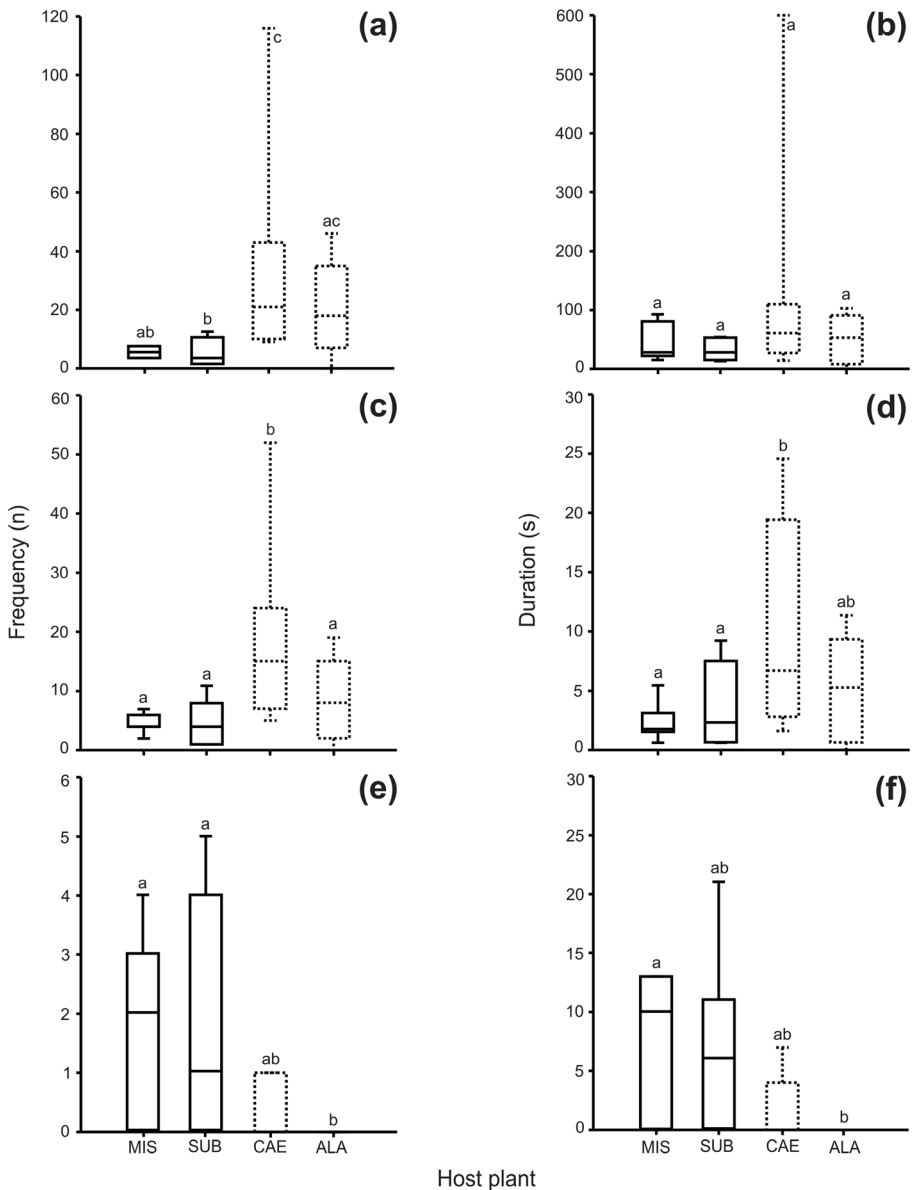
Fig. 4 Percent of time spent performing each behavioral event during the total testing time of *Heliconius erato phyllis* in relation to different hosts, in a single-choice situation (*n* = 9) (M = *Passiflora misera*, S = *Passiflora suberosa*, C = *Passiflora caerulea* and A = *Passiflora alata*) (median ± interquartile range). (**a**) inspecting flight; (**b**) drumming; (**c**) abdomen touching; (**d**) flight and (**e**) resting. Solid bars denote preferred hosts; dashed bars, non-preferred hosts. For each panel, different letters above boxplots denote significant differences among hosts (Tukey's multiple comparison tests for inspecting flight; Dunn's multiple comparison tests for the remaining behavioral events, *P* < 0.05)

P. caerulea and *P. alata* compared to *P. misera* and *P. suberosa* (Friedman statistic = 19.30, *P* < 0.001 Dunn's multiple comparison tests, *P* < 0.05) (Fig. 4e).

The absolute frequency of inspecting flights differed significantly among hosts ($F_{3,24}$ = 10.03, *P* < 0.001). Females of *H. erato phyllis* inspected *P. misera* significantly less than *P. caerulea*, and also inspected *P. suberosa* significantly less than *P. caerulea* and *P. alata* (Tukey's multiple comparison tests, *P* < 0.05) (Fig. 5a). In contrast, the time durations of inspecting flights did not vary among hosts (Friedman statistic = 4.55, *P* = 0.21) (Fig. 5b).

For all hosts, females of *H. erato phyllis* performed drumming in a matter of seconds (mean ± SE, *P. misera*: 2.26 ± 0.47; *P. suberosa*: 3.24 ± 1.04; *P. caerulea*: 10.04 ± 2.65; *P. alata*: 4.79 ± 1.33). Drumming frequency was significantly higher on *P. caerulea* compared to all other hosts ($F_{3,24}$ = 8.31, *P* < 0.001 followed by Tukey's multiple comparison tests, *P* < 0.05 for all comparisons involving this host). Drumming duration differed significantly among hosts ($F_{3,24}$ = 6.15, *P* < 0.01). Females drummed significantly less on *P. misera* and *P. suberosa* compared to *P. caerulea* (Tukey's multiple comparison tests, *P* < 0.05) (Fig. 5c, d). Females differed significantly in abdomen-touching behavior, depending on the host (Friedman statistic = 14.75 and 15.77 for absolute frequency and time duration, respectively; *P* < 0.01 for both parameters). Abdomen touching occurred more frequently on *P. misera* and *P. suberosa* than on *P. alata* (Dunn's multiple comparison tests, *P* < 0.05) (Fig. 5e), and the time spent displaying this behavior was significantly longer on *P. misera* than on *P. alata* (Dunn's multiple comparison tests, *P* < 0.05). The latter parameter did not differ between
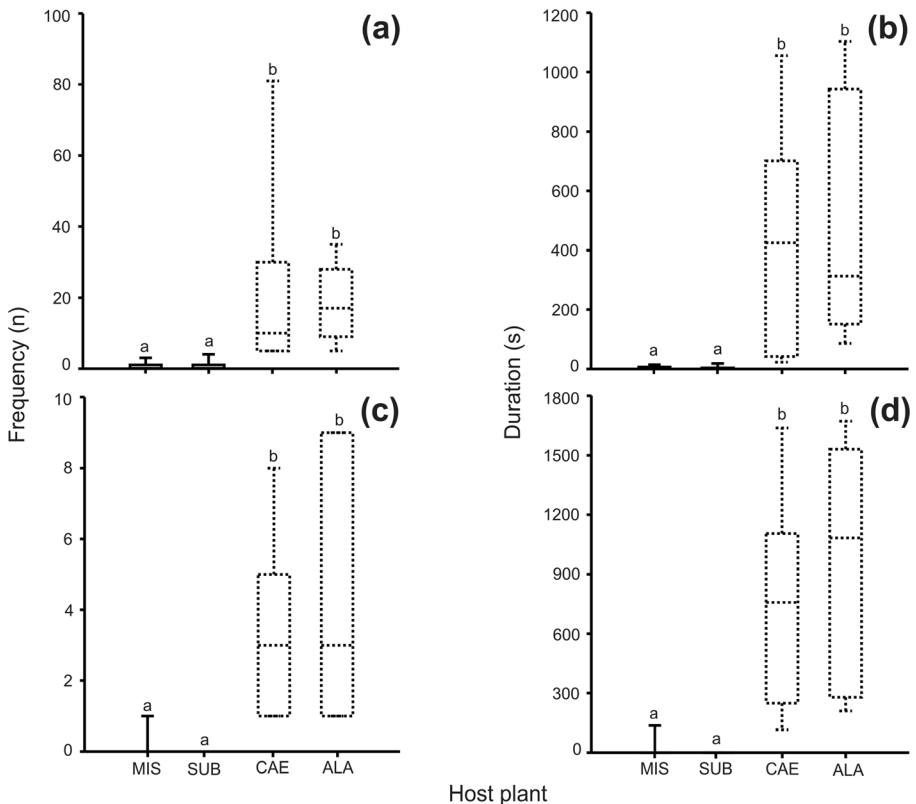
Fig. 5 Behavioral events involved in the oviposition process of *Heliconius erato phyllis*, that are associated with egg deposition in relation to different hosts, in a single-choice situation (*n* = 9) (MIS = *Passiflora misera*, SUB = *Passiflora suberosa*, CAE = *Passiflora caerulea* and ALA = *Passiflora alata*) (median ± interquartile range). Left column refers to the absolute frequency of each behavioral event; right column, duration of each behavioral event. (**a**) and (**b**), inspecting flight; (**c**) and (**d**), drumming; (**e**) and (**f**), abdomen touching. Solid bars denote preferred hosts; dashed bars, non-preferred hosts. For each panel, different letters above boxplots denote significant differences (Tukey's multiple comparison tests for absolute frequency of inspecting flight, and absolute frequency and duration of drumming; Dunn's multiple comparison tests for the remaining behavioral events, *P* < 0.05)

P. suberosa and *P. caerulea*, as well as when both hosts were compared to *P. misera* and *P. alata* (Dunn's multiple comparison tests, *P* > 0.05 in all cases) (Fig. 5f).

Flight patterns of *H. erato phyllis* differed significantly among hosts (Friedman statistic = 22.91, $P < 0.0001$ for both absolute frequency and duration). When tested on *P. misera* and *P. suberosa*, females flew significantly less and for shorter periods of time than when tested on *P. caerulea* and *P. alata* (Dunn's multiple comparison tests, $P < 0.05$ for both parameters) (Fig. 6a, b). A similar pattern was observed for resting behavior (Friedman statistic = 23.14 for absolute frequency and 23.78 for duration; $P < 0.0001$ for both parameters), which occurred less often and for shorter periods of time on *P. misera* and *P. suberosa* than on *P. caerulea* and *P. alata* (Dunn's multiple comparison tests, $P < 0.05$ for both parameters) (Fig. 6c, d).

Multiple-Choice Tests

In half of the cases, eggs were laid on *P. suberosa*, followed by *P. misera* (40 %) and *P. caerulea* (10 %). No eggs were laid on *P. alata*. When the hosts were offered simultaneously to females, there was no difference among the hosts in female activity time

Fig. 6 Behavioral events involved in the oviposition process of *H. erato phyllis*, that are not associated with egg deposition in relation to different hosts, in a single-choice situation ($n = 9$) (MIS = *Passiflora misera*, SUB = *Passiflora suberosa*, CAE = *Passiflora caerulea* and ALA = *Passiflora alata*) (median ± interquartile range). Left column refers to the absolute frequency of each behavioral event; right column, duration of each behavioral event. (**a**) and (**b**), flight; (**c**) and (**d**), resting. Solid bars denote preferred hosts; dashed bars, non-preferred hosts. For each panel, different letters above boxplots denote significant differences (Dunn's multiple comparison tests, $P < 0.05$)

(minimum and maximum seconds, *P. misera*: 0.00–49.16; *P. suberosa*: 0.00–118.80; *P. caerulea*: 0.00–31.36; *P. alata*: 0.00–21.40) (Friedman statistic = 7.70, $P = 0.052$).

Inspecting-flight duration, absolute frequency of drumming behavior, and drumming duration did not differ among hosts (Friedman statistic = 7.70, 7.79 and 6.16, respectively; $P > 0.05$ for all parameters) (Fig. 7b, c, d). Absolute frequency of inspecting flight, as well as absolute frequency and abdomen touching duration differed significantly in a multiple-choice situation (Friedman statistic = 8.54, 8.40 and 9.43 respectively; $P = 0.036$, $P = 0.038$ and $P = 0.024$, respectively). However, when the hosts were then compared pairwise, no significant differences were found (Dunn's multiple comparison tests, $P > 0.05$ for all parameters) (Fig. 7a, e, f).

Discussion

Our results clearly show that drumming behavior plays a crucial role in host-plant selection by *H. erato phyllis*. In both single- and multiple-choice tests, drumming occurred in all trials, and differed markedly in terms of absolute frequency and time duration. In addition, when only one option was available, the *H. erato phyllis* behavioral repertoire, including drumming, differed on preferred hosts from that seen on non-preferred hosts. In general, behaviors that are associated with egg deposition predominated when butterflies interacted with preferred hosts; conversely, in non-preferred hosts, behaviors that do not indicate association with egg deposition were prominent.

For lepidopterans, behavioral events that lead to oviposition generally follow a sequence that begins with searching and finding the hosts, followed by landing, contact evaluation, and finally host acceptance or rejection (Renwick and Chew 1994). When *H. erato phyllis* females were tested on preferred hosts, all the behavioral events occurred in this sequence, and ended with host acceptance. However, behavioral events associated with egg deposition occur repeatedly or intercalated with those non-associated with egg deposition. Harris and Miller (1991) described a similar pattern for Anthomyiidae flies, whose sequences among pre oviposition behaviors can vary and even be regressive. On preferred hosts, progressive transitions predominated in *H. erato phyllis* (e.g. inspecting flight to drumming; drumming to abdomen touching; abdomen touching to oviposition; drumming to oviposition; inspecting flight to oviposition), and oviposition took place shortly after the females performed drumming for the first time. The opposite occurred with non-preferred hosts, in which the females flew and rested repeatedly, and regressive behavioral transitions occur in high frequencies (e.g. drumming to inspecting flight; drumming to flight; drumming to resting). In these hosts, the trials always ended with no oviposition. As a consequence, when females of *H. erato phyllis* were faced with non-preferred hosts, decision-making processes took longer compared to the situations in which preferred hosts were available.

In phytophagous insects, finding potential hosts occurs through both olfactory and visual cues (Prokopy and Owens 1983; Renwick 1989). For all hosts examined here, the *H. erato phyllis* females first performed searching and inspecting behaviors, with no contact with the host-plant surface. As mentioned above, inspecting flights occur at a
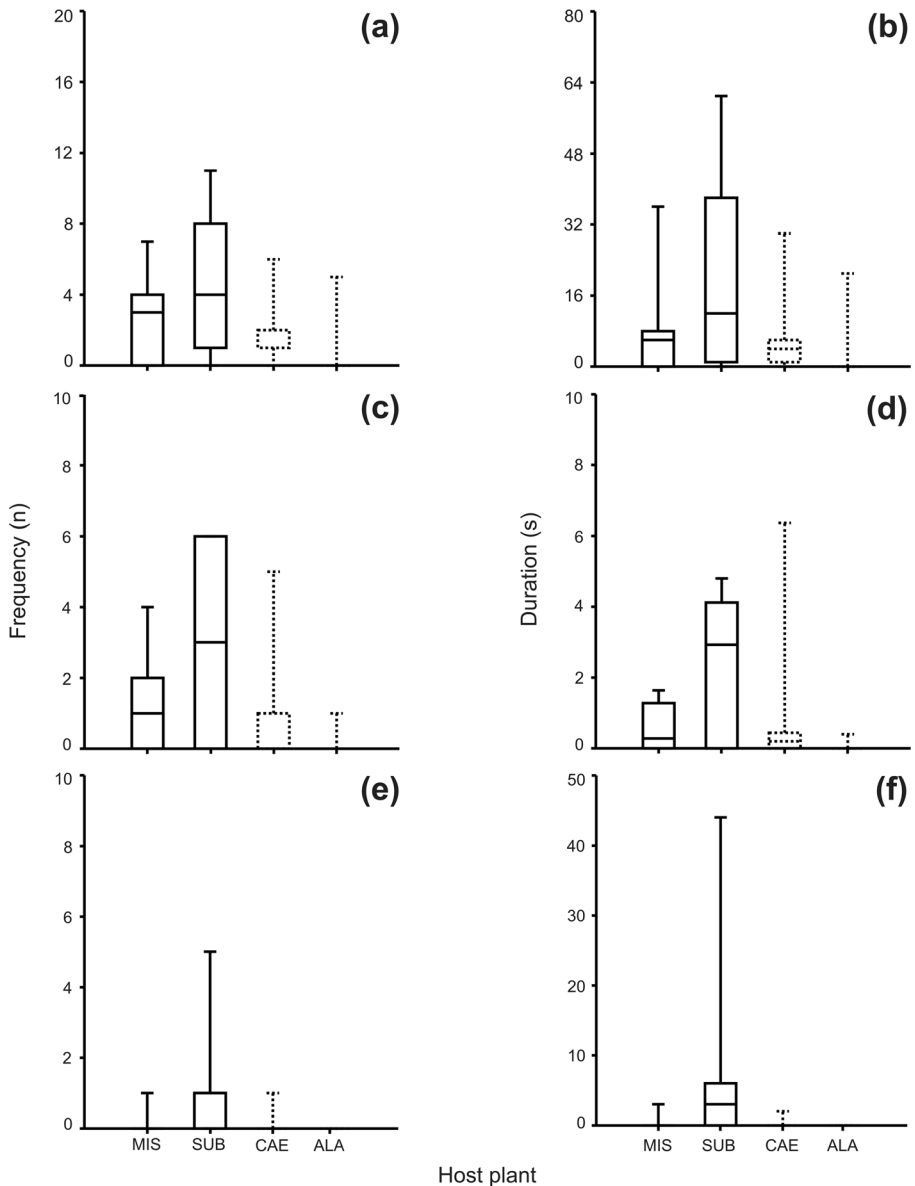
Fig. 7 Behavioral events involved in the oviposition process of *H. erato phyllis*, that are associated with egg deposition in relation to different hosts in a multiple-choice situation (*n* = 10) (MIS = *Passiflora misera*, SUB = *Passiflora suberosa*, CAE = *Passiflora caerulea* and ALA = *Passiflora alata*) (median ± interquartile range). Left column refers to the absolute frequency of each behavioral event; right column, duration of each behavioral event. (**a**) and (**b**), inspecting flight; (**c**) and (**d**), drumming; (**e**) and (**f**), abdomen touching. Continuous bars denote preferred hosts; dashed bars, non-preferred hosts

maximum radius of 20 cm and, in some cases, females were only a few millimeters away from the host. Although *H. erato phyllis* spent a similar amount of time inspecting all host plants, the frequency of this behavior was remarkably higher on non-preferred hosts, which probably reflects their poor suitability for larval feeding.

After inspecting flights, *H. erato phyllis* females performed drumming behavior. While they are drumming, butterfly females evaluate the oviposition substrate by assessing both stimulant and deterrent compounds (Nishida 2005). *Heliconius erato phyllis* drummed less frequently on *P. misera*, *P. suberosa* and *P. alata* compared to *P. caerulea*. In nature, *P. misera* and *P. suberosa* are widely used by this species, and use of *P. caerulea* is occasional (Périco 1995; Rodrigues and Moreira 2002; Kerpel and Moreira 2005). As a consequence, the decision-making process for oviposition may be facilitated because of the stimulating compounds present in these two high-quality hosts. On *P. alata*, drumming was less pronounced than on *P. caerulea*, similarly as seen in the preferred hosts. Although the presence of *H. erato phyllis* eggs on *P. alata* has been recorded (Dell'Erba et al. 2005), these instances are likely rare or accidental, as this host is lethal and leads to no viable offspring in southern Brazil (Périco 1995; Silva et al. 2014). Females probably recognize this lethal host through deterrent compounds, which are detected by the females in a few short drumming events. Although several stimuli can influence oviposition in phytophagous insects, including visual ones, *H. erato phyllis* do not lay eggs when foretarsi are experimentally inactivated, which increases the body of evidence that drumming is crucial for oviposition to take place (Silva 2015).

The host plants used in this study have distinct chemical profiles, at least with respect to flavonoids and saponins (Birk et al. 2005), and these compounds are known to be important for host selection by herbivorous insects, including lepidopterans (Gershenzon and Croteau 1991; Hartmann 1991). *Passiflora caerulea* can be considered an alternative host to *H. erato phyllis*, as oviposition records exist (see Dell'Erba et al. 2005), but performance is poor in terms of longer larval development time and smaller adult size compared to *P. misera* and *P. suberosa* (Périco 1995; Silva et al. 2014). The higher frequency of inspecting flights and drumming behavior on *P. caerulea* compared to all other hosts indicates that *H. erato phyllis* probably needs additional time to decide either to accept or to reject this host, due to its intermediate quality. In contrast, decision-making for laying an egg is rapid when females encounter high-quality resources such as *P. misera* and *P. suberosa*, as well as abandoning the host in the case of the lethal *P. alata*. Similarly to our study organism, Cecidomyiidae females show regressive behavioral transitions when they encounter a suboptimal host. In this case, females thoroughly rather than rapidly inspect these hosts. Because of the high predation risk to adults and slow flight due to unfavorable weather and small body size, the probabilities of finding superior host plants in case females leave the suboptimal host are low. As a consequence, it is advantageous taking time to assess suboptimal host plants instead of leaving them (Ganehiarachchi and Harris 2009).

After evaluating preferred hosts through drumming, *H. erato phyllis* females inspected the host-plant surface using their ovipositor. Although the presence of sensilla on the *H. erato phyllis* ovipositor has not yet been confirmed, abdomen touching has been considered important to evaluate plant compounds during oviposition (Chadha and Roome 1980; Renwick and Chew 1994). With respect to non-preferred hosts, a few females assessed the plant surface with the ovipositor on *P. caerulea*, but this behavior did not occur for *P. alata*. As suggested above, deterrent compounds detected during drumming behavior likely arrests the sequence of oviposition behaviors and prevents further contact investigation through abdomen touching on the lethal *P. alata*.

In a multiple-choice scenario, no differences were detected in the behaviors that are associated with egg deposition. Bernays (2001) pointed out that, because of neural limitations, decision-making in phytophagous insects is faster when the choices are limited, and the time tends to increase as the universe of options also increases. In the present study, most females physically interacted with both preferred and non-preferred hosts before laying eggs on a preferred host. Passion vines show intraspecific morphological variation (Benson et al. 1975), and this variation may make it difficult for females to visually discriminate among hosts from a distance. Thus, females of *H. erato phyllis* must inspect hosts from close range, through inspecting flight and drumming. Although the behaviors did not differ in a multiple-choice situation in terms of the number of events and time duration, eggs were mostly laid on the preferred hosts; only one oviposition event was recorded on *P. caerulea*, and no cases occurred on *P. alata*. In short, although the difficulty level increases with the number of options, the final decision by *H. erato phyllis* does not differ from that seen in a single-choice situation.

Conclusions

Although drumming behavior has long been considered a key factor for host-plant acceptance or rejection in butterflies, for the first time this behavior is shown by images, as well as quantitatively described in terms of absolute frequency and duration on hosts that differ in quality. At least in the case of *H. erato phyllis*, drumming cannot be clearly seen with the naked eye, and for the first time close-up video images are available (Online Resource 2).

For high-quality hosts, plant information is acquired through brief inspecting flights and a few drumming events, followed by abdomen touching. This last behavior is of paramount importance for the final decision. When the host confers poor larval performance, females perform inspecting flights and drumming many times, interspersing these behaviors with resting and flying. This behavior pattern leads to a long decision-making process. *Heliconius erato phyllis* are long-lived probably because of their diet, that includes pollen, which allows them to choose proper hosts for their offspring. When females encounter a lethal host, rejection occurs after only a few drumming events, which prevents abdomen touching and, as a consequence, oviposition.

Further studies would be helpful to examine whether foreleg movement, and in turn the signal produced during drumming, varies on different hosts in terms of amplitude, duration and frequency. Swihart (1967) showed that *H. erato* has very sensitive hearing organs at the base of the hindwings. Thus, in addition to perception of vibration of substrate-borne vibrations, if any, one could expect that females are able to hear the airborne sounds produced by their legs during drumming. These signals may also vary depending on the host plant, a matter that deserves further investigation.

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