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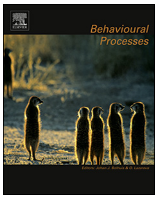

The specialization continuum: Decision-making in butterflies with different diet requirements

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ABSTRACT

Differences in diet requirements may be reflected in how floral visitors make decisions when probing nectar sources that differ in chemical composition. We examined decision-making in butterflies that form a specialization continuum involving pyrrolizidine alkaloids (PAs) when interacting with PA and non-PA plants: *Agraulis vanillae* (non-specialist), *Danaus erippus* (low demanding PA-specialist) and *D. gilippus* (high demanding PA-specialist). In addition, we assessed whether experience affected decision-making. Butterflies were tested on either *Tridax procumbens* (absence of PAs in nectar) or *Ageratum conyzoides* flowers (presence of PAs in nectar). *Agraulis vanillae* showed more acceptance for *T. procumbens* and more rejection for *A. conyzoides*; no differences were recorded for both *Danaus* species. *Agraulis vanillae* fed less on *A. conyzoides* than both *Danaus* species, which did not differ in this regard. In all butterfly species, experience on PA flowers did not affect feeding time. In the field, butterflies rarely visited PA flowers, regardless of the specialization degree. Our findings reveal that the specialization continuum seen in butterflies explains, at least in part, decision-making processes related to feeding. Additional factors as local adaptation mediated by the use of alternative nectar sources can affect flower visitation by specialist butterflies.

1. Introduction

Flower visitors encounter a wide array of nectar sources while foraging. Thus these visitors are supposed to be able to evaluate nectar features and make advantageous decisions according to their diet requirements (Papaj and Lewis, 1993; Weiss, 2001; Pyke, 2016; Parachnowitsch et al., 2018). Nectar composition, including the specialized metabolites, can affect nectar feeding by floral visitors (Baker, 1977; Rhoades and Bergdahl, 1981; Adler, 2000; Stevenson et al., 2017). Visitors that neither require nor are adapted to certain specialized metabolites (i.e., generalist or non-specialist visitors) are expected to be repelled or deterred by these compounds. In contrast, specialists are attracted to flowers that contain the specialized metabolites they need, sequestering and storing them in their body tissues to their own benefit (Baker and Baker, 1975; Bernays and Chapman, 1987; Nishida, 1995; Trigo, 2000; Manson et al., 2010; Nishida, 2014).

Specialization and generalization denote extremes of a continuum, as generalist herbivores (hereafter non-specialists) and specialists can vary in their responses with respect to specialized metabolites due to life history traits, cognitive abilities, detoxification and assimilation capacities, as well as spatial and temporal distribution of resources (Harborne, 1988; Bernays and Wcislo, 1994; Bernays, 2001; Ali and Agrawal, 2012; Barrett and Heil, 2012). For example, the monarch butterfly *Danaus plexippus* L. (Nymphalidae: Danainae) shows oviposition preferences for species of *Asclepias* (Apocynaceae) with intermediate levels of cardenolides (cardiac glycosides) (Oyeyele and Zalucki, 1990; Zalucki et al., 1990; Jones and Agrawal, 2016) because, although larvae are specialized in sequestering these chemical compounds (Brower et al., 1967; for a review, see Opitz and Müller, 2009), growth and survivorship in this life stage is impaired when cardenolides are consumed in high concentrations (Zalucki et al., 1990; Zalucki and Brower, 1992; Zalucki and Malcolm, 1999; Zalucki et al., 2001). *Bombus impatiens* Cresson, 1863 (Hymenoptera: Apidae), a generalist pollinator, is not deterred by the alkaloid gelsemine in nectar of *Gelsemium sempervirens* (L.) W.T. Aiton (Gelsemiaceae). Nevertheless, this bumblebee species can change its foraging strategies when other nectar sources are available, in order to avoid high concentrations of gelsemine (Gegear et al., 2007).

Pyrrolizidine alkaloids (PAs) occur in several plant families (e.g., Asteraceae: Senecioneae, Eupatorieae; Boraginaceae; Fabaceae:

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Crotalarieae; Apocynaceae: *Parsonsia* and Echiteae; Orchidaceae and Convolvulaceae) and are distributed in roots, leaves, flowers, nectar and seeds (Brown, 1984b; Hartmann and Witte, 1995; Hartmann, 1999; Hartmann and Ober, 2008). These alkaloids are well-studied specialized metabolites in the context of interactions between herbivores and plants (Boppré, 1986; Hartmann and Witte, 1995; Hartmann, 1999; Opitz and Müller, 2009; Boppré, 2011; Macel, 2011; Trigo, 2011). PAs have been recorded to be deterrent and / or toxic to non-specialist herbivores. For example, PAs in *Crotalaria pallida* Blanco (Fabaceae) have a negative effect on growth and survivorship of *Heliothis virences* (Fabricius) larvae (Lepidoptera: Noctuidae) (Cogni and Trigo, 2016). In *Apis mellifera* L. (Hymenoptera: Apidae), it was shown that high PA concentrations in the nectar have both toxic and deterrent effects (Reinhard et al., 2009). On the other side, some herbivorous insects are PA specialists, using these compounds as a defense against natural enemies and / or sexual communication (Macel, 2011; Trigo, 2011). Singer et al. (2004) have shown that larvae of the tiger moth *Grammia geneura* Strecker (Lepidoptera: Erebidae) use PAs of their hosts for self-medication and to reduce parasitism by tachinid flies. Other examples of PA specialists include *Oreina* and *Platyphora* leaf-beetles (Coleoptera: Chrysomelidae) (Pasteels and Hartmann, 2004), *Zonocerus variegatus* L. (Orthoptera: Pyrgomorphidae) (Bernays et al., 1977), *Aphis jacobaeae* Schrank (Hemiptera: Aphididae) (Witte et al., 1990), *Largus rufipennis* Laporte bug (Hemiptera: Largidae) and *Chauliognathus fallax* (German) soldier beetle (Coleoptera: Cantharidae) (Klitzke and Trigo, 2000) and arctiini moths as, for example, *Utetheisa ornatrix* L. (Lepidoptera: Erebidae) (Martins et al., 2015).

Danaine butterflies are among the most known PA specialists (Nymphalidae: Danainae: Danaini and Ithomiini) (Boppré, 1978; Ackery and Vane-Wright, 1984; Boppré, 1990). For most species of Danainae, PA sequestering is restricted to the adult stage (Edgar et al., 1974; Ackery and Vane-Wright, 1984; Boppré, 1990; Trigo et al., 1996). Adults of some species have been reported visiting vegetative tissues and nectar sources of PA plants in a collective way (Edgar and Culvenor, 1975; Pliske, 1975b, c; Boppré, 1983; Ackery and Vane-Wright, 1984). When visiting PA plants, these butterflies actively access PA sources by using the proboscis and the mesothoracic legs (Boppré, 1983). PAs are used by Danaine butterflies for sexual pheromone biosynthesis [dihidropyrrolizidine], as well as defense against natural enemies (Meinwald et al., 1969; Brown, 1984a; Boppré, 1990; Masters, 1990; Cardoso, 1997). PA concentration in the body tissues of adults varies according to species and sex (Edgar et al., 1976, 1979; Brown, 1984b). In general, males have highest concentrations of PAs and visit PA sources more than females (Boppré, 1983; Brown, 1984b). These differences in PA concentration indicate that Danaine butterflies have different PA demands and/or tolerance levels, suggesting that they might also vary in their use of flowers containing PAs in the nectar. In this ‘PA specialization continuum’, butterflies with the highest PA concentration in body tissues are expected to accept and feed on PA flowers more intensely than those with low or no PA concentration in body tissues, which remains to be tested.

In this study, we examined decision-making in three butterfly species that form a specialization continuum involving PAs when interacting with both PA and non-PA plants: *Agraulis vanillae* (Linnaeus, 1758) (Nymphalidae: Heliconiinae) (non-specialist) (Masters, 1991; Landolt and Lenczewski, 1993), *Danaus erippus* (Cramer, 1775) (Nymphalidae: Danainae) (specialist with low PA demand) and *D. gilippus* (Cramer, 1775) (Nymphalidae: Danainae) (specialist with high PA demand). The distinct PA demands of these two species of *Danaus* are based on the PA concentrations in the body tissues (*D. erippus*: 0.41 µg/ mg and 0.21 µg/ mg for males and females, respectively; *D. gilippus:* 4.5 µg/ mg for both sexes) (Brown, 1984b; Lopez, 2016). In addition, under insectary conditions, *D. erippus* are able to mate and to lay eggs without consuming PAs, while *D. gilippus* are not (MV Oliveira, PPS Ferreira, D Rodrigues, JR Trigo, BCM Ramos, personal observations) (Pliske and Eisner, 1969). PA sequestering by both *Danaus* species is restricted to the adult stage (Ackery and Vane-Wright, 1984). We also investigated how experience on PA and non-PA flowers affects decision-making in each butterfly species. We hypothesized that: 1) decision-making related to non-PA flowers did not differ among butterfly species, 2) decision-making related to PA flowers differs among butterflies depending on the PA specialization continuum, and 3) experience on PA flowers affects decision-making of *A. vanillae* and *D. gilippus* on these nectar sources. To test these hypotheses we offered a monotypic array composed by either *Tridax procumbens* (absence of PAs in nectar) or *Ageratum conyzoides* (presence of PAs in nectar) to individual butterflies on four consecutive days, and assessed attraction, acceptance, rejection, and feeding time on these plants. We predicted that the three butterfly species accept non-PA flowers at similar rates, since no PAs occur in the nectar. It is expected that *A. vanillae* shows reduced attraction and lower feeding time on PA flowers than both species of *Danaus*. With respect to the specialist species, we predicted that lower attraction and feeding time on PA flowers occurs in *D. erippus* compared to *D. gilippus*. In addition, males of the specialist species would be attracted to PA flowers more intensely compared to females. Finally, we predicted that experience in PA sources decreases feeding time by *A. vanillae*, and increases in *D. gilippus*.

2. Material and methods

2.1. Study system

2.1.1. Butterflies

PA non-specialist: *Agraulis vanillae* is distributed from USA to South America (Emsley, 1963; DeVries, 1987). It is common in open areas (Brown, 1992) and uses *Passiflora* (Passifloraceae) as larval host plants (Benson et al., 1975; Brown, 1992; Périco, 1995). No PAs have been recorded in *Passiflora*. Under laboratory conditions, *A. vanillae* adults have a lifespan ranging from 14 to 27 days (Arbogast, 1965). No information on longevity was found for this species in nature. *Agraulis vanillae* has an intermediate body size (May, 1985; DeVries, 1987) and a generalist diet (May, 1985, 1992; Masters, 1991; Landolt and Lenczewski, 1993; Weiss, 1995). Learning abilities were demonstrated for *A. vanillae* when using artificial flowers, as well as in the context of oviposition (Masters, 1991; Landolt and Lenczewski, 1993; Weiss, 1995; Kroutov et al., 1999). We collected eggs of *A. vanillae* from *Passiflora edulis* Sims (Passifloraceae) at Horto da Prefeitura Universitária of Universidade Federal do Rio de Janeiro (UFRJ), Cidade Universitária, Rio de Janeiro, RJ (22°54′S; 43°13′O).

PA specialists: *Danaus erippus* and *D. gilippus* occur in South America and in the Neotropical region, respectively (Ackery and Vane-Wright, 1984; Smith et al., 2005; Slager and Malcolm, 2015). Both species are common in disturbed, open areas (Slager and Malcolm, 2015). In Brazil, the main larval host plant of these species is *Asclepias curassavica* L. (Apocynaceae) (Becalloni et al., 2008), which has high levels of cardenolides in its leaves (Rasmann and Agrawal, 2011). No PAs have been recorded in *A. curassavica*. *Danaus erippus* and *D. gilippus* have long life spans (*Danaus*: from 2 to 4 months under field conditions) (Ackery and Vane-Wright, 1984), and both species range from medium to large-sized adults (Ackery and Vane-Wright, 1984; Brown, 1992; Ferreira, 2017). Studies on learning abilities using artificial and real flowers have shown that *D. erippus* have cognitive abilities in the context of feeding (de Oliveira et al., 2015; Rodrigues, 2016; Ramos et al., 2017). In contrast, insectary and laboratory trials have shown that *D. gilippus* do not visit artificial flowers (D. Rodrigues, personal communication). Eggs of both species of *Danaus* were collected from Reserva Ecológica de Guapiaçu (REGUA), Cachoeiras de Macacu, Rio de Janeiro, RJ (22°50′S; 42°88′O) and Reserva Biológica do Japi, Jundiaí, São Paulo (23°11′S; 46°53′O).

After collection, eggs of the three butterfly species were transferred to Petri dishes with moistened filter paper, and were monitored daily until larval hatching or egg failure. Newly-hatched larvae were transferred to transparent plastic pots (1 l) and reared at 25 °C (± 1 °C) under a 14 h: 10 h light:dark cycle with *ad libitum* leaves of *A. curassavica* (*D. erippus* and *D. gilippus*) and *P. edulis* (*A. vanillae*) until pupation. Leaves were washed with tap water and treated with germicidal light (654 nm) for 20 min to prevent mortality by pathogens. Pupae were transferred to outdoor insectaries for emergence and acclimation. Twenty-four hours after emergence, all adults were individually fed for 1 min at the artificial flower. To do so, each butterfly had its proboscis gently unrolled with a histological pin moistened with 20% aqueous sucrose, and placed on the nectar well. The artificial flower was constructed with a black concentric EVA circle with 4.0 cm in diameter. The EVA circle was pierced in the center with a 2 mL Eppendorf filled with artificial nectar. Artificial nectar consisted of 20% aqueous sucrose.

2.1.2. Plants

No-PA plant: *Tridax procumbens* L. (Asteraceae: Heliantheae) is an annual herb native to Central America (Lorenzi, 2000) and distributed in both tropical and subtropical regions (Powell, 1965). This species shows pistillate ray flowers, bilabiate corolla whose colors vary from cream to yellow arranged into capitulum-like inflorescences (Hattori and Nakajima, 2008) (Figure S1A- Supplementary material). A single inflorescence has a display varying from 7 to 15 mm diameter (Powell, 1965). The sucrose concentration of *T. procumbens* is 20%, and the average daily nectar production is 0.21 ± 0.05 µl per flower (Varalakshmi and Raju, 2013). No PAs have been recorded in this species (Jude et al., 2009). The inflorescences are commonly visited by several butterfly and bee species (Varalakshmi and Raju, 2013).

PA-plant: *Ageratum conyzoides* L. (Asteraceae: Eupatorieae) is native to South America and distributed across both tropical and subtropical regions (Johnson, 1971; Lorenzi, 2000; Kohli et al., 2006). It is an annual herb with lilac and white flowers arranged into corymbiform inflorescences (Lorenzi, 2000; Moreira and Bragança, 2011), and a single inflorescence is ca 5 mm diameter (Johnson, 1971; Bosi, 2012) (Figure S1B- Supplementary material). This plant species possesses the PA lycopsamine in both vegetative and floral parts (Brown, 1984b; Trigo, 1987; Wiedenfeld and Röder, 1991; Trigo et al., 1996; Bosi, 2012). Inflorescences of *A. conyzoides* have PAs of the lycopsamine type, which are open-chain monoesters or diesters containing at least one unit of 2-isopropyl-2,3-dihydroxybutyrate, the unique C_7-necic acid (Brown, 1984b; Orr et al., 1996; Trigo et al., 1996; Hartmann and Ober, 2008; Amadi et al., 2012; Bosi et al., 2012). Because of the minute size of individual flowers, no method was successful in collecting nectar from *A. conyzoides* (JR Trigo, personal observation). As a consequence, presence of PAs in the nectar of this plant is treated as an assumption. *Ageratum conyzoides* is one of the preferred PA sources by danaines (Orr et al., 1996; Trigo et al., 1996), and field surveys have shown *D. plexippus* and *D. erippus* visiting *A. conyzoides* flowers (Brown, 1984b; Trigo, 1987; Trigo et al., 1996; Morris and Kline, 2015). To our knowledge, there is no information on sucrose concentration and daily nectar production in *A. conyzoides*.

Seedlings of *T. procumbens* and *A. conyzoides* were collected from different localities at Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro City. The seedlings were planted in soil with organic moisture and watered once a day in greenhouses at Horto of UFRJ.

2.2. Experimental design

We performed single choice experiments using either *T. procumbens* or *A. conyzoides* inflorescences (n = 20 individuals/ butterfly species/ plant species). Butterflies were tested individually in an outdoor insectary (2.0 × 2.0 x 2.5 m) with one potted plant of *T. procumbens* or *A. conyzoides* having open inflorescences (n = 1 potted plant/ butterfly species/ day). In each test, 1 to 3 inflorescences of *T. procumbens* were offered to butterflies; as for *A. conyzoides*, 20 to 35 inflorescences composed each potted plant used in the trials. For both nectar sources, there was no indication that number of inflorescences affected the interaction with the three butterfly species (BCM Ramos, personal observation). Each potted plant was placed at the insectary's center, and those that were already visited by butterflies were not used again in further tests. Tests began two days after butterfly emergence. Butterflies were continuously observed during the trials and were allowed to probe only one inflorescence per trial (Martin and Bateson, 2007). Each trial had a maximum duration of 16 min. To stimulate butterflies to forage for nectar, before trials butterflies were fed using the artificial flower for 5 s. Such a procedure is standard in studies on learning in lepidopteran floral visitors (e.g., Rodrigues et al., 2010; Blackiston et al., 2011; Cepero et al., 2015; Ramos et al., 2017). Adults that were not attracted to the inflorescences after 7 min were captured and then offered an artificial flower for 5 s; afterwards, butterflies were gently placed on an inflorescence. This procedure was done to stimulate the butterflies to probe flowers, as spontaneous landing on the inflorescences was low under some circumstances (see results). In case a given butterfly did not probe flowers 15 min after trials commenced, the artificial flower was offered once again for 5 s. Afterwards, the butterfly was released back into the testing insectary and observed for one additional minute. After each trial, all butterflies were fed using the artificial flower for 1 min in order to standardize levels of satiation. To determine the effects of experience on decision-making, each trial was conducted once a day during four consecutive days. During the trials, we recorded the following parameters: attraction (percentage of individuals that spontaneously landed on the inflorescence followed by proboscis extension), rejection (number of individuals that performed proboscis extension inside the inflorescence followed by its removal for 1 s or less), acceptance (number of individuals that performed proboscis extension followed by its insertion inside the inflorescence for more than 1 s) and feeding time (total proboscis' extension and insertion times). Observations were made from 8 h 30 min to 15 h.

2.3. Statistical analyses

For each butterfly species, the number of individuals that either rejected or accepted the nectar source in the first trial was compared through Fisher's exact tests (Zar, 1999) by using GraphPad software (available in: https://www.graphpad.com/quickcalcs/contingency2/). Data on feeding time were log-transformed (log N) to obtain data normality and variance homoscedasticity. We used a generalized linear mixed-effects model with normal distribution (link function: identity). Butterfly species, sex and experience (trial day) were considered fixed factors, and individuals were considered a random factor. We also calculated the interaction between butterfly species and plant species. We analyzed the data using the lmer function in the "lme4″ package (Bolker et al., 2009) and glht function in the "multcomp" package, to perform multiple comparisons (Tukey contrasts) whenever applicable (Hothorn et al., 2008). For model selection, we used Akaike's Information Criterion through the AICtab function in the "bbmle" package (Burnham and Anderson, 2002). Only butterflies that visited inflorescences in at least three out of the four trials were included in the analyses. The analyses were run in R environment, v.3.1.3 (R Development Core Team, 2015).

2.4. Flower visitation in the field

To examine the flower visitation of *A. vanillae* and *Danaus* spp. under field conditions, a survey was conducted from 10 to 11 May 2017, in a 1330m-long and 5m-wide trail located in a pasture area of Itatiaia county, RJ (22°21′58.35″S; 44°29′48.59″O). Both butterflies and nectar sources were common in the study area. The area is surrounded by forest patches and grasslands. During the two days, we recorded all plant species showing open flowers (regardless of being a PA plant or not) and their corresponding abundance. To record abundance, individuals were delimited by modules located at least 1-m apart

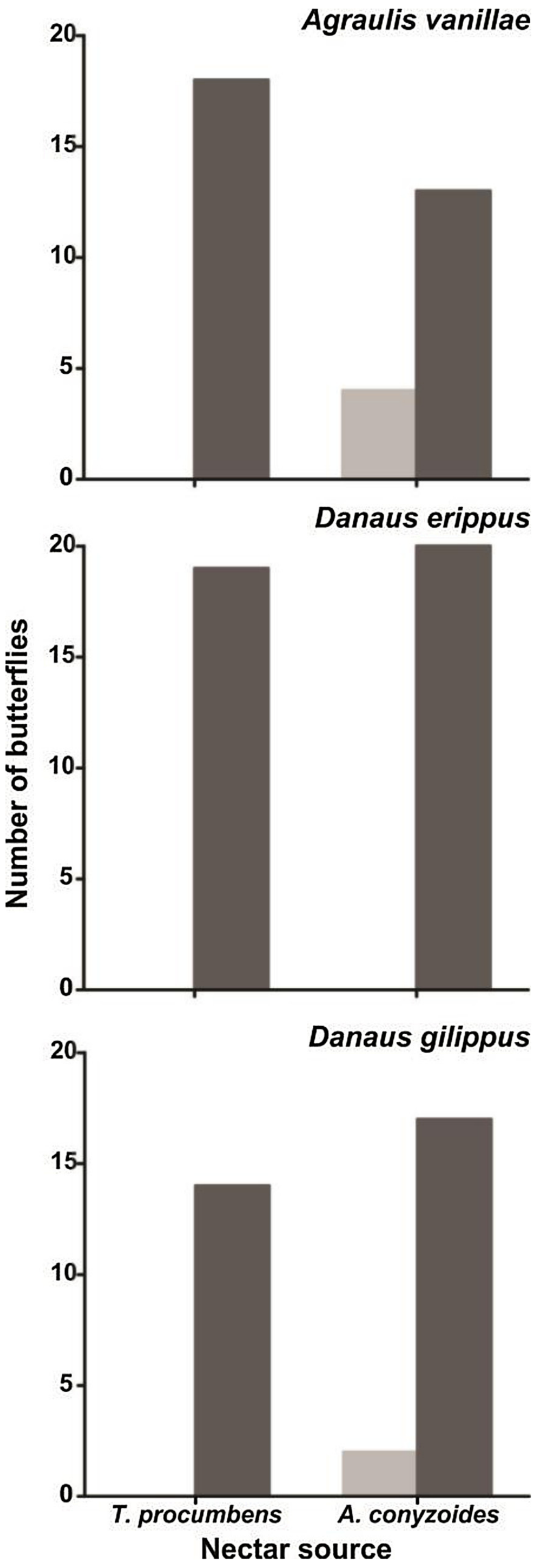

Fig. 1. Responses of butterflies in the first proboscis extension event in *Tridax procumbens* flowers (non-PA source) and *Ageratum conyzoides* (PA source). Rejection (light gray bars) and acceptance (dark gray bars).

from each other. The number of flower visits (hereafter feeding events) and flying events by the three butterfly species was recorded through a visual survey (Kearns and Inouye, 1993; Lehner, 1996). Inspection flight is defined as flying around the flower within a maximum radius of ca. 20 cm (Dinesh and Venkatesha, 2013). This survey was made from 8 h to 14 h by three observers. Butterflies are active foragers and are able to change their feeding responses in a matter of minutes (May, 1992; Masters, 1991; Weiss, 1995; de Oliveira et al., 2015; Rodrigues, 2016; Ramos et al., 2017; Ramos, 2018), so that the time duration devoted to this field survey is enough to examine use of preferred nectar sources by *Danaus* spp. and *A. vanillae*. Plants were identified at the species level; voucher specimens are deposited at the herbarium of UFRJ.

3. Results

3.1. Decision-making and feeding time

When probing flowers for the first time, rates of acceptance and rejection of *A. vanillae* differed between *T. procumbens* and *A. conyzoides* (Fisher's exact tests: $P = 0.046$) (Fig. 1). As for *D. erippus* and *D. gilippus*, there was no significant difference between rejection and acceptance of both nectar sources (*D. erippus*, Fisher's exact tests: $P = 1.000$; *D. gilippus*, $P = 0.490$) (Fig. 1).

For both nectar sources, there was a significant effect of butterfly species on feeding time (*T. procumbens*: $\chi^2 = 6.53$, df = 2, $P = 0.038$; *A. conyzoides*: $\chi^2 = 19.62$, df = 2, $P < 0.0001$) (Fig. 2; Tables 1 and 2; Table S1, S2, S3- Supplementary material). In addition, to *A. conyzoides* the interaction between sex and butterfly species was significant ($\chi^2 = 11.02$, df = 2, $P = 0.004$), but there was no sex effect ($\chi^2 = 0.13$, df = 1, $P = 0.723$). The interaction between butterfly species and plant species was significant ($\chi^2 = 23.69$, df = 2, $P < 0.0001$).

3.2. Experience on T. procumbens inflorescences

The percent of butterflies that showed attraction to *T. procumbens* and distended the proboscis on its inflorescences varied according to species (Table 3).

3.2.1. Agraulis vanillae

There was a significant effect of experience on feeding time on *T. procumbens* inflorescences ($\chi^2 = 5.78$, df = 1, $P = 0.016$). The median feeding time was 14 s (mean ± SD = 22.0 ± 19.3) in the first trial and 10 s (mean ± SD = 13.82 ± 10.32) in the fourth trial. There was no sex effect on feeding time (Table S4, S5- Supplementary material).

3.2.2. Danaus erippus

There was no experience effect on feeding time (out from the most parsimonious model Table S6, S7- Supplementary material). The median feeding time was 13.5 s (mean ± SD = 20.30 ± 21.86) in the first trial and 7 s (mean ± SD = 9.73 ± 7.57) in the fourth trial. There was no sex effect on feeding time ($\chi^2 = 0.05$, df = 1, $P = 0.810$).

3.2.3. Danaus gilippus

There was no experience effect on feeding time (out from the most parsimonious Table S8, S9- Supplementary material). The median feeding time was 5.5 s (mean ± SD = 11.38 ± 12.66) in the first trial and 5 s (mean ± SD = 13.91 ± 20.48) in the fourth trial. There was no sex effect on feeding time ($\chi^2 = 0.94$, df = 1, $P = 0.330$).

3.3. Experience on A. conyzoides inflorescences

The percent of butterflies that showed attraction to *A. conyzoides* and distended the proboscis on its inflorescences varied according to species (Table 4).

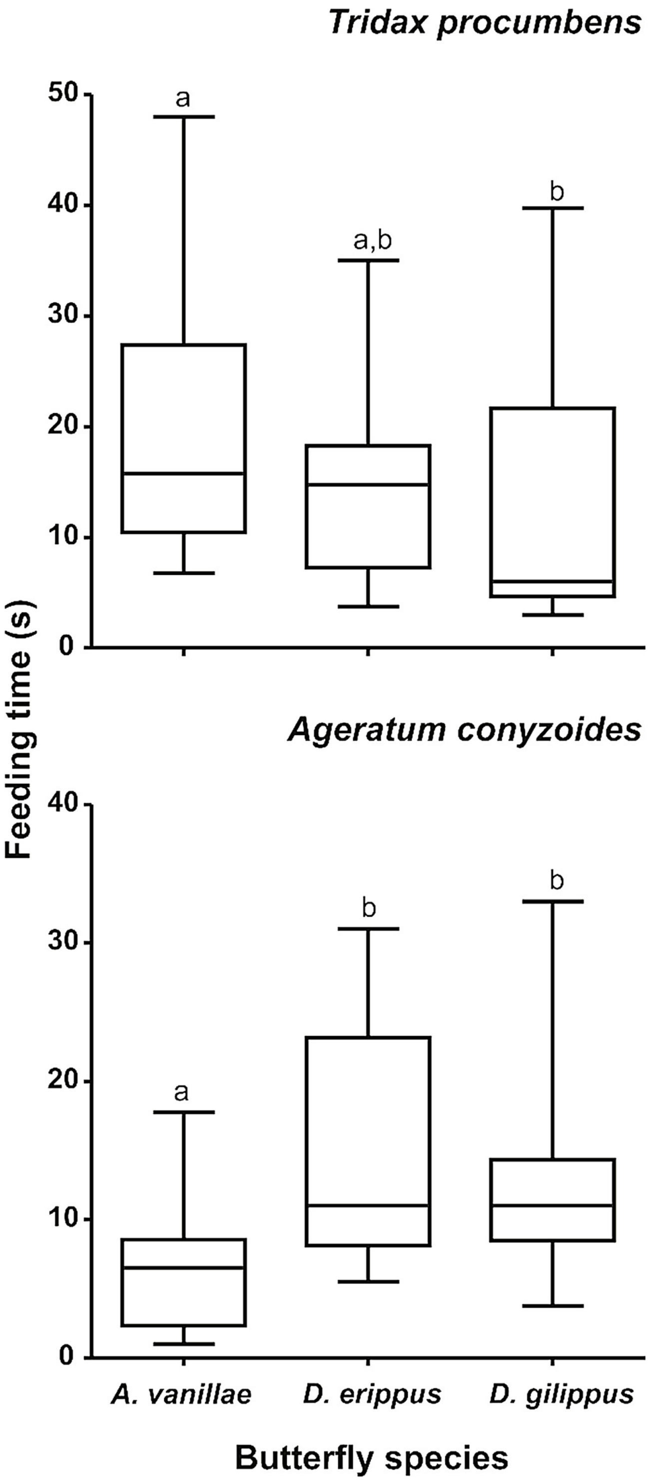

Fig. 2. Feeding time (s; median ± interquartile range) of *Agraulis vanillae*, *Danaus erippus* and *D. gilippus* on *Tridax procumbens* (non-PA source) and *Ageratum conyzoides* (PA source). In each plant species, different letters above the bars indicate significant differences in feeding time ($P < 0.05$).

Table 1
Multiple comparisons (Tukey contrasts) for feeding time (s; ln) among *Agraulis vanillae*, *Danaus erippus* and *D. gilippus* on *Tridax procumbens* inflorescences [non-PA source] (based on the best model predicting "feeding time"; general linear mixed-effects model). Significant differences in bold ($P < 0.05$).

Butterfly species	Estimate (± SE)	Z	P
A. vanillae			
D. erippus	−0.41 (0.21)	−1.94	0.12
D. gilippus	**−0.57 (0.24)**	**−2.36**	**0.047**
D. erippus			
D. gilippus	−0.15 (0.24)	−0.64	0.79

Table 2
Multiple comparisons (Tukey contrasts) for feeding time (s; ln) among *Agraulis vanillae*, *Danaus erippus* and *D. gilippus* on *Ageratum conyzoides* inflorescences [PA source] (based on the best model predicting "feeding time"; general linear mixed-effects model). Significant differences in bold ($P < 0.05$).

Butterfly species	Estimate (± SE)	Z	P
A. vanillae			
D. erippus	**0.93 (0.21)**	**4.31**	**< 0.001**
D. gilippus	**0.71 (0.22)**	**3.14**	**0.004**
D. erippus			
D. gilippus	−0.22 (0.21)	−1.03	0.55

Table 3
Total number of individuals that interacted with *Tridax procumbens* inflorescences (non-PA source) in different contexts during the insectary experiment. Percentage of individuals are in parentheses.

Butterfly species	Attraction	Proboscis extension
Agraulis vanillae	12 (60)	18 (90)
Danaus erippus	5 (25)	19 (95)
Danaus gilippus	1 (5)	14 (70)

Table 4
Total number of individuals that interacted with *Ageratum conyzoides* inflorescences (PA source) in different contexts during the insectary experiment. Percentage of individuals are in parentheses.

Butterfly species	Attraction	Proboscis extension
Agraulis vanillae	1 (5)	17 (85)
Danaus erippus	14 (70)	20 (100)
Danaus gilippus	5 (25)	19 (95)

3.3.1. *Agraulis vanillae*

There was no experience effect on feeding time (out from the most parsimonious model Table S10, S11- Supplementary material). The median feeding time was 5.5 s (mean ± SD = 10.71 ± 15.67) in the first trial and 2 s (mean ± SD = 4.25 ± 5.36) in the fourth trial. There was not a significant sex effect on feeding time ($\chi^2 = 3.67$, df = 1, $P = 0.055$; female = intercept: 1.60, SE: 0.23, *t*: 6.84; male = intercept: 0.85, SE: 0.39, *t*: -1.92).

3.3.2. *Danaus erippus*

There was no experience effect on feeding time (out from the most parsimonious model Table S12, S13- Supplementary material). The median feeding time was 11 s (mean ± SD = 19.50 ± 19.19) in the first trial and 10 s (mean ± SD = 13.53 ± 13.98) in the fourth trial. There was no sex effect on feeding time ($\chi^2 = 0.54$, df = 1, $P = 0.450$).

3.3.3. *Danaus gilippus*

There was no experience effect on feeding time (out from the most parsimonious model Table S14, S15- Supplementary material). The median feeding time was 3 s (mean ± SD = 4.14 ± 3.67) in the first trial and 8 s (mean ± SD = 10.54 ± 9.01) in the fourth trial. There was a sex effect on feeding time ($\chi^2 = 5.35$, df = 1, $P = 0.021$; female = intercept: 1.73, SE: 0.20, *t*: 8.59; male = intercept: 2.41, SE: 0.29, *t*: 2.31).

3.4. Flower visitation in the field

We recorded seven PA species (n = 211 individuals) and 17 plant species deprived of PAs (n = 380 individuals) (Table S16-Supplementary material). Individuals of *D. erippus* were observed visiting *A. curassavica* (n = 18 feeding events), *Urena lobata* L. (Malvaceae) (n = 2 feeding events), *Chromolaena maximilianii* (Schrad.) R. M. King & Rob. (Eupatorieae; PA-flower) (n = 1 landing event) and flying (n = 24 events). As for *D. gilippus*, we observed individuals interacting with the flowers of *A. curassavica* (n = 9 feeding events), *C. laevigata* (Lam.) R.M. King & H. Rob. (Eupatorieae; PA-flower) (n = 1 feeding event; male), *Austroeupatorium inulaefolium* (Kunth) R.M.King & H.Rob. (Eupatorieae; PA-flower) (n = 1 female; inspection flight), *C. maximilianii* (n = 1 feeding event; female) and flying (n = 17 events). Some individuals of *Danaus* were not possible to identify at the species level (7 feeding events and 33 flying events). No individuals of *A. vanillae* were observed feeding; one individual of this species were seen flying.

4. Discussion

Butterflies are selective foragers (Lewis and Lipani, 1990), and diet requirements can incur distinct decisions depending on the nectar source (Weiss, 2001; Krenn, 2010). Our results have shown that, when considering the ends of the continuum, non-specialist and specialist PA species differ with respect to their decisions when finding PA-flowers. In contrast, it is similar among the specialists (*Danaus*). Interestingly, experience on PA-flowers did not affect decision-making in both specialist and non-specialist butterflies.

Tridax procumbens is a nectar source widely used by butterflies, bees and thrips (Corlett, 2004; Chandra et al., 2013; Varalakshmi and Raju, 2013). We have predicted that both *Agraulis* and *Danaus* would interact with the inflorescences of *T. procumbens* similarly. However, *D. gilippus* showed a reduced feeding time on *T. procumbens* than *A. vanillae*. In addition, as *A. vanillae* individuals became experienced regarding the inflorescences of *T. procumbens*, feeding time has decreased on this nectar source. The reason for this reduction in feeding time is unclear, as there are no records of deterrent or toxic compounds in the nectar of *T. procumbens*. In contrast, experience on *T. procumbens* did not change feeding time in both *Danaus* species. We suggest that these results reflect nutritive rather than reproductive and / or defensive demands of these butterfly species, a matter that needs further investigation.

We have demonstrated that *A. conyzoides* inflorescences are less attractive to the non-specialist *A. vanillae* compared to both *Danaus* species. Thus, we suggest that PAs in the nectar of *A. conyzoides* is deterrent to *A. vanillae*. Indeed, nectar chemistry composition can affect time spent in each flower visit, as well as floral constancy (Chittka et al., 1999; Manson et al., 2013; Parachnowitsch and Manson, 2015). The presence of alkaloids in the nectar of *Gelsemium sempervirens* (L.) W.T. Aiton (Gelsemiaceae), for example, decreases the time visiting flowers by hymenopteran pollinators (Adler and Irwin, 2005). However, we did not observe a decrease in feeding activity of *A. vanillae* on *A. conyzoides* as they became experienced in this PA plant. A possible explanation for this finding is that these alkaloids are not toxic for these butterfly species, and thus nectar of *A. conyzoides* can be consumed by *A. vanillae* when other nectar sources are not available. Two studies on feeding of *A. vanillae* on PAs in the artificial nectar have shown contrasting results. Masters (1991) noticed a deterrent effect of monocrotaline, and Landolt and Lenczeski (1993), in a shorter time scale, did not find a deterrent effect of this alkaloid on *A. vanillae* feeding. As a consequence, we suggest that visiting PA flowers by *A. vanillae* can be due to the concentration range of PAs in nature, which is not enough to promote aversive learning (Bernays, 1993, 2001). For example, when sucrose concentrations are higher than aversive compounds, *A. mellifera* is not able to detect the former and thus consume nectar sources with these compounds, leading to lethality in some cases (Ayestaran et al., 2010). Presence of other chemical compounds in the nectar of A. *conyzoides* may also affect decision-making and feeding in *A. vanillae*, *D. erippus* and *D. gilippus*, as well as other flower visitors. For example, Amadi et al. (2012) have found low concentrations of glycosides in *A. conyzoides* flowers. In case these specialized metabolites occur in the nectar of this PA plant as well, they might act as deterrent against certain floral visitors (Stevenson et al., 2017).

Sequestered PAs can vary in concentration in Danaine butterflies according to species and sex (Brown, 1984b). Males of Danainae visit more often PA sources than females (Pliske, 1975b, c; Boppré, 1990) and release sex pheromones through the androconial organs (Brower et al., 1965; Myers and Brower, 1969; Pliske, 1975a). As stated, *D. erippus* has less dependency for PAs than *D. gilippus*. However, both Danaine species did not differ in the visitation pattern of *A. conyzoides* inflorescences; sexes did not differ as well. De Oliveira et al. (2015) have shown that *D. erippus* did not develop learned preferences for PA flowers. In the monarch butterfly, which is the sister species of *D. erippus*, there are records of field populations having no PAs in their body tissues (Edgar et al., 1976). Indeed, these alkaloids do not have a pivotal role in monarchs' courtship success (Pliske, 1975a). With respect to *D. gilippus,* reduced PA consumption negatively impacts courtship (Pliske and Eisner, 1969). Indeed, contrary to *D. erippus*, our data have shown that males of *D. gilippus* invested more time on the flowers of *A. conyzoides* compared to females. It is also important to notice that *D. gilippus* individuals exposed to *A. conyzoides* were more active than those assigned to *T. procumbens*. Taken together, our results and the previous studies above reveal the dependence of PAs by *D. gilippus*. Contrary to our expectations, *D. gilippus* did not increase feeding time on PA flowers according to experience on this nectar source. In the monarch butterfly, experience on artificial flowers having different cardenolide levels did not affect feeding (Jones and Agrawal, 2016). We suggest that the contrasting PA requirements seen in *D. erippus* and *D. gilippus* (i.e. PA concentration in the body tissues and dependency of this compound for reproduction) may be mediated by assimilation capabilities, and not behavioral processes.

Our field data indicated that *D. erippus and D. gilippus* rarely visit PA flowers, and *A. curassavica* is the main nectar source. This finding strongly differs from Malcolm and Slager (2015), which have observed thousands of *D. erippus* visiting *C. arnottiana* (Griseb.) R.M. King & H. Rob. (Asteraceae: Eupatorieae) flowers in Argentina. These authors have also recorded use of flowers belonging to six species of *Asclepias* by *D. erippus* that migrate across Argentina and Bolivia. In Venezuela, Pliske (1975c) observed that mostly Ithomiini and Arctiini visited PA sources as *H. indicum* and *C. xestolepis* (B. L. Rob.) R. M. King & H. Rob. (Asteraceae). As for *Danaus*, *D. gilippus xanthippus* (n = 10), *D. plexippus* (n = 2) and *D. eresimus* (n = 8) were observed on the inflorescences of *H. indicum*. A possible explanation for the low visitation of PAs flowers in the field by both *Danaus* species observed in our study is the high availability of *A. curassavica*. The nectar of *A. curassavica* has low cardenolide concentration (Manson et al., 2012) and 44% sucrose concentration (Percival, 1974). We suggest that *D. erippus* and *D. gilippus* individuals from the Itatiaia population are a case of local adaptation, so that consuming nectar with cardenolides in this site could influence PA dependency in these butterfly species. Cardenolides are sequestered in the larval stage and are retained in the subsequent stages for protection against natural enemies (Brower et al., 1967, 1972; 1982). To date, the role of PAs in butterfly defense has been experimentally demonstrated for *D. affinis* (Ackery and Vane-Wright, 1984; Orr et al., 1996) and, according to Lopez (2016), a microevolutionary trade-off involving allocation of chemical defenses occurs in the genus *Danaus*. In other words, species that show higher allocation in cardenolides as defenses invest less in PA allocation, and vice-versa (Lopez, 2016).

5. Conclusions

To our knowledge, this is the first study that examines decision-making in floral visitors that present a specialization continuum, as well as its possible effects according to experience. Our findings reveal that the specialization continuum seen in butterflies can explain, at least in part, decision-making processes in nectarivorous butterflies. In addition, other factors as local adaptation mediated by the use of local nectar sources can influence flower selection of specialist butterflies in natural environments. The literature states that flowers having PAs in the nectar are avoided by non-specialists and actively visited by specialists (Pliske, 1975b; Masters, 1991; Trigo, 2011). Indeed, the non-specialist *A. vanillae* is less attracted to PA flowers than both specialist *Danaus* species. In turn, *D. erippus* and *D. gilippus* did not differ in attractiveness and use of PA sources. The fitness consequences of nectar consumption of PA and non-PA plants by both non-specialist and non-specialist butterflies, as well as the physiological constraints associated to detoxification and assimilation of specialized compounds, constitute further venues of research.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.beproc.2019.06.006.

References

Ackery, P.R., Vane-Wright, R.I., 1984. Milkweed Butterflies - Their Cladistics and Biology, first ed. Cornell University Press, New York.

Adler, L.S., 2000. The ecological significance of toxic nectar. Oikos 91, 409–420.

Adler, L.S., Irwin, R.E., 2005. Ecological costs and benefits of defenses in nectar. Ecology 86, 2968–2978.

Ali, J.G., Agrawal, A.A., 2012. Specialist versus generalist insect herbivores and plant defense. Trends Plant Sci. 17, 293–302.

Amadi, B.A., Duru, M.K.C., Agomuo, E.N., 2012. Chemical profile of leaf, stem, root and flower of Ageratum conyzoides. Asian J. Plant Sci. Res. 2, 428–432.

Arbogast, R.T., 1965. Biology and Migratory Behavior of *Agraulis vanillae* (L) (Lepidoptera, Nymphalidae). University of Florida unpublished Dissertation.

Ayestaran, A., Giurfa, M., Sanchez, M.G.B., 2010. Toxic but drank: gustatory aversive compounds induce post-ingestional malaise in harnessed honeybees. PLoS One 5, e15000.

Barrett, L.G., Heil, M., 2012. Unifying concepts and mechanisms in the specificity of plant-enemy interactions. Trends Plant Sci. 17, 282–292.

Baker, H.G., 1977. Non-sugar chemical constituents of nectar. Apidologie 8, 349–356.

Baker, H.G., Baker, I., 1975. Studies of nectar-constitution and pollinator-plant coevolution. In: Gilbert, L.E., Raven, P.H. (Eds.), Coevolution of Animals and Plants. University of Texas Press, Austin, pp. 100–140.

Becalloni, G.W., Viloria, A.L., Hall, S.K., Robinson, G.S., 2008. Catalogue of the Hostplants of the Neotropical Butterflies, first ed. Monografías Tercer Milenio, Zaragoza.

Benson, W.W., Brown, K.S., Gilbert, L.E., 1975. Coevolution of plants and herbivores: passion flower butterflies. Evolution 29, 659–680.

Bernays, E.A., 1993. Aversion learning and feeding. In: Papaj, D.R., Lewis, A.C. (Eds.), Insect Learning: Ecology and Evolutionary Perspectives. Chapman & Hall, London, pp. 1–17.

Bernays, E.A., 2001. Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. Ann. Rev. Entomol. 46, 703–727.

Bernays, E.A., Chapman, R., 1987. The evolution of deterrent responses in plant-feeding insects. In: Chapman, R.F., Bernays, E.A., Stoffolano JrJ.G. (Eds.), Perspectives in Chemoreception and Behavior. Springer Verlag, New York, pp. 159–173.

Bernays, E.A., Wcislo, W.T., 1994. Sensory capabilities, information processing, and resource specialization. Q. Rev. Biol. 69, 187–204.

Bernays, E.A., Edgar, J.A., Rothschild, M., 1977. Pyrrolizidine alkaloids sequestered and stored by the aposematic grashopper, *Zonocerus variegatus*. J. Zool. 182, 85–87.

Blackiston, D., Briscoe, A.D., Weiss, M.R., 2011. Color vision and learning in the monarch butterfly, *Danaus plexippus* (Nymphalidae). Exp. Biol. 214, 509–520.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., Whitte, J.S.S., 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24, 127–135.

Boppré, M., 1978. Chemical communication, plant relationships, and mimicry in the evolution of danaid butterflies. Entomol. Exp. Appl. 24, 64–77.

Boppré, M., 1983. Leaf-scratching: a specialized behaviour of danaine butterflies (Lepidoptera) for gathering secondary plant substances. Oecologia 59, 414–416.

Boppré, M., 1986. Insects pharmacophagously utilizing defensive plant chemicals (pyrrolizidine alkaloids). Naturwissenschaften 73, 17–26.

Boppré, M., 1990. Lepidoptera and pyrrolizidine alkaloids – exemplification of complexity in chemical ecology. J. Chem. Ecol. 16, 165–185.

Boppré, M., 2011. The ecological context of pyrrolizidine alkaloids in food, feed and forage: an overview. Food Add. Contam. A 28, 260–281.

Bosi, C.F., 2012. Presença de alcalóides pyrrolizidine em Ageratum conyzoides L., Asteraceae. Universidade Federal de Santa Catarina Unpublished thesis.

Bosi, C.F., Rosa, D.W., Grougnet, R., Lemonakis, N., Halabalaki, M., Skaltsounis, A.L., Biavatti, M.W., 2012. Pyrrolizidine alkaloids in medicinal tea of *Ageratum conyzoides*. Rev. Bras. Farmacogn. 23, 425–432.

Brower, L.P., Brower, J.V.Z., Cranston, F.P., 1965. Courtship behavior of the queen butterfly, *Danaus gilippus* Berenice (cramer). Zoologica-N. Y. 50, 1–24.

Brower, L.P., Bower, J.V.Z., Corvino, J.M., 1967. Plant poisons in a terrestrial food chain. P. Natl. Acad. Sci. U. S. A. 57, 893–898.

Brower, L.P., McEvoy, P.B., Williamson, K.L., Flannery, M.A., 1972. Variation in cardiac glycoside content of monarch butterflies from natural populations in eastern North America. Science 177, 426–429.

Brower, L.P., Seiber, J.N., Nelson, C.J., Lynch, S.P., Tuskes, P.M., 1982. Plant-determined variation in the cardenolide content, thin-layer chromatography profiles, and emetic potency of monarch butterflies, *Danaus plexippus*, reared on the milkweed, *Asclepias eriocarpa* in California. J. Chem. Ecol. 8, 579–633.

Brown, K.S.J., 1984a. Adult-obtained pyrrolizidine alkaloids defend ithomiine butterflies against a spider predator. Nature 21, 707–709.

Brown, K.S.J., 1984b. Chemical ecology of dehydropyrrolizidine alkaloids in adult Ithomiinae (Lepidoptera: nymphalidae). Rev. Bras. Biol. 44, 435–460.

Brown, K.S.J., 1992. Borboletas da Serra do Japi: diversidade, habitats, recursos alimentares e variação temporal. In: Morellato, L.P.C. (Ed.), História natural da Serra do Japi- ecologia e preservação de uma área florestal no sudeste do Brasil. Unicamp, Campinas, pp. 142–186.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference: a Practical Information-theoretic Approach, first ed. Springer, New York.

Cardoso, M.Z., 1997. Testing chemical defence based on pyrrolizidine alkaloids. Anim. Behav. 54, 985–991.

Cepero, L.C., Rosenwald, L.C., Weiss, M.R., 2015. The relative importance of flower color and shape for the foraging monarch butterfly (Lepidoptera: nymphalidae). J. Insect Behav. 28, 499–511.

Chandra, P.H., Ramana, K.V., Krishna, R., Rani, D.S., Rao, S.P., Raju, A.J.S., 2013. A study on interactions of insects with herbaceous plants in Visakhapatnam. In: Prof. Raju, A.J.S. (Ed.), Advances in Pollen Spore Research. Today & Tomorrow's Printers and Publishers, New Delhi, pp. 69–79.

Chittka, L., Thomson, J.D., Waser, N.M., 1999. Flower constancy, insect psychology, and plant evolution. Naturwissenschaften 86, 361–377.

Cogni, R., Trigo, J.R., 2016. Pyrrolizidine alkaloids negatively affect a generalist herbivore feeding on the chemically protected legume Crotalaria pallida. Neotrop. Entomol. 45, 252–257.

Corlett, R.T., 2004. Flower visitors and pollination in the Oriental (Indomalayan) Region. Biol. Rev. 79, 497–532.

de Oliveira, M.V., Trigo, J.R., Rodrigues, D., 2015. Southern monarchs do not develop learned preferences for flowers with pyrrolizidine alkaloids. J. Chem. Ecol. 41, 662–669.

DeVries, P.J., 1987. The Butterflies of Costa rica and Their Natural History: Papilionidae, Pieridae, Nymphalidae, first ed. Princeton University Press, New Jersey.

Dinesh, A.S., Venkatesha, M.G., 2013. A quantified ethogram for oviposition behavior and oviposition preference in the hemipterophagous butterfly*Spalgis epius* (Westwood) (Lepidoptera: Lycaenidae). J. Ethol. 31, 71–77.

Edgar, J.A., Culvenor, C.C.J., Pliske, T.E., 1974. Coevolution of danaid butterflies with their host plants. Nature 250, 646–648.

Edgar, J.A., Culvenor, C.C.J., 1975. Pyrrolizidine alkaloids in *Parsonsia* species (Family Apocynaceae) which attract danaid butterflies. Experientia 31, 393–504.

Edgar, J.A., Cockrum, P.A., Frahn, J.L., 1976. Pyrrolizidine alkaloids in *Danaus plexippus* L. and *Danaus chrysippus* l. Specialia 32, 1535–1537.

Edgar, J.A., Boppré, M., Schneider, D., 1979. Pyrrolizidine alkaloids storage in African and Australian danaid butterflies. Experientia 35, 1447–1448.

Emsley, M., 1963. A morphological study of imagine Heliconiinae (Lepidoptera, Nymphalidae) with consideration of the evolutionary relationships within the group. Zoologica N. Y. 48, 85–130.

Ferreira, P.P.S., 2017. História de vida e comportamento de duas espécies neotropicais de Danainae (Lepidoptera: Nymphalidae) em apocináceas nativas e exóticas. Universidade Federal do Rio de Janeiro Unpublished thesis.

Gegear, R.J., Manson, J.S., Thomson, J.D., 2007. Ecological context influences pollinator deterrence by alkaloids in floral nectar. Ecol. Lett. 10, 375–382.

Harborne, J.B., 1988. Introduction to Ecological Biochemistry, third ed. Academic Press, San Diego.

Hartmann, T., 1999. Chemical ecology of pyrrolizidine alkaloids. Planta 207, 483–495.

Hartmann, T., Ober, D., 2008. Defense by pyrrolizidine alkaloids: developed by plants and recruited by insects. In: Schaller, A. (Ed.), Induced Plant Resistance to Herbivory. Springer, New York, pp. 213–231.

Hartmann, T., Witte, L., 1995. Pyrrolizidine alkaloids: chemical, biological and chemoecological aspects. In: Pelletier, S.W. (Ed.), Alkaloids: Chemical and Biological Perspectives. Pergamon Press, Oxford, pp. 155–233.

Hattori, E.K.O., Nakajima, J.N., 2008. A família Asteraceae na estação de pesquisa e desenvolvimento ambiental galheiro, perdizes, Minas Gerais, Brasil. Rodriguésia 59, 687–749.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 50, 346–363.

Johnson, M.F., 1971. A monograph of the genus *Ageratum* L. (Compositae-Eupatorieae). Ann. Mo. Bot. Gard. 58, 6–88.

Jones, P.L., Agrawal, A.A., 2016. Consequences of toxic secondary compounds in nectar for mutualist bees and antagonist butterflies. Ecology 97, 2570–2579.

Jude, C.I., Catherine, C.I., Ngozi, M.I., 2009. Chemical profile of *Tridax procumbens* Linn. Pak. J. Nutr. 8, 548–550.

Kearns, C.A., Inouye, D.W., 1993. Techniques for Pollination Biologists, first ed. University Press of Colorado, Colorado.

Klitzke, C.F., Trigo, J.R., 2000. New records of pyrrolizidine alkaloid-feeding insects. Hemiptera and Coleoptera on *Senecio brasiliensis*. Biochem. Syst. Ecol. 28, 313–318.

Kohli, R.K., Batish, D.R., Singh, H.P., Dogra, K.S., 2006. Status, invasiveness and environmental threats of three tropical American invasive weeds (*Parthenium hysterophorus* L., *Ageratum conyzoides* L., *Lantana camara* L.) in India. Biol. Invasions 8, 1501–1510.

Krenn, H.W., 2010. Feeding mechanisms of adult Lepidoptera: structure, function, and evolution of the mouthparts. Ann. Rev. Entomol. 55, 307–327.

Kroutov, V., Mayers, M.S., Emmel, T.C., 1999. Olfactory conditioning of the butterfly *Agraulis vanillae* (L.) (Lepidoptera, Nymphalidae) to floral but not host-plant odors. J. Insect Behav. 12, 833–843.

Landolt, P.J., Lenczewski, B., 1993. Lack of evidence for the toxic nectar hypothesis: a plant alkaloid did not deter nectar feeding by Lepidoptera. Fla. Entomol. 76, 556–566.

Lehner, P.N., 1996. Handbook of Ethological Methods, second ed. Cambridge University Press, New York.

Lewis, A.C., Lipani, G.A., 1990. Learning and flower use in butterflies: hypotheses from honey bees. In: Bernays, E.A. (Ed.), Insect-Plant Interactions. CRC Press, Boca Raton, pp. 95–110.

Lopez, L.M., 2016. Demandas conflitantes entre defesas químicas: cardenolidas e alcaloides pirrolizidínicos em *Danaus erippus* e *Danaus gilippus* (Lepidoptera: Danainae). Universidade Estadual de Campinas Unpublished thesis.

Lorenzi, H., 2000. Plantas daninhas do Brasil: terrestres, aquáticas, parasitas e tóxicas, third ed. Instituto Plantarum, Nova Odessa.

Macel, M., 2011. Attract and deter: a dual role for pyrrolizidine alkaloids in plant-insect interactions. Phytochem. Rev. 10, 75–82.

Malcolm, S.B., Slager, B.H., 2015. Migration and host plant use by the southern monarch butterfly, *Danaus erippus*. In: Oberhauser, K.A., Altizer, S., Nail, K. (Eds.), Monarchs in a Changing World: Biology and Conservation of an Iconic Insect. Cornell University Press, Ithaca, pp. 225–235.

Manson, J.S., Cook, D., Gardner, D.R., Irwin, R.E., 2013. Dose-dependent effects of nectar alkaloids in a montane plant-pollinator community. J. Ecol. 101, 1604–1612.

Manson, J.S., Otterstatter, M.C., Thomson, J.D., 2010. Consumption of a nectar alkaloid reduces pathogen load in bumble bees. Oecologia 162, 81–89.

Manson, J.S., Rasmann, S., Halitschke, R., Thomson, J.D., Agrawal, A.A., 2012. Cardenolides in nectar may be more than a consequence of allocation to other plant parts: a phylogenetic study of *Asclepias*. Funct. Ecol. 26, 1100–1110.

Martin, P., Bateson, P., 2007. Measuring Behavior – an Introductory Guide, third ed. Cambridge University Press, New York.

Martins, C.H.Z., Cunha, B.P., Solferini, V.N., Trigo, J.R., 2015. Feeding on host plants with different concentrations and structure of pyrrolizidine alkaloids impacts the chemical defense effectiveness of a specialist herbivore. PLoS One 10, e0141480.

Masters, A.R., 1990. Pyrrolizidine alkaloids in artificial nectar protect adult ithomiine butterflies from a spider predator. Biotropica 22, 298–304.

Masters, A.R., 1991. Dual role of pyrrolizidine alkaloids in nectar. J. Chem. Ecol. 17, 195–205.

May, P.G., 1992. Flower selection and dynamics of lipid reserve in two nectarivorous butterflies. Ecology 73, 2181–2191.

May, P.G., 1985. Foraging Selectivity in Adult Butterflies: Morphological, Ecological, and Physiological Factors Affecting Flower Choice. University of Florida unpublished Dissertation.

Meinwald, J., Meinwald, Y.C., Mazzocchi, P.H., 1969. Sex pheromone of the queen butterfly: chemistry. Science 164, 1174–1175.

Moreira, H.J.C., Bragança, H.B.N., 2011. Manual de identificação de plantas infestantes: hortifrúti, first ed. FMC Agricultural Products, Campinas.

Morris, G.M., Kline, C., 2015. Status of *Danaus plexippus* population in Arizona. J. Lepid. Soc. 69, 91–107.

Myers, J., Brower, L.P., 1969. A behavioural analysis of the courtship pheromone receptors of the queen butterfly, *Danaus gilippus berenice*. J. Insect Physiol. 15, 2117–2130.

Nishida, R., 1995. Sequestration of plant secondary compounds by butterflies and moths. Chemoecology 4, 127–138.

Nishida, R., 2014. Chemical ecology of insect-plant interactions: ecological significance of plant secondary metabolites. Biosci. Biotechnol. Biochem. 78, 1–13.

Opitz, S.E.W., Müller, C., 2009. Plant chemistry and insect sequestration. Chemoecology 19, 117–154.

Orr, A., Trigo, J.R., Witte, L., Hartmann, T., 1996. Sequestration of pyrrolizidine alkaloids by larvae of *Tellervo zoilus* (Lepidoptera: ithomiinae) and their role in the chemical protection of adults against the spider *Nephila maculate* (Araneidae). Chemoecology 7, 68–73.

Oyeyele, S.O., Zalucki, M.P., 1990. Cardiac glycosides and oviposition by *Danaus plexippus* on *Asclepias fruticosa* in south-east Queensland (Australia), with notes on the effect of plant nitrogen content. Ecol. Entomol. 15, 177–185.

Papaj, D.R., Lewis, A.C., 1993. Insect Learning: Ecological and Evolutionary Perspectives, first ed. Chapman & Hall, New York.

Parachnowitsch, A.L., Manson, J.S., 2015. The chemical ecology of plant pollinator interactions: recent advances and future directions. Curr. Opin. Insect Sci. 8, 41–46.

Parachnowitsch, A.L., Manson, J.S., Sletvold, N., 2018. Evolutionary ecology of nectar. Ann. Bot. London mcy 132. https://doi.org/10.1093/aob/mcy132.

Pasteels, J.M., Hartmann, T., 2004. Sequestration of pyrrolizidine alkaloids in *Oreina* and *Platyphora* leaf-beetles: physiological, ecological and evolutionary aspects. In: Jolivet, P., Santiago-Blay, J.A., Schmitt, M. (Eds.), New Developments in the Biology of Chrysomelidae. SPB, The Hague, pp. 677–691.

Percival, M., 1974. Floral ecology of coastal scrub in Southeast Jamaica. Biotropica 6, 104–129.

Périco, E., 1995. Interação entre quatro espécies de Heliconiini (Lepidoptera: Nymphalidae) e suas plantas hospedeiras (Passifloraceae) em uma floresta secundária do Rio Grande do Sul, Brasil. Biociências 3, 3–18.

Pliske, T.E., 1975a. Courtship behavior of the monarch butterfly, *Danaus plexippus* L. Ann. Entomol. Soc. Am. 68, 143–151.

Pliske, T.E., 1975b. Attraction of Lepidoptera to plants containing pyrrolizidine alkaloids. Environ. Entomol. 4, 455–473.

Pliske, T.E., 1975c. Pollination of pyrrolizidine alkaloid-containing plants by male Lepidoptera. Environ. Entomol. 4, 474–479.

Pliske, T.E., Eisner, T.E., 1969. Sex pheromone of the queen butterfly: biology. Science 164, 1170–1172.

Powell, A.M., 1965. Taxonomy of *Tridax* (Compositae). Brittonia 17, 47–96.

Pyke, G.H., 2016. Floral nectar: pollinator attraction or manipulation? Trends Ecol. Evol. 31, 339–341.

Ramos, B.C.M., Rodríguez-Gironés, M.A., Rodrigues, D., 2017. Learning in two butterfly species when using flowers of the tropical milkweed *Asclepias curassavica*: no benefits for pollination. Am. J. Bot. 104, 1168–1178.

Rasmann, S., Agrawal, A.A., 2011. Latitudinal patterns in plant defense: evolution of cardenolides, their toxicity and induction following herbivory. Ecol. Lett. 14, 476–483.

Reinhard, A., Janke, M., von der Ohe, W., Kempf, M., Theuring, C., Hartmann, T., Schreier, P., Beuerle, T., 2009. Feeding deterrence and detrimental effects of pyrrolizidine alkaloids fed to honey bees (*Apis mellifera*). J. Chem. Ecol. 35, 1086–1095.

Rhoades, D.F., Bergdahl, J.C., 1981. Adaptive significance of toxic nectar. Am. Nat. 117, 798–803.

Rodrigues, D., 2016. Both associative learning and speed accuracy trade-off occur in the southern monarch butterfly when visiting flowers. Ethol. Ecol. Evol. 28, 30–41.

Rodrigues, D., Goodner, B.W., Weiss, M.R., 2010. Reversal learning and risk-averse behavior in the monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae). Ethology 116, 270–280.

RStudio Team, 2015. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA Website http://www.rstudio.com/.

Singer, M.S., Carrière, Y., Theuring, C., Hartmann, T., 2004. Disentangling food quality from resistance against parasitoids: diet choice by a generalist caterpillar. Am. Nat. 164, 423–429.

Slager, B.H., Malcolm, S.B., 2015. Evidence for partial migration in the southern monarch butterfly, *Danaus erippus*, in Bolivia and Argentina. Biotropica 47, 355–362.

Smith, D.A.S., Lushai, G., Allen, J.A., 2005. A classification of *Danaus* butterflies (Lepidoptera: Nymphalidae) based upon data from morphology and DNA. Zool. J. Linn. Soc. Lond. 144, 191–212.

Stevenson, P.C., Nicolson, S.W., Wright, G.A., 2017. Plant secondary metabolites in nectar: impacts on pollinators and ecological functions. Funct. Ecol. 31, 65–75.

Trigo, J.R., 1987. Ecologia química na interação Ithominae (Lepidoptera: Nymphalidae) / Echitoideae (Angiospermae: Apocynaceae). Universidade Estadual de Campinas Unpublished thesis.

Trigo, J.R., 2000. The chemistry of antipredator defense by secondary compounds in Neotropical Lepidoptera: facts, perspectives and caveats. J. Braz. Chem. Soc. 6, 551–561.

Trigo, J.R., 2011. Effects of pyrrolizidine alkaloids through different trophic levels. Phytochem. Rev. 10, 83–98.

Trigo, J.R., Brown, K.S., Witte, L., Hartmann, T., Ernst, L., Barata, L.E.S., 1996. Pyrrolizidine alkaloids: different acquisition and use patterns in Apocynaceae and Solanaceae feeding ithomiine butterflies (Lepidoptera: Nymphalidae). Biol. J. Linn. Soc. 58, 99–123.

Varalakshmi, P., Raju, J.S., 2013. Psychophilous and melittophilous pollination syndrome in *Tridax procumbens* L. (Asteraceae). Taprobanica 5, 124–130.

Weiss, M.R., 1995. Associative colour learning in nymphalid butterfly. Ecol. Entomol. 20, 298–301.

Weiss, M.R., 2001. Vision and learning in some neglected pollinators: beetles, flies, moths, and, butterflies. In: Chittka, L., Thomson, J.D. (Eds.), Cognitive Ecology of Evolution: Animal Behavior and Floral Evolution. Cambridge University Press, Cambridge, pp. 171–190.

Wiedenfeld, H., Röder, E.T., 1991. Pyrrolizidine alkaloids from *Ageratum conyzoides*. Planta Med. 57, 578–579.

Witte, L., Ehmke, A., Hartmann, T., 1990. Interespecific flow of pyrrolizidine alkaloids from plants via aphids to ladybirds. Naturwissenchaften 77, 540–543.

Zalucki, M.P., Brower, L.P., 1992. Survival of first instar larvae of *Danaus plexippus* (Lepidoptera: danainae) in relation to cardiac glycoside and latex content of *Asclepias humistrata* (Asclepiadaceae). Chemoecology 3, 81–93.

Zalucki, M.P., Malcolm, S.B., 1999. Plant latex and first-instar monarch larval growth and survival on three North American milkweed species. J. Chem. Ecol. 25, 1827–1842.

Zalucki, M.P., Brower, L.P., Alonso, M.A., 2001. Detrimental effects of latex and cardiac glycosides on survival and growth of first-instar monarch butterfly larvae *Danaus plexippus* feeding on the sandhill milkweed *Asclepias humistrata*. Ecol. Entomol. 26, 212–224.

Zalucki, M.P., Brower, L.P., Malcolm, B., 1990. Oviposition by *Danaus plexippus* in relation to cardenolide content of three *Asclepias* species in the southeastern U.S.A. Ecol. Entomol. 15, 231–240.

Zar, J.H., 1999. Biostatistical Analysis, fourth ed. Prentice Hall, Upper Saddle River.