Observations on the behavior and diet of the Harlequin *Taxila haquinus haquinus* Fabricius 1793 (Lepidoptera: Riodinidae) in Singapore

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Abstract: *Taxila haquinus haquinus* (the Harlequin) is a moderately rare species in the butterfly family Riodinidae which occurs in Singapore. There is a general lack of information on the autecology of Riodinidae globally, including this species. A series of observations and experiments was therefore conducted to investigate the behavior and diet of larvae and adults of the Harlequin in its natural habitat and in captivity, to provide insights into its autecology and to aid in the design of recovery programmes targeted at this species. We found that resting was the main activity performed by adults of both sexes, with this behavior perhaps partly functioning in thermoregulation. Males spent a larger proportion of their time feeding and in flight, while females 'hopped' more than males, in which the butterfly performed a quick, short flight with a maximum duration of 3 seconds, and then alighted on the surface of a plant. Behavioral differences observed between sexes may reflect the priorities for males to search for mates and females to search for suitable oviposition sites. This study is the first to document a female Harlequin ovipositing on a non-host plant species and an interaction between a male and a female in their natural habitat, possibly an aspect of courtship behavior. Adult Harlequin were not recorded feeding on flowers but they were observed extending their proboscis to touch the leaf surface of multiple plant species, although it is not clear whether this behavior represents feeding. Caterpillars, regardless of their instars and the age of the leaf, appeared to be less receptive to feeding on non-host plant species belonging to the same plant family as the known host plant. During a leaf diet test, many individuals died between one and seven days following a switch to non-host plant species. Caterpillars that fed and pupated on non-host plant species also failed to eclose successfully as adults.

Key words: Behavior, diet, Lepidoptera, Riodinidae, Singapore, *Taxila haquinus haquinus*

INTRODUCTION

The Harlequin *Taxila haquinus haquinus* Fabricius 1793 is a moderately rare butterfly in Singapore (Khew, 2015). It is a member of the family Riodinidae, which are commonly known as Metalmarks for the small, metallic-looking spots on their wings (Fig. 1). Although distributed globally, riodinids are most often associated with the Neotropics, where over 90% of species are found (Hall & Willmott, 2000). In Singapore, seven species from this family have been recorded, of which five are currently extant (Jain *et al.*, 2018). The Harlequin is widely distributed across India and southeast Asia, with many subspecies, and our local subspecies *Taxila haquinus haquinus* is also found in south Thailand and Peninsular Malaysia (Kirton, 2014).

The Harlequin is sexually dimorphic, with the sexes showing differences on both upperside and underside (Fig. 1). Males have a dark brown upperside, with a reddish-brown sub-apical portion of the forewings. Females have small black metallic spots throughout their reddish-brown upperside and a prominent white band not crossed by dark veins in the sub-apical portion of the forewings. In both sexes, the reddish-brown underside is marked by multiple black and silvery-white spots and white-edged black spots. However, the white sub-apical patch on the forewings of females is broader and more prominent than in males. Like all Riodinidae, the forelegs of male are highly reduced and appear more like hair tufts, leaving them with only four walking legs. In contrast, females possess six fully developed legs for walking (Khew, 2015).

In Singapore, The Harlequin inhabits lowland forests where its host plant, the native tropical shrub *Ardisia elliptica* Thunb. (Primulaceae) is present (Flora & Fauna Web, 2019a; Yong, 1983; Khew, 2015). The Harlequin is generally found in shaded understorey where it tends to rest for extended periods of time on the leaf surfaces of shrubs with partially opened wings (Khew, 2015). Occasionally, the butterfly may fly up into the canopy. It is not known to exhibit any migratory behavior and rarely ventures beyond the vicinity of its host plant (Khew, 2007).

To date, in Singapore, the Harlequin has been recorded in the Central Catchment Nature Reserve, Western Catchment and adjacent wooded areas, as well as the offshore island of Pulau Tekong (Khew, 2011). Although *A. elliptica* has been classified as Nationally Endangered in Singapore, the plant has been widely cultivated in parks and nature areas in recent years (Flora & Fauna Web, 2019a; Tan, 2016). Despite this, the Harlequin has a highly localized distribution and the factors

Fig. 1. Both male (a) and female (b) have white-edged black metallic spots throughout their reddish-brown underside. Like the underside, male (c) and female (d) are sexually dimorphic on the upperside.

affecting the dispersal and distribution of the species remain unknown. Given the restricted distribution of the butterfly, it is vulnerable to habitat loss and degradation and is presently classified as Nationally Endangered (Davison *et al.*, 2008).

The Harlequin is one of the species on the Singapore National Parks Board's Species Recovery Programme. Under this programme, extensive efforts are underway to translocate this butterfly to suitable habitats, as well as to establish a viable captive population (National Parks Board, 2017). However, recovery efforts have been hindered by the general lack of information on the autecology of Riodinidae. As such, a series of *in situ* and *ex situ* studies were carried out over a period of five months to investigate the biology, ecology and behavior of this poorly studied species. In particular, we were interested in the following: 1) Behavior of the Harlequin in its natural environment, 2) Potential alternative species of host plants that caterpillars may feed on, and 3) Potential food for captive-raised adults.

MATERIALS AND METHODS

In situ *behavioral study*

Study site

The study was conducted between September 2018 and February 2019 at a young secondary forest fragment (approximately 9,500 m^2) in the west of mainland Singapore (Fig. 2). Most observations were carried out within a 3,160 m^2 plot (approximately 35% of the study site) within the forest as the species was commonly found here. The forest floor was dominated by the native herbaceous creeper *Piper sarmentosum* Roxb. (Piperaceae) while the understorey comprised a mixture of native and exotic species. Native species included *Dillenia suffruticosa* (Griff.) Martelli (Dilleniaceae), *Caryota mitis* Lour. (Arecaceae), *Leea indica* (Burm.f.) Merr. (Vitaceae), *Cinnamomum iners* Reinw. ex Blume (Lauraceae), *Stenochlaena*

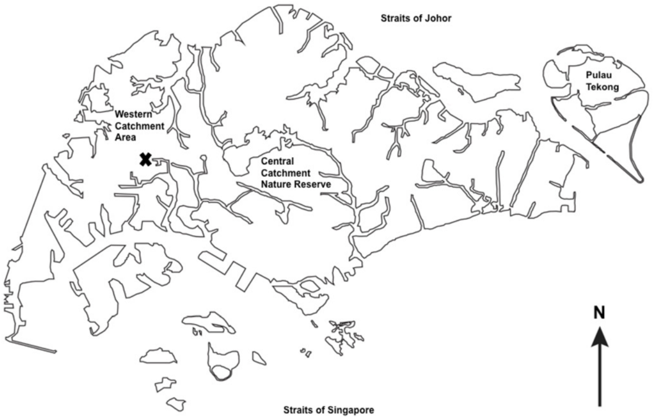

Fig. 2. Map of Singapore, with location of study site indicated by 'X'.

palustris (Burm. f.) Bedd. (Blechnaceae), *Syzygium polyanthum* (Wight) Walp. (Myrtaceae), *Syzygium grande* (Wight) Walp. (Myrtaceae), *Nephelium lappaceum* L. (Sapindaceae), *Cayratia trifolia* (L.) Domin (Vitaceae) and *Ardisia elliptica*, the host plant of the Harlequin. Non-native species consisted of *Andira inermis* (Wright) DC. (Leguminosae), *Ptychosperma macarthurii* (H.Wendl. ex H.J. Veitch) (Arecaceae) and *Elaeis guineensis* Jacq. (Arecaceae).

Observation methodology

Each observation session consisted of at least two observers and there was usually a one-week interval between the sessions. Observers would follow a fixed transect route and upon encountering a butterfly, observations would commence. The study utilized focal-animal sampling, where an observer monitored the behavior of a single butterfly for as long as possible until the observer lost sight of it (Altmann, 1974). Most observations were conducted between 10:00 and 13:00 when the butterflies were most active (Khew, 2015). Occasionally, two observation sessions were carried out in a single day. We assumed that any butterflies sighted near where individuals had been observed during earlier sessions were the same individuals. We did not record the location of each butterfly using GPS because of the small size of the study site and because butterflies were occasionally too close to one another to be assigned different coordinates.

Observations were made from a distance as close to the butterflies as possible without noticeably affecting their behavior. The weather condition, sex and an easily visible aspect of wing condition (intact or damaged) of the observation subject were recorded. Wing condition was assumed to be indicative of the age of a butterfly, with older butterflies generally assumed to have more tattered wings (Takeuchi, 2006). Behavioral observations were recorded using the following predetermined categories: resting, moving, hopping (a quick, short flight with a maximum duration of 3 seconds, followed by the butterfly alighting on the surface of a plant), flight, interacting with plant, copulation and oviposition (Table 1). Initially, we defined the extension of the proboscis by a butterfly to touch the surface of a plant as feeding. However, as butterflies may extend their proboscis to inspect for suitable substances on which to feed, we re-categorized this behavior as interacting with plants. Any plant species with which a butterfly was observed interacting was identified and recorded. Due to the difficulty in distinguishing *S. grande* from *S. polyanthum* in the field, these species were recorded as *Syzygium* sp. In the event where multiple activities overlapped, only the main activity was recorded. For example, when a butterfly interacted with a plant and moved concurrently, only the interaction with the plant was recorded. In addition, any interesting inter- and intra-specific interactions (eg. courtship behavior) were also recorded.

The duration of each behavior was calculated by recording the time when the behavior started (Time start) and the time when the next behavior began (Time end). For each butterfly, the proportion of time spent on a single activity, *P*(activity) was then calculated. The average proportion of time spent on a single activity and the standard error (SE) were then calculated for each sex for all wing conditions combined. The average proportion of time spent on a single activity was also analyzed according to wing condition for each sex. To determine whether the butterflies displayed preference for any plant species during their interaction with plants, the average proportion of interaction time was calculated for each plant species for each sex.

Ex situ *diet studies*

The *ex situ* studies consisted of two components: 1) Investigating the feeding behavior of Harlequin caterpillars across different instars and, 2) investigating the feeding behavior of adult butterflies. These experiments were conducted with caterpillars and adults obtained from captive stock and placed in standard round (approximately 13 cm × 7 cm, D × H) or rectangular (approximately 15 cm × 10 cm × 6 cm, L × B × H) plastic containers. Similar types of container have been used to successfully rear Harlequin caterpillars from egg to adult and to house the freshly-emerged adults.

Leaf test for caterpillars

To determine the dietary breadth of Harlequin larvae, caterpillars ranging from 1st to 5th instars were given leaves of several plant species in Primulaceae, the family of the natural host plant, *A. elliptica*. These species included *Ardisia crenata* Sims, *A. sanguinolenta* Blume, *Ardisia purpurea* Reinw. ex Blume and *Embelia ribes* Burm.f., with *A. elliptica* serving as the control. *Embelia ribes* is known to be the host plant for the caterpillars of other riodinid species in Singapore, such as *Abisara* species (Khew, 2015), which is a member of the same subtribe as the Harlequin (Seraphim *et al*., 2018). These plant species are all native to Singapore (Chong *et al*., 2009). For each species of plant, two caterpillars of similar instar were placed in a plastic container that had either a young or old leaf. Daily observations were made on the presence of grazing or bite marks on leaves, number of surviving caterpillars, instar of caterpillars, as well as characteristics of their frass such as

Table 1. Behavior categories used in this study.

Activity	**Description**
Flight	The butterfly flies off and remains in flight for more than 3s (i.e. long flight).
Hop	A short flight with a maximum duration of 3s.
Rest	Butterfly remains stationary and rests on the leaf surface or any part of a plant.
Moving	Any non-flight movement like walking and turning on leaf surfaces or any part of a plant.
Interacting with plants	Extension of proboscis to touch the leaf surface or any part of a plant.
Oviposition	The process where the butterfly bends its abdomen and touches the leaf surface on either side with its abdominal tip.
Copulation	The process where the abdomen of the male butterfly curves around and clasps the tip of the female abdomen (Guppy & Shepard, 2001).

coloration, wetness, quantity and shape.

Prior to the start of each leaf test, the leaves were digitally scanned and leaf surface area was determined from the scanned images using ImageJ. The leaves were digitally scanned again on consecutive experiment days to quantify daily changes in leaf surface area. The difference in leaf surface area was then divided by the number of days between each scanning session and the number of caterpillars alive to calculate the 'Area of leaf consumed per day per caterpillar' (refer to equation below).

$$\text{Changes in leaf surface area per day per caterpillar} = \frac{\text{Leaf surface area}_{\text{Day } N\text{-}1} - \text{Leaf surface area}_{\text{Day } N}}{\text{Number of days between Day } N\text{-}1 \text{ and Day } N} \div \text{Number of caterpillars alive on Day } N\text{-}1$$

For each leaf test, the mean of the 'Area of leaf consumed per day per caterpillar' was calculated. As the surface area of leaves with no new feeding marks could be affected by wilting, we only calculated the 'Area of leaf consumed per day per caterpillar' when we observed new feeding/bite marks on the leaves. Estimated reduction in leaf surface area due to wilting ranged from 0.8% of *A. elliptica* leaf surface (0.2 cm^2/day) to 6.7% (9.6 cm^2/day) for *A. sanguinolenta* and 14.1% for *E.ribes* (1.4 cm^2/day). Although wilting appeared to cause relatively large reduction in leaf surface area, leaf surface reduction due to wilting could be overestimated as wilted leaves were often wrinkled and appeared smaller than their actual size. Leaves were replaced either when the caterpillars had finished consuming them or when they were no longer fresh. Containers were kept clean by removing dead caterpillars and waste material daily. The study was conducted between February and March 2019.

Diet test for adults

To determine possible dietary requirements of adult Harlequin, adults were provided with four food types: 10% fructose solution, 10% salt solution, rotten banana and rotten fish. Dechlorinated water was used as a control. The choice of 10% fructose solution was adapted from Morton (1991), who observed that naturally short-lived butterflies were able to survive well on 10% sugar solution in captivity. More concentrated sugar solution was discouraged as the sugars might crystallize in the gut, which is potentially fatal. Rotten banana, rotten fish and 10% salt solution were included since an earlier study reported Neotropical riodinids feeding on rotting fruits, rotting carrion and puddling on damp soil (Hall & Willmott, 2000). Paper strips were soaked in various food samples and placed on the walls of the container where the butterflies were kept. When the butterflies extended their proboscis to touch a paper strip, it was considered a sign of feeding.

Data analysis

For the *in situ* behavioral study, we attempted to analyze the proportion of time spent for each activity using Dirichlet Regression for continuous proportion using R statistical software (ver. 3.4.3). However, according to Douma & Weedon (2019), small sample size could lead to bias during analysis. Our study had a total of 44 butterflies (16 males, 28 females) and this sample size was too small for us to run the Dirichlet Regression model containing up to three-way interactions terms (Sex:Weather condition:Wing condition). While we have sufficient sample size to run up to two interaction terms, failing to consider the three-way interaction terms could lead to bias in our analysis. Hence, we decided to proceed with qualitative analysis of the data through comparison of the percentages and their standard errors. Similarly, due to the small sample size of the leaf test for caterpillars and *ex situ* diet test for adults, we were unable to carry out statistical analysis and decided to proceed with qualitative analysis of the data.

RESULTS

In situ *behavioral study*

A total of 44 adult butterflies (16 males, 28 females) were observed, with a total sampling effort of 33 hours 43 minutes. Analyzed data on the average proportion of time spent on each activity by sex and wing condition, the list of plant species with which the Harlequin was observed interacting with and the average proportion of interaction time spent on each plant species by adults can be found in the Supporting Information.

Activity profile of the Harlequin

Adult Harlequin spent the largest percentage of observation time in resting, regardless of sex, wing and weather conditions. Butterflies of different sex and wing condition spent overlapping proportions of time resting, ranging from 82.6 ± 4.5% (female) to 85.4 ± 5.2% (male) for individuals with intact wings and from 85.1 ± 5.4% (female) to 85.2 ± 11.6% (male) for individuals with damaged wings (refer to Supporting Information 1). When all wing conditions were combined, males spent an average of 81.9 ± 5.6% and females an average of 83.0 ± 3.9% of time resting (Fig. 3). The longest time recorded for resting within one observation session was approximately 54.8 min by a female with intact wings. Most records of resting that exceeded 30 minutes, ranging from 34.4 min to 54.8 min, were by females.

The next most commonly observed behavior was moving (male: 9.3 ± 4.5% vs female: 9.8 ± 3.1%) and interacting with plants (male: 6.9 ± 2.8% vs female: 5.5 ± 2.0%) (Fig. 3). As compared to females, males spent a larger proportion of time interacting with plants and in flight (0.9 ± 0.8%) (vs female: 0.2 ± 0.1%). In contrast, females hopped (1.2 ± 0.2%) more than males (0.9 ± 0.2%).

Throughout the study, there were two occurrences of oviposition and both were performed by the same female during the same observation period. Both occurrences were relatively short events that lasted 6 s and 10 s. The female first oviposited on the underside of an *A. elliptica* leaf, before hopping around and ovipositing a second time on the underside of a *P. sarmentosum* leaf, which was in close proximity to a young *A. elliptica*.

Observation of a potential mating attempt by an adult male

There was one recorded interaction between a male and a female during the *in situ* behavioral study. This observation also featured interactions between the butterflies and an ant. The observation subject, a male, was initially resting on a *D. suffruticosa* leaf while a female was on a nearby leaf. The female responded to an approaching ant by hopping onto the leaf on which the male was resting. In response, the male hopped onto the leaf on which the female was originally resting. This mutual switching of positions continued a few times before both individuals were back on their original leaves. When the ant approached the female again, the female responded by moving towards the *D. suffruticosa* leaf on which the male was resting. The male then hopped onto the female's leaf. No interaction between the sexes was observed for the next 20 s as they rested. As the ant approached the female butterfly, both butterflies moved but resumed resting soon after. As the ant re-approached the female, the butterfly responded by quickly turning such that her posterior end faced the ant. Thereafter, the female resumed resting.

After resting for a short period of time, both butterflies started moving. As the male moved, the female appeared to be monitoring the male's movements by constantly turning to face him. The female also slowly approached the male. Upon disturbance by an approaching ant, the female quickly turned such that her posterior end faced the ant and incidentally the male. The male then responded by turning to face the female. This was followed by a short chase on the leaf surface that lasted 3 s. As the male approached the female during the chase, he was observed lowering his hindlegs and in the process, the tornus of both hindwings were pressed against the leaf. The male then appeared to use his forelegs to attempt to hold onto the female's abdomen as she was moving away. Following this, the female rapidly turned around to chase the male away, which responded by hopping away. The male attempted to approach the female again but was unable to do so. A third and final attempt was made by the male to approach the female, but he was also unsuccessful. The male then flew to another leaf.

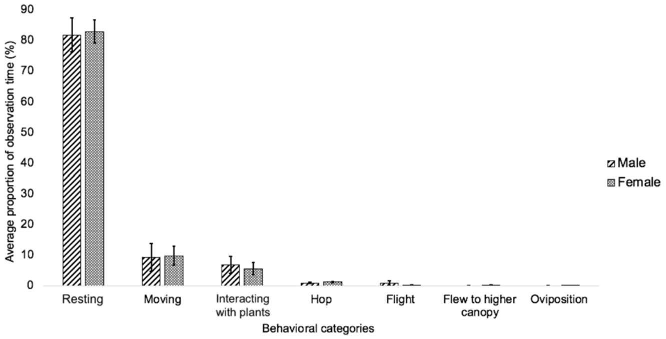

Fig. 3. Average (± SE) proportion of time spent on each behavior by male and female butterflies for all wing conditions combined.

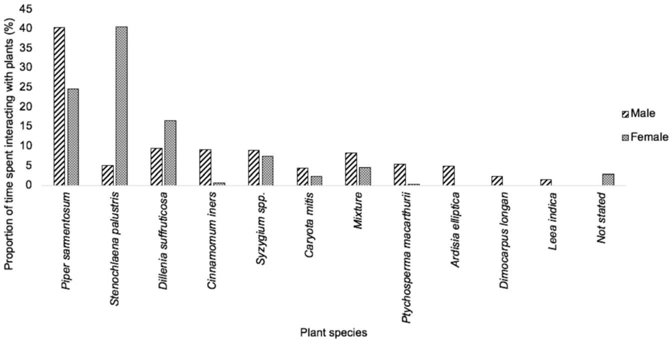

Fig. 4. Average proportion of interaction time spent on each plant species by male and female. *Syzygium* spp. refers to either *Syzygium grande* or *Syzygium polyanthum*. For 'Mixture', the butterflies were observed interacting with multiple plant species and the interaction duration for each species was not recorded. It consisted of additional plant species such as *Cayratia trifolia* for males and *Leea indica* for females.

Harlequin interaction with various plant species

Adult Harlequin would usually move or hop around the adaxial surface of the leaf with their proboscis extended on the leaf surface. The butterflies were observed interacting with a variety of plant species belonging to different families during the *in situ* behavioral study (refer to Supporting Information 2 to 4). Males interacted with slightly more plant species (11 species, which included *C. trifolia* and *D. longan*) than females (8 species). Males were also observed interacting more with *P. sarmentosum* (40.4% of interaction time with plants) while females spent a greater proportion of their interaction time with *S. palustris* (40.5%), followed by *P. sarmentosum* (24.7%) (Fig. 4). Another plant species with which Harlequins spent a considerable amount of time interacting was *D. suffruticosa* (male: 9.4% and female: 16.5%). In addition to the upper surface of a leaf, one female was also observed extending her proboscis on the stem of *D. suffruticosa* as well as on the surface of a vinyl tape found on a tree branch at the study site.

Ex situ *diet studies*

Leaf test for caterpillars

Regardless of caterpillar instar and the age of leaf provided, caterpillars consumed substantially smaller quantities of non-*A. elliptica* leaves as compared to the *A. elliptica* control (Table 2). An exception to this was when two 3rd instar caterpillars consumed a larger area of old *A. sanguinolenta* leaf (1.8cm^2/day/caterpillar) than two different 3rd instar caterpillars fed with old *A. elliptica* leaf (1.2cm^2/day/caterpillar). However, the caterpillars fed with the old *A. sanguinolenta* leaf eventually died within one week of the leaf test without moulting, while the caterpillars provided with *A. elliptica* leaf eventually became pre-pupae at the end of the leaf test.

The frass produced by caterpillars fed with non-*A. elliptica* leaves differed in quantity, shape, size and colour from those produced by caterpillars in the *A. elliptica* control tests (Table 2). With the exception of 3rd instar caterpillars fed with old *A. sanguinolenta*, most caterpillars provided with non-*A. elliptica* leaves (eg. *E. ribes*, *A. crenata*, young *A. sanguinolenta* and old *A. purpurea*) produced smaller quantities of frass than control tests. Occasionally, the frass produced by caterpillars in the non-*A. elliptica* tests were also more irregular in shape than the controls. Size of the frass could also vary from being smaller to being larger than the frass produced by control caterpillars. In addition, the colour of frass produced by caterpillars fed with non-*A. elliptica* leaves varied from black to brown, or a mixture of black and brown. Occasionally, caterpillars in non-control tests produced wet frass. Of the three recorded occurrences of wet frass, two happened on the day the caterpillars died (5th

Table 2. Summary of results from the caterpillar leaf test. Calculations have been rounded off to the nearest one decimal figure.

Leaf test	Plant species	Instar of caterpillar at start of test	Caterpillar eclosion success	Mean area of leaf consumed per day per caterpillar (cm^2/day/caterpillar)	Characteristics of frass
Young leaf and 5th instar caterpillars	*Ardisia elliptica*	5th instar	2 out of 2 caterpillars eclosed successfully into adults	10.2	Produced large quantity of dry brown frass throughout entire test duration
	Embelia ribes	5th instar	0 out of 2 caterpillars eclosed successfully into adults: One died as pre-pupa and one died as pupa	0.6	Compared to *A. elliptica* tests, produced smaller quantity of dry black frass; frass irregular in shape. Produced slightly wet frass on one occasion
Old leaf and 5th instar caterpillars	*Ardisia elliptica*	5th instar	2 out of 2 caterpillars eclosed successfully into adults	12.5	Produced large quantity of dry brown frass throughout entire test duration
	Embelia ribes	5th instar	0 out of 2 caterpillars eclosed successfully into adults: Both died within seven days of leaf test as 5th instar	1.5	Compared to *A. elliptica* tests, produced smaller quantity of dry black frass; frass irregular in shape. Produced wet frass on Day 5 of leaf test
Old leaf and 5th instar caterpillars	*Ardisia sanguinolenta*	5th instar	0 out of 1 caterpillar eclosed successfully into adult: Pupa was deformed and eventually died within 11 days of leaf test	Unable to measure as caterpillar pupated on Day 3 of leaf test but caterpillar consumed a large area of leaf before pupating	Produced dry brown frass; frass slightly larger in size than *A. elliptica* tests
Young leaf and early instar caterpillars	*Ardisia elliptica*	1st instar	One 3rd instar and one 5th instar at the end of leaf test	0.7	Produced large quantity of dry brown frass throughout entire test duration
	Embelia ribes	2nd instar	0 out 2 caterpillars eclosed successfully into adults: Both died within five days of leaf test as one 2nd and one 3rd instar	0.2	Produced very small quantity of dry black frass
	Ardisia crenata	3rd instar	0 out 2 caterpillars eclosed successfully into adults: Both died within one day of leaf test as 3rd instar	No prominent grazing mark	Compared to *A. elliptica* tests, produced smaller quantity of a mixture of dry brown and black frass; frass irregular in shape and smaller in size
	Ardisia sanguinolenta	3rd instar	0 out of 2 caterpillars eclosed successfully into adults: Both died within two days of leaf test as 3rd instar	No prominent grazing mark	Compared to *A. elliptica* tests, produced smaller quantity of dry brown frass; shape of frass similar to that of *A. elliptica* tests
Old leaf and early instar caterpillars	*Ardisia elliptica*	1st instar	2 out of 2 caterpillars became pre-pupa at the end of leaf test	1.2	Produced large quantity of dry brown frass, except on few days
	Embelia ribes	2nd instar	0 out of 2 caterpillars eclosed successfully into adults: Both died within five days of leaf test as 2nd instar	No prominent grazing mark	Produced very small quantity of dry black frass
	Ardisia crenata	3rd instar	0 out 2 caterpillars eclosed successfully into adults: Both died within two days of leaf test as 3rd instar	0.3	Compared to *A. elliptica* tests, produced smaller quantity of a mixture of dry brown and black frass; frass irregular in shape and smaller in size
	Ardisia sanguinolenta	3rd instar	0 out of 2 caterpillars eclosed successfully into adults: Both died within seven days of leaf test as 3rd instar	1.8	Produced large quantity of dry brown frass; shape of frass similar to that of *A. elliptica* tests
	Ardisia purpurea	3rd instar	0 out of 2 caterpillars eclosed successfully into adults: Both died within five days of leaf test as 3rd instar	0.1	Compared to *A. elliptica* tests, produced smaller quantity of dry black and brown frass; size of frass slightly larger than *A. elliptica* tests. Produced wet frass on Day 5 of leaf test

instar caterpillar fed with old *E. ribes* and 3rd instar caterpillars fed with old *A. purpurea*).

No other leaf tests apart from the *A. elliptica* control tests resulted in caterpillars successfully eclosing into adults. Among the earlier instars, most caterpillars died within a few days of the leaf test without moulting to the next instar (Table 2). The shortest leaf test lasted for a day where two 3rd instar caterpillars were fed with young *A. crenata* leaf. The longest leaf test was seven days where 3rd instar caterpillars were provided with old *A. sanguinolenta* leaf. Among 5th instars fed with non-*A. elliptica* leaves that reached pre-pupal or pupal stages, all individuals eventually died. Although the 5th instar caterpillar fed with old *A. sanguinolenta* leaf pupated, it was deformed and died within 11 days of the leaf test.

Diet test for adults

Adults subjected to the diet test were observed to feed on all food samples (10% fructose solution, 10% salt solution, rotten banana, rotten fish and dechlorinated water (control)) presented on paper strips that were placed on the container's walls without any particular preference. Due to the small sample size, we were unable to carry out any statistical test for any dietary differences amongst the 4 food types.

DISCUSSION

For both sexes, the largest proportion of observation time was spent resting. Harlequins were commonly observed interspersing resting with a sequence of more energetically intensive activities such as moving, hopping, flying and interacting with plants. Being ectothermic, butterflies rely on a variety of morphological, physiological and behavioral adaptations to regulate their temperatures (Clench, 1966). Tsai *et al.* (2020) found that the Lycaenid *Satyrium caryaevorus* almost always basks in the sun following a flight, as its wing temperature, unlike the thoracic temperature which remains high (30.9 ± 0.7°C) due to metabolic heat generated by the flight muscles when flying, often drops close to the ambient temperature (only 0.7 ± 0.5°C above ambient temperature range of 25 to 28°C). Resting may allow butterflies, including the Harlequin, to regulate their body and wing temperatures to be within their optimal temperature range.

Male riodinids are known to rest, singly or in aggregations, on distinctive topographic sites that include hilltops, vegetation and forest edges, to locate females (Hall & Harvey, 2002; Alcock & Dodson, 2008; Sáfián, 2016). Such behavior is referred to as 'perching'. However, the lack of observed conspecific interactions throughout the behavioral study, particularly the absence of male-male interactions, the observed resting behavior of male Harlequin may not represent true territorial or lekking behavior. Whether Harlequin males might perch in different localities, such as specific microhabitats or in the tree canopy, remains unknown.

Compared to females, males spent a substantially larger proportion of time in flight. In addition to lekking, males may also search for females by patrolling or flying almost continuously over large areas. Spending longer duration in flight may increase their success in finding a suitable mate (Rutowski, 1991; Pfeifer *et al.*, 2000). The longest duration of a male in flight was 110s, vis-à-vis a female's maximum flight duration of 39s. In contrast, females were observed to spend a slightly larger proportion of observation time hopping than males. Possible reasons for this include searching for suitable oviposition sites and food sources, as well as in response to disturbances. According to Pfeifer *et al.* (2000), male *Glaucopsyche nausithous* were observed spending twice the amount of time in flight as compared to females, while females engaged in flights more frequently than males, and this resulted in a shorter duration per flight for females. Similarly, female Harlequin were observed engaging in shorter flights (which was defined as 'Hop' in this study) more frequently than males (average 14.6 hops per female vs 11 hops per male). The average flight duration of male is 20.3 ± 7.0s, which was double the average duration of female in flight (10.2 ± 8.7s). It should however be noted that flight duration in this study may be an underestimation due to difficulties in monitoring flying butterflies, especially those that flew to a higher or inaccessible location (pers. obs.).

Throughout the study period we observed only two oviposition events, performed by the same female. The lack of observed oviposition events could be because females generally remain stationary at an area after copulation to avoid detection by other male conspecifics or predators (Rutowski, 1991). Hence, it is possible that oviposition events might have gone undetected.

Interestingly, the female oviposited on a non-host plant, *P. sarmentosum*, during one oviposition event, and this was the second occurrence of a Harlequin female ovipositing on a non-host plant species. The first observation occurred outside of the *in situ* behavioral study and the female was observed ovipositing on a climbing plant *Derris trifoliata* Lour. (Leguminosae) that grew on a *A. elliptica* (pers. obs.). Following this observation, a preliminary *ex situ* leaf test where caterpillars were given *P. sarmentosum* leaves was conducted. We observed small caterpillar bite marks on the *P. sarmentosum* leaves. However, when *A. elliptica* leaves were given, the caterpillars switched to feeding on the *A. elliptica* leaves. Egg-laying behavior on non-host plant substrates, including rocks and crevices in tree trunks, has been reported in multiple butterfly species across different families (Wiklund, 1984; De-Silva *et al.*, 2011; Wick *et al.*, 2012; Freitas & Kaminski, 2014). Female butterflies may seek to reduce predation or parasitism pressures on eggs by depositing them on substrates where they are less detectable by natural enemies (De-Silva *et al.*, 2011; Wick *et al.*, 2012). Eggs will usually be deposited on a non-host plant substrate that is located close to the host plant. Occurrence of this behavior would mean that the benefits of ovipositing away from the host plant outweigh the costs associated with searching for the host plant by the caterpillars. De-Silva *et al.* (2011) reported that average survival of eggs laid away from the host plant (56 ± 6%) was significantly higher than those laid on the host plant (37 ± 5%) for *Oleria onega*. Similarly, the Harlequin was also observed ovipositing on a non-host plant species that was in close proximity to an *A. elliptica* in both occurrences. Since oviposition on non-host plant substrate is not frequently reported in the tropics (De-Silva *et al.*, 2011; Freitas & Kaminski, 2014), these records of the Harlequin ovipositing on a non-host plant substrate serve as potentially important findings for not just the riodinids, but also for the behavioral biology of tropical butterfly species.

It is possible that the male-female interaction observed in this study is part of the Harlequin's courtship behavior. During the interaction, the male was observed lowering his hindlegs and the tornus of both hindwings were pressed against the leaf surface. Similar behavior was seen in the Neotropical riodinid species *Periplacis hebrus* (Cramer, 1777), which placed its wings downwards so that they touched the leaf surface while curling its extended abdomen over its thorax (Hall & Harvey, 2002). According to Hall & Harvey (2002), at least 25% of riodinid species possess androconial organs or specialized scales involved in the dissemination of pheromones utilized during courtship. Depending on the tribe and genus, these scales can be present on the wings, abdomens and appendages. By pressing the tornus of both hindwings on the leaf surface, the male Harlequin may be attempting to disseminate pheromones to increase the receptiveness of the nearby female to mating. Following this, the male attempted to hold onto the female's abdomen using his forelegs. We hypothesize this to be analogous to Pliske (1975)'s definitions of "aerial pounce" and "aerial takedown" for the courtship sequence in *Danaus* *plexippus* (Linnaeus, 1758), in which the male lands and grabs onto a female's dorsum to hinder her flight and facilitate copulation. In response, the female Harlequin attempted to outdistance the male, before subsequently chasing the male away. The resistance of mating by females was documented by both Pliske (1975) and Frey (1999), who also found that females that were already mated were more likely to resist mating than virgin females, as they would be more interested in searching for suitable oviposition sites (Rutowski, 1982).

Based on the *ex situ* leaf tests conducted on different instars of Harlequin caterpillars, the caterpillars did not appear to be receptive to feeding on other plant species belonging to the same family as their known host plant (*A. elliptica*). Furthermore, since the caterpillars were not subjected to starvation prior to the leaf tests, some documented survival of caterpillars on novel hosts could be due to them being sustained by nutrients from their earlier diet. Although there are studies that have successfully induced caterpillars to feed on novel plant species, in other cases larvae were also observed not to feed on novel plant species and would eventually starve to death, even when the alternative plant species was known to be suitable for their development (Bernays & Weiss, 1996). As Harlequin caterpillars in this study were fed their host plant species immediately after hatching, they could have been imprinted to accept *A. elliptica* as their only preferred food plant and may have therefore refused to feed on alternative plant species (Bernays & Weiss, 1996; Heard, 1999). In other words, freshly-hatched caterpillars that have not been fed the host plant species may be more receptive to consuming a novel plant species. Imprinting with a particular plant species provides a possible explanation for the absence of grazing marks on the leaves of young *A. crenata*, young *A. sanguinolenta* and old *E. ribes* when provided to the early instar caterpillars that had previously been fed with *A. elliptica*. The 5th instar Harlequin caterpillars, in contrast, were observed feeding on non-*A. elliptica* species in this study. However, the quantity of leaves consumed by 5th instar caterpillars was likely insufficient to sustain their survival and successful eclosion into adults. In addition to their prior feeding experiences with *A. elliptica*, the receptivity of Harlequin caterpillars to novel plant species could be affected by differing physiology in different instars, and this may affect their abilities to feed and digest alternative plant species. Earlier studies found that the diet breadth of riodinid larvae ranged from monophagous to polyphagous (DeVries & Chacón, 1992; Nielsen & Kaminski, 2018), and more studies are needed to investigate the diet breadth of Harlequin caterpillars.

During the *in situ* behavioral study, adult Harlequins were observed interacting with 11 plant species from different families (refer to Supporting Information 2). Among nectar-feeding butterfly species, it has been suggested that the diet breadth of butterflies could be influenced by the species composition and abundance of flowering plants in a habitat (Jain *et al.*, 2016). Both male and female Harlequins were observed spending a large proportion of their time interacting with *P. sarmentosum*, a herbaceous creeper that grew extensively throughout the study site. Only males were observed interacting with *A. elliptica*, *D. longan* and *C. trifolia*, which were less well distributed at the study site. As males may wander more widely throughout the study site during their search for mates, they were more likely to encounter less common plant species. Although limitations in the current study's observation methodology do not allow us to confirm whether butterflies were feeding during their interactions with the plants, during the *ex-situ* diet test we observed that adult Harlequins did not actively search for paper strips with specific food samples but instead fed on the paper strip closest to them (pers. obs.). This suggests that adults could be opportunistic feeders, which may explain the wide variety of plant species with which Harlequins were observed to be interacting during the *in situ* behavioral study.

Riodinids are known to feed on flowers, rotting carrion, and rotting fruit, as well as puddling on damp soil. It has been hypothesized that butterflies feed on rotting carrion and puddle to obtain the sodium ions needed to maintain activities such as flight, copulation and reproduction (Hall & Willmott, 2000). It has also been proposed that the tendency to seek out sodium is associated with life strategy adaptations that can be broadly measured by the ratio of the butterfly's wing area to thoracic volume (WA:TV ratio), due to differences in flight speed and metabolic rate among butterflies with different WA:TV ratios. In the case of adult Harlequins, the butterflies were not once spotted interacting with flowers throughout the study duration during the *in situ* behavioral study and do not appear to be nectar feeders. Instead, the constant testing of leaf surfaces suggests feeding on microscopic substances found on leaves (Khew, 2007; Khew, 2011). Although we were unable to confirm whether Harlequins take up sodium ions in this study, we have observed instances outside of the study where captive-reared Harlequins extended their proboscis on our perspiration as well as on the surface of rubber boots (pers. obs.).

Given the difficulty of observing the Harlequin's feeding behavior in the field, alternative methods for studying the food preference of the adults could involve closer observations of the feeding behavior of wild and captive adults via video recordings. To determine whether sodium ions are taken up during feeding, adults could be provided with leaves washed in salt water as well as leaves treated to remove microscopic substances present on the surface in future diet experiments. In addition, future behavioral studies should be carried out across a longer observation period (e.g. whole-day observations) as well as aim to provide more detailed descriptions of certain behaviors such as resting (e.g. wing angle, wing and body orientation to sun, above-ground height). Such studies will provide more details about the daily activity pattern of adult Harlequin, which in turn may provide more insights about the mating-locating, courtship and mating behavior of these highly secretive butterflies.

CONCLUSIONS

The Riodinidae remains one of the most poorly studied Lepidoptera families, partly because of the challenges associated with studying these butterflies due to their frequent low densities in their natural habitats (Nielsen & Kaminski, 2018). The presence of the Harlequin in a small young secondary forest fragment that contained a relatively high density of their host plant species thus provided a suitable opportunity to study the behavior and biology of this species. Our *in situ* behavioral study found that both males and females spent a large proportion of time resting. The proportion of time spent in flight and hopping also differed between the sexes. The first occurrence of a female ovipositing on a non-host plant species as well as interactions between a male and a female were documented. These observations may provide further clues about the courtship and oviposition behavior of these secretive butterflies. The *in situ* behavioral study, reinforced by a series of diet tests conducted on caterpillars and adults in captivity, found that caterpillars had poor receptivity to novel plant species while adults may feed on microscopic substances present on the leaf surfaces of various plant species.

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Supporting Information

1. Average proportion of time spent on each activity, *P*(activity), by sex and wing condition. *n* represents the sample size. One male was removed from the analysis as the wing condition was not recorded.

Average *P*(activity)	Female + Damaged (*n* = 5) Average ± SE	Male + Damaged (*n* = 4) Average ± SE	Female + Intact (*n* = 24) Average ± SE	Male + Intact (*n* = 11) Average ± SE
Resting	85.1 ± 5.4%	85.2 ± 11.6%	82.6 ± 4.5%	85.4 ± 5.2%
Moving	7.6 ± 3.5%	8.4 ± 6.1%	10.2 ± 3.7%	4.2 ± 2.4%
Interacting with plants	6.1 ± 2.1%	5.7 ± 5.1%	5.4 ± 2.4%	8.0 ± 3.7%
Hop	0.8 ± 0.3%	0.7 ± 0.4%	1.3 ± 0.3%	1.0 ± 0.3%
Flight	0.4 ± 0.3%	0.0 ± 0.0%	0.2 ± 0.1%	1.4 ± 1.1%
Flew to higher canopy	0.0 ± 0.0%	0.0 ± 0.0%	0.2 ± 0.2%	0.0 ± 0.0%
Oviposition	0.0 ± 0.0%	-	0.1 ± 0.1%	-

2. List of plant species with which adult Harlequin were observed interacting during the *in situ* behavioral study. Information on native and threat status of the species were obtained from Flora & Fauna Web (2019b).

Plant species	Family	Native status	Threat
Piper sarmentosum	Piperaceae	Native	Common
Stenochlaena palustris	Blechnaceae	Native	Common
Dillenia suffruticosa	Dilleniaceae	Native	-
Syzygium spp. (*Syzygium grande* and *Syzygium polyanthum*)	Myrtaceae	Native	*S. grande*: Common *S. polyanthum*: Vulnerable
Caryota mitis	Arecaceae	Native	Common
Cinnamomum iners	Lauraceae	Native	-
Ptychosperma macarthurii	Arecaceae	Not-native	-
Ardisia elliptica	Primulaceae	Native	Endangered
Dimocarpus longan	Sapindaceae	Uncertain	-
Leea indica	Vitaceae	Native	Common
Cayratia trifolia	Vitaceae	Native	Vulnerable

3. Average proportion of time spent interacting with each plant species by adult female Harlequin during the *in situ* behavioral study.

Plant species	Average proportion of time spent interacting with plants
Stenochlaena palustris	40.5%
Piper sarmentosum	24.7%
Dillenia suffruticosa	16.5%
Syzygium spp. (*Syzygium grande* and *Syzygium polyanthum*)	7.4%
Caryota mitis	2.4%
Cinnamomum iners	0.7%
Ptychosperma macarthurii	0.4%
Mixture of species (includes *Leea indica*)	4.5%
Not stated	2.9%

4. Average proportion of time spent interacting with each plant species by adult male Harlequin during the *in situ* behavioral study.

Plant species	Average proportion of time spent interacting with plants
Piper sarmentosum	40.4%
Dillenia suffruticosa	9.4%
Cinnamomum iners	9.2%
Syzygium spp. (*Syzygium grande* and *Syzygium polyanthum*)	8.9%
Ptychosperma macarthurii	5.5%
Stenochlaena palustris	5.1%
Ardisia elliptica	5.0%
Caryota mitis	4.5%
Dimocarpus longan	2.3%
Leea indica	1.6%
Mixture (includes *Cayratia trifolia*)	8.3%