BEHAVIORAL TRAITS EXPRESSED DURING *HELICONIUS* BUTTERFLIES ROOST-ASSEMBLY

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Abstract - Several species within the genus *Heliconius* display nocturnal communal roosting. The butterflies have permanent roost sites that are used night after night. A few hours before sunset the butterflies start navigating towards the roost sites. When several individuals meet at these places, a series of behavioral interactions occur before they are all perched gregariously. Although some of these interactions have been previously reported, there are no data supporting these interactions as defined and consistently expressed behavioral traits. Using natural and in-cage roosting aggregations of *H. sara* and *H. erato*, from Costa Rica, Panama and Peru, and modern behavior analysis tools, the present work studied roost-assembly behavioral traits to establish whether these traits are consistently expressed or not, to provide a detailed description of them, and to detect interactions never reported before.

Key words: communal roosting, behavior

INTRODUCTION

Several species from the butterfly genus *Heliconius* display communal nocturnal roosting. The most gregarious species include *H. erato*, *H. sara*, *H. hecale*, *H. charithonia*, and *H. hewitsoni* (Mallet and Gilbert 1995). *Heliconius* butterflies roost gregariously in selected roost sites night after night (Brown Jr. 1981; Cook et al. 1976; Mallet and Jackson 1980; Turner 1981). Roosting sites are usually under vegetation mats, in man-made live fences, or forest edges, sometimes near streams, and in areas with almost no wind, where dead hanging vines, leaves or twigs provide a roosting perch. Roost sites are fairly stable (Mallet 1986) unless the perch is destroyed by a natural disturbance or by human intervention. When a roosting perch is physically altered, the butterflies do not come back. Instead, they spread out, and perch in nearby sites until a new stable perch site is found. Once a new perch is found, they gradually start perching gregariously again. The recruiting mechanisms are unknown.

In the typical situation, the butterflies start navigating towards their roost sites as early as 3 h before sunset (Mallet 1986, pers. obs.). Individuals navigate along forest edges and clearings until they arrive to the roost site. After arrival to the site, they express a pre-roosting behavior, which comprises sitting in nearby areas (3-5 m), brief air-chases, and occasional basking. Once light levels start dropping rapidly, approximately 30-45 min before sunset, the butterflies fly towards the perch and start expressing a set of roost-assembly behavioral interactions. During the course of these interactions, where physical contact between individuals is frequent, individuals start to perch until they are all perched gregariously. At night, the butterflies remain motionless and conserve their location in the perch until the next day (Salcedo 2006). At sunrise they disband, usually one by one, unless one of them disturbs closely perched neighbors or a physical disturbance of the perch occurs, in which case several individuals leave simultaneously (Mallet 1986, pers. obs.).

Behavioral traits of *Heliconius* exhibiting roost-assembly behavior were first addressed by Mallet (1986). Using *H. erato* from Costa Rica and Colombia, Mallet (1986) used the term "fanning" to describe a behavior that involved various levels of interest in roosted conspecifics, ranging from brief approaches to hovering closely with probable antennal and wing contact; he also used the term "clutching" to describe grasping at the perch or the wings of a roosted butterfly with the tarsal claws, and "fending-off" to describe vigorous fluttering, without letting go of the perch, of a perched individual when fanned or clutched by another individual.

Using natural and in-cage roosting aggregations of *H. sara* and *H. erato*, from Costa Rica, Panama, and Peru, and modern behavioral analytical tools, such as low speed digital photography, digital video, and behavior analysis software, the present study examined roost-assembly behavioral traits to establish whether these traits are consistently expressed, to provide a better description of such traits, and to detect interactions hitherto unreported.

METHODS

Ethogram production: Identification of behavioral traits was done by observing and photographing behavioral interactions for each species for least 7 days at dusk for approximately 40-60 min. High-speed photography by a Pentax® K100D Super SLR digital camera was used to identify specific contact surfaces during interactions between individuals. In-cage observations included a group of 10 males and 10 females of *Heliconius erato* from Peru and a group of 4 males and 4 females of *H. erato* from Costa Rica. Field observations included roost sites of *H. sara* from Costa Rica, *H. erato* from Panama and *H. charithonia* from Florida, USA.

Frequency of behavioral traits: Frequencies were studied using captive *H. erato* (10 males and 10 females) and wild *H. sara* (five males and three females). In order to determine if a behavioral trait was consistently expressed, its frequency (number of times a behavioral trait was expressed per minute) was calculated. Recordings were made with a SONY DCR-SR220 handheld camera. To accurately videotape the behavioral traits, the camera field of view covered the entire roost site and whenever an interaction was detected, the observer zoomed-in and recorded the interaction. This technique proved to be the most efficient because in preliminary trials, when the camera field of view covered the entire roost site permanently, the identification of each behavioral trait was difficult during the posterior video analysis due to lack of resolution. The videos were analyzed using specialized software (Observer XT by Noldus). In total, 300 minutes of roosting behavioral interactions were videotaped.

RESULTS AND DISCUSSION

Mallet (1986) included probable antennal and wing contact as part of fanning (*sensu lato*) behavior. In this work, fanning will be considered only as hovering over a perched individual (*sensu stricto*). The results show that there are five clearly defined behavioral traits that are consistently expressed during *Heliconius* roost-assembly. A repeated sequence in the expression of the traits was not found. The identified traits include fanning *sensu stricto*, approach, fending-off, clutching or leg-wing contact, and antenna-wing contact (Figure 1). It is important to note that the *H. erato* group that was used to estimate frequencies had a 1:1 sex ratio and was held in an enclosure while the *H. sara* group was studied in the wild. Full data on observations and frequency of each behavioral trait are reported in Table 1.

Fanning is hovering above a perched individual with no physical contact (Figure 1). Fanning was described by Mallet (1986) from his observations of *H. erato* roosting aggregations. Both sexes express fanning, but males express it more than females (Mallet 1986). Fanning is not expressed exclusively during roosting interactions. It is more frequently expressed when butterflies are identifying flowers for feeding, when females search for a suitable oviposition site, and when males court females for mating (Klein and de Araújo, 2010). The results show that in *H. erato* and *H. sara*, fanning behavior is highly expressed: 2.54 events/min and 5.51 events/min, respectively. Fanning involves fluttering of the wings while holding a somewhat stationary position in front of the individual (5-15 cm from it) and hence generates air movement. This airflow may facilitate the dispersion of volatile chemical compounds that reach chemosensillae in the antennae and other parts of the fanned individual.

Approach involves direct flight towards an individual. Approach behavior is probably an approximation for inspection of an individual or subject and is triggered from a distance (0.8-

Fig. 1. Behavioral interactions expressed during *Heliconius* roost formation. A: *H. erato* antenna-wing, B: *H. charithonia* clutching, C: *H. erato* fanning, D: *H. sara* fending-off.

Fig. 2. *Heliconius erato* fending-off: individuals perched on the wire flutter their wings "fending-off" approaching individuals.

4 m) by a color cue (wing color pattern for inter or intraspecific interactions). This behavior is also expressed in courtship and when approaching flowers in the forest. The results show that it is highly expressed in the captive *H. erato* group (2.75 events/min) and far less (about 90% less frequent) in the wild *H. sara* group (0.28 events/min). Approach is the only behavior that does not occur exclusively at close range (0-15 cm). This suggests that *H. sara* individuals can identify conspecifics more efficiently from distance and do not need numerous approaches like *H. erato*. This may be achieved ultimately through more efficient wing pattern detection or by highly attractive volatile chemical cues. In addition, by fanning *H. sara* may "compensate" for this reduced number of approaches. Alternatively a higher number of approaches could be the result of having *H. erato* in an enclosure, where the probability of approaching perched individuals would be increased by the lack of space, compared to free flying *H. sara* in the wild.

Clutching involves brief contacts between an approaching individual's tarsal claws and the wings of a perched individual (Figure 1). Usually, the contact involves the mesothoracic legs, but the metathoracic pair is sometimes involved. The term "clutching" was coined by Mallet (1986). Clutching behavior occurs also during courtship and mating (Klein and de Araújo 2010), but with the clutched butterfly in a standing position (Mallet 1986). This behavior is sometimes expressed during fanning. The results show that clutching behavior had a high frequency in *H. sara* (2.97 events/min), 10 more times than in *H. erato* (0.31 events/min). Repeated physical contact with tarsal claws in general, suggests detection of contact chemical cues. Scanning electron microscopy revealed several contact chemosensillae in the first 4 tarsal segments of *H. erato* males and females (Salcedo unpub. data). The higher frequency of clutching in *H. sara* may suggest that contact chemical cues are more important in their roost assembly interactions compared to *H. erato*.

Fending-off behavior includes vigorous fluttering of the wings by one or several individuals upon fanning and/or clutching an approaching individual who is seeking space to perch (Figure 1). During fending-off, *Heliconius* males flutter their wings and expose androconial patches on their hind wings; females flutter their wings and expose their abdominal "stink clubs" (scent glands) (Mallet 1986) in a behavior similar to that used in diurnal rejection of males (Gilbert 1976). The results show that fending-off is expressed 10 times more frequently in *H. sara* than in *H. erato* (0.66 vs. 0.07 events/minute). This trend may be related to roost spatial structure. *H. erato* aggregations are known to include several sub clusters of 3-5 individuals (Mallet 1986, Salcedo 2006). Perhaps a lower fending-off frequency can contribute to this sub cluster formation. In addition, fending-off may also contribute to the dissemination of volatile chemical cues that may serve for intraspecific recognition or as spacing pheromones.

Antenna-wing behavior consists of an approaching individual that touches a perched individual wing with the antennae (Figure 1). The results show that antenna-wing occurs at a very low frequency in *H. erato* and *H. sara* (0.09 and 0.02 event/min). Antenna-wing may occur concurrently with wing-wing contact. It may occur also incidentally as a result of an individual trying to touch another when clutching. Sensillae in the *Heliconius* antennae are probably used primarily to detect

Table 1. Frequency (occurrences per minute of interaction) of behavioral traits of *Heliconius erato* and *H. sara* during roosting aggregations formation.

Observation period (min)	Behavioral trait - number of interactions observed				
	fanning	approach	fending-off	clutching	antenna-wing
H. erato-captive 10 males 10 females					
10.57	38	20	0	8	6
10.67	43	45	1	0	0
36.25	110	172	4	10	5
45.68	180	202	7	34	4
65.80	58	25	0	1	1
Frequency (#/min)	2.54	2.75	0.07	0.31	0.09
H. sara-wild 5 males 3 females					
12.27	102	10	6	46	0
12.23	150	12	4	72	2
29	45	6	36	51	0
51.98	286	6	27	116	1
16.20	88	0	7	76	0
Frequency (#/min)	5.51	0.28	0.66	2.97	0.02

non-contact volatile chemical cues (Andersson and Dobson 2003) but the detection of contact chemical cues cannot be discarded. Additionally, this behavior may be related to an auditory interaction. Some species of *Heliconius* have been reported to produce sound (Hay-Roe and Mankin 2004) and the greatest number of auditory receptors in *Heliconius* is located at the optic lobes near the base of the antennae (Swihart 1967).

This work clearly shows that *Heliconius* roosting behavior has a set of defined behavioral traits that are consistently expressed to successfully form roosting aggregations. Further research is needed to determine if there are differences in the frequency of expression of such traits between all the different *Heliconius* roosting species, to determine if these traits are involved with roost spatial structure, and to study the role of contact and non-contact chemical cues in these behavioral interactions.

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