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Gregarious Oviposition and Clutch Size Adjustment by a *Heliconius* Butterfly[1]

ABSTRACT

Female *Heliconius hewitsoni* butterflies were found to aggregate during oviposition, producing multi-parent egg clutches. This behavior occurred when host plants were locally plentiful, indicating that females chose to oviposit gregariously. Collective clutch size correlated with host growth rate and with the number of females contributing to a clutch. Eggs did not positively bias host plant growth. Collective clutch size adjustment may represent a mechanism for facilitating larval aggregation while reducing intraspecific competition.

Key words: *communal oviposition; intraspecific competition; larval aggregation;* Passiflora; *social oviposition*

BUTTERFLIES ARE AN IMPORTANT SYSTEM FOR STUDYING THE BEHAVIORAL ECOLOGY of clutch laying and larval aggregation (Stamp 1980, Godfray *et al.* 1991). An exceptional aspect of butterfly clutch laying is that some butterflies produce collective egg clutches through gregarious oviposition behavior (Mallet & Jackson 1980, Godfray *et al.* 1991, Sourakov 1997, Prokopy & Roitberg 2001). Previous descriptions of this behavior, however, consist of only a few observations with quantitative data. This study documents gregarious oviposition in the butterfly *Heliconius hewitsoni* in terms of spatial and temporal aggregation of ovipositing females in nature, and relates these data to population-wide patterns of collective clutch laying in the context of host plant availability.

Heliconius hewitsoni occurs in lowland rain forests of southwestern Costa Rica and western Panama (Longino 1984, DeVries 1987). Individuals have small home ranges and display predictable patterns of daily movement (Reed, pers. obs.). Larvae of *H. hewitsoni* feed solely on the liana *Passiflora pittieri*, and gregarious oviposition behavior has been previously noted in this species (Longino 1984; Duckett 1989; L. E. Gilbert, pers. comm.).

This study was conducted near La Sirena field station, Corcovado National Park, Costa Rica, where I observed butterflies at four *P. pittieri* sites (8°28.68′N, 83°35.41′W; 8°29.04′N, 83°35.48′W; 8°28.83′N, 83°35.46′W; and 8°28.81′N, 83°35.11′W) during the wet season of July and August 2001. I numbered all females with a Sharpie pen and recorded the identity of ovipositing individuals, the total number of eggs laid during an event, shoot length at the time of oviposition, the time of the first oviposition, the approximate time of the final oviposition, and, for a subset of females, individual clutch contributions.

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TABLE 1. *A summary of observed* H. hewitsoni *oviposition events.*

Date	Approx. time (h)	Number of females	Collective clutch size	Shoot	Shoot length (cm)	Unexploited shoots[a]	Mean eggs per female	Approx. duration (h)
12 July	0907–<1420[b]	≥2[b]	34	Natural	1.6	≥4	≤17.0[b]	?[b]
25 July	1000–1034	4	63	Natural	7.7	≥5	15.8	0.57
25 July	0920–1230	5	48	Natural	0.9	≥5	9.6	3.17
25 July	1228–1245	3	62	Natural	0.4	≥5	20.7	0.28
1 Aug.	0815–0900	7	191	Cutting	4.5	≥0	27.3	0.75
1 Aug.	0855–0955	6	75	Cutting	3.4	≥0	12.5	1.00
2 Aug.	0921–1018	3	39	Cutting	6.2	≥2	13.0	0.95
3 Aug.	1237–1335	1	23	Cutting	3.1	≥4	23.0	0.97
4 Aug.	1144–1217	2	35	Cutting	4.3	≥6	17.5	0.55
5 Aug.	0844–1040	10	156	Cutting	2	≥6	15.6	1.93
5 Aug.	0843–0940	4	30	Natural	0.9	≥6	7.5	1.05
5 Aug.	0938–?[b]	≥2[b]	44	Natural	2.1	≥6	≤22.0[b]	?[b]

[a] This refers to the number of identified young host plant shoots in the vicinity that did not bear eggs at the end of the day, but were within the acceptable length range and were oviposited on before the end of the study.

[b] Uncertainty is because eggs were added to the clutch after the observation period.

Females were most active at host plant sites between 0800 and 1300 h, except during heavy rain (Table 1), and were frequently observed revisiting the same site over a period of many days. Thirteen different females revisited the same host plant site regularly over a period of a week or more, and one female revisited the same host plant site during a 25-day period. Eighteen cases of a female visiting the same host plant site over two or more consecutive days were noted. There were seven observations of females ovipositing on two consecutive days at the same site, and one observation of three consecutive days of oviposition at the same site. These observations and previous studies (Ehrlich & Gilbert 1973, Mallet 1986) suggest that most females visited specific host plant sites on an almost daily basis and were capable of ovipositing at any time.

Initiation of gregarious oviposition was a striking event. Every morning, multiple females would visit a host plant site, inspecting every shoot in the vicinity several times before leaving the area or deciding to oviposit. Once a female had initiated oviposition, other nearby females would fly to the shoot where oviposition was taking place and either oviposit simultaneously or alternately with the other female(s). Females joining an oviposition group would often probe the other freshly laid eggs with their probosces.

To justify the use of the term "gregarious" in describing *H. hewitsoni* oviposition behavior, I sought to determine if individuals were spatially and temporally aggregating independent of resource availability. Ten complete and two partial oviposition events were observed at 8°28.68′N, 83°35.41′W, where two mature (>15-yr-old) *P. pittieri* plants and many small clones occurred within a 3 m^2 area. An oviposition event was defined as the time between a shoot receiving the first egg and the final egg over a 24-hour period. Six oviposition events took place on naturally growing host plant shoots and six occurred on greenhouse cuttings placed at the host plant site during morning hours (Table 1). The use of shoot cuttings placed within easy viewing range facilitated observation of oviposition behavior in the field. The mean number of females that contributed eggs to a discrete clutch during an oviposition event was 4.5 (N = 10, SD = 2.64, range = 1–10) and only one case of a single female being the sole contributor to a clutch was witnessed directly (Table 1). The mean length of natural shoots at the time of oviposition was 1.5 cm (N = 19, SD = 0.75, range = 0.4–3.2 cm). These observations of multiple females clearly demonstrated spatial aggregation during oviposition.

To assess the temporal aspect of oviposition, I determined the durations of ten complete oviposition events (Table 1). The average length of an oviposition event was 1.12 hours (N = 10, SD = 0.84, range = 0.28–3.17 h) even though oviposition could potentially occur over a five-hour window of time on a given day (0815–1335 h; Table 1). These records provide strong circumstantial evidence for temporal aggregation. In particular, the observations of seven females producing a 191-egg clutch within 45 minutes, and of three females producing a 62-egg clutch within 17 minutes (Table 1) strongly suggest that females did not add eggs to clutches at random intervals over an activity window of several hours.

To determine if eggs were being added to clutches one or more days after the initial oviposition event

TABLE 2. *Females chose to oviposit on only a subset of acceptable host plant shoots in the population.*

Date	Total shoots w/o eggs	Acceptable shoots[a]	Shoots rec'd eggs (%)
12 July	5	4	1 (25)
16 July	4	2	1 (50)
17 July	4	3	0
18 July	4	3	1 (33)
19 July	6	5	3 (60)
21 July	3	1	0
22 July	5	3	1 (33)
23 July	3	2	1 (50)
24 July	10	5	3 (60)
25 July	11	5	3 (60)
26 July	11	3	3 (100)
3 Aug.	6	4	0
4 Aug.	8	6	1 (17)
5 Aug.	8	6	3 (50)

[a] Shoots that did not bear eggs, were within the acceptable size range, and were oviposited on before the end of the study.

I tracked clutch sizes on 36 natural host plant shoots spread along the four sites. Of the 36 naturally growing clutch-bearing *P. pittieri* shoots, only 3 shoots (8.3%) were subject to secondary oviposition. The three secondary clutches were unusual because they were laid in mats on the basal leaves of mature shoots instead of shoot tips. Placement of secondary clutches was not directly witnessed and they may have represented a heliconiine species other than *H. hewitsoni*, in which case the secondary oviposition rate would have been less than 8.3 percent. Species identification of the secondary clutches was not verified because larvae from the earlier clutches completely defoliated the shoots and destroyed the secondary clutches.

One possible explanation for aggregation behavior is that the population density of females is greater than the available host plant resource, and females aggregate and oviposit in groups due to a paucity of suitable host plant shoots. This scenario would predict a negative correlation between the number of host plant shoots and oviposition group size or collective clutch size. To assess this, I determined the daily supply of acceptable shoots to compare to oviposition group size and collective clutch size. In this case, "acceptable shoots" refers to shoots that were not oviposited on during an observation day, were of appropriate size for detection and oviposition (inferred from the observed range of shoot lengths at oviposition in Table 1), were located at sites that were visited and utilized by the observed females, and were eventually used for oviposition during the study period. This definition was an attempt to preclude counting shoots that were unacceptable for oviposition. The number of available acceptable shoots had no correlation with oviposition group size ($P > 0.10$, $N = 10$, $r = -0.17$) or collective clutch size ($P > 0.10$, $N = 10$, $r = -0.38$). Therefore, there was no evidence that aggregation was associated with resource availability.

For 14 days, an average of 38.4 percent of acceptable shoots at the four field sites received eggs each day (Table 2) despite consistent daily inspection by females. There were 3 days when no shoots received eggs. The daily supply of shoots was rarely saturated and females almost always had a choice to oviposit singly or gregariously. For 10 of the 12 observed oviposition events in Table 1, females contributed to collective eggs clutches even though they had the choice to oviposit individually on unused shoots.

Heliconius hewitsoni collective clutch sizes varied from shoot to shoot both within and between individual host plants. Previous studies have demonstrated that females may adjust clutch sizes with respect to leaf bud size (Pilson & Rausher 1988), leaf area (Pilson & Rausher 1988, Kagata & Ohgushi 2001), and number of leaves (Pilson & Rausher 1988, Vasconcellos-Neto & Monteiro 1993, Tsukabi 1995). It was not possible to analyze these metrics here because *H. hewitsoni* oviposition occurred when *P. pittieri* shoots were small buds. Therefore, I used mean shoot growth rate and shoot length at oviposition to assess shoot quality relative to clutch size. Natural *P. pittieri* shoot lengths were measured daily to calculate mean growth rates for individual shoots. For each shoot, at least four daily measurements

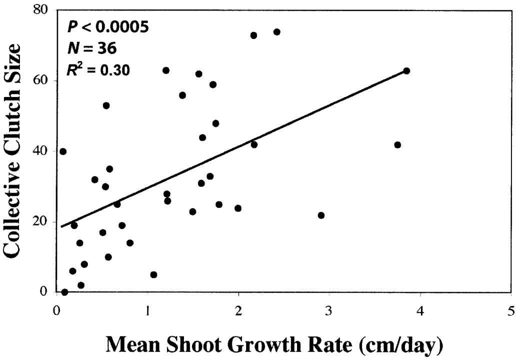

FIGURE 1. Collective clutch size was correlated with the mean growth rate of individual host plants shoots.

were used to calculate a least squares regression, the slope of which provided the mean shoot growth rate. No measurements were used from shoots being fed on by *H. hewitsoni* larvae. Growth trajectories were linear for *P. pittieri* shoots, with R^2 values averaging 0.93 ($N = 36$, SD = 0.052, range = 0.81–0.99) and the correlation coefficient between shoot length and time averaging 0.96 ($N = 36$, SD = 0.027, range = 0.90–0.99). Collective clutch size was not correlated with shoot length at oviposition ($P > 0.10$, $N = 22$, $r = 0.15$); however, collective clutch size and mean growth rate of individual shoots was correlated ($P < 0.0005$, $N = 36$, $r = 0.55$; Fig. 1), as was collective clutch size and shoot length at hatching ($P < 0.05$, $N = 24$, $r = 0.42$).

To determine if eggs themselves positively biased host growth, I compared mean growth rates between egg-bearing shoots and experimental egg-free shoots. I applied net bags to 13 natural *P. pittieri* shoots that were interspersed among plants also containing unbagged, egg-bearing shoots. This scheme permitted control of variables related to shoot microenvironment and interplant variation. The net bags prevented butterfly oviposition but did not exclude ants, flea beetles, or other insects associated with *P. pittieri.* The mean growth rate of net-covered egg-free shoots was 1.5 cm/day ($N = 13$, SD = 0.83, range = 0.41–2.96 cm/day), which was slightly greater than the mean growth rate of natural egg-bearing shoots at 1.3 cm/day ($N = 36$, SD = 0.96, Range = 0.06–3.74 cm/day). Therefore, there was no evidence that the presence of *H. hewitsoni* eggs positively biased shoot growth rate.

There are two potential mechanisms for clutch size adjustment: individual females may vary their contributions to collective clutches, and/or the number of females participating in an oviposition event may vary. There was no significant correlation between clutch size and the number of eggs contributed to a clutch by individual females ($P > 0.10$, $N = 21$, $r = 0.051$). There was, however, a significant correlation between collective clutch size and the number of contributing females ($P < 0.005$, $N = 10$,

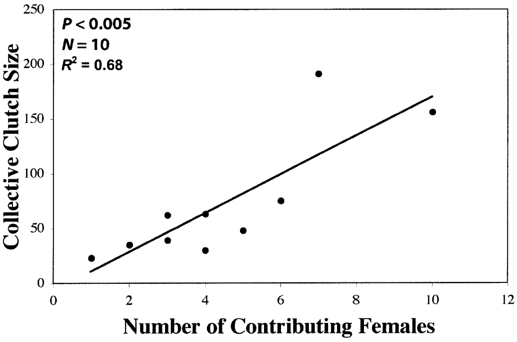

FIGURE 2. Collective clutch size was correlated with the number of egg-contributing females.

$r = 0.83$; Fig. 2). Together, these data suggest that clutch size adjustment in *H. hewitsoni* occurs through variation in the number of females that choose to participate in an oviposition event.

The observations here illustrate that *H. hewitsoni* females aggregate to produce collective eggs clutches independent of resource availability, and suggest that gregarious oviposition in this species may be a mechanism for clutch size adjustment. It is reasonable to speculate that this behavior is a mechanism for assessing shoot quality to facilitate larval "selfish herds" while minimizing intraspecific competition. Despite the fact that gregarious oviposition has been observed in a variety of insects (Browne *et al.* 1969, New 1985, Martens 2000), the ecological and evolutionary implications of this unusual behavior remain poorly understood and deserve further investigation.

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