

Consequences of caterpillar-egg cannibalism on the ontogeny of *Heliconius erato phyllis* (Fabricius, 1775) (Lepidoptera, Nymphalidae)

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Resumo

Consequências do canibalismo lagarta-ovo na ontogenia de *Heliconius erato phyllis* (Fabricius, 1775) (Lepidoptera, Nymphalidae). Lagartas recém-eclodidas de *Heliconius erato phyllis* (Fabricius, 1775) podem ser canibais ou não-canibais; além disso, elas são capazes de reconhecer parentes, preferencialmente canibalizando ovos não relacionados. Este estudo analisou diferenças no desenvolvimento e sobrevivência até a maturidade de lagartas canibais e não-canibais desta borboleta, como estimativas dos custos e benefícios do comportamento canibal e não-canibal. As variáveis analisadas foram peso e volume do ovo, número de dias desde a eclosão até a idade adulta, taxa de crescimento, peso pupal, área da asa, mortalidade de imaturos e sobrevivência de imaturos sob regime de fome. Houve diferenças significativas para o volume e peso do ovo (canibal > não-canibal) e para a duração do primeiro ínstar e pupa (canibal < não-canibal). A mortalidade entre canibais e não-canibais não foi diferente, mas a sobrevivência dos canibais sob fome foi significativamente maior. Irmãos de canibais e não-canibais não diferem nas mesmas variáveis. Os não-canibais têm o benefício de deixar mais irmãos, mas sofrem o custo de um desenvolvimento um pouco mais lento. Uma breve discussão sobre os custos e benefícios do canibalismo e não-canibalismo neste estudo, bem como na literatura disponível, está incluída.

Palavras-chave: Benefícios; Custos; Desenvolvimento

Abstract

Newly hatched caterpillars from a clutch of *Heliconius erato phyllis* (Fabricius, 1775) eggs can be cannibals or non-cannibals. Further, they are able to recognize kin and preferentially cannibalize unrelated eggs. This study analyzed differences in the development and survival to maturity of cannibal and non-cannibal caterpillars of this butterfly, as estimates of the costs and benefits of cannibalistic and non-cannibalistic behavior. The variables analyzed were egg weight and volume, number of days from hatching to adulthood, growth rate, pupal weight,



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wing area, mortality of immatures, and survival of immatures under starvation. There were significant differences for egg volume and weight (cannibal > non-cannibal) and for duration of the first instar and pupa (cannibal < non-cannibal). Mortality among cannibals and non-cannibals was not different, but survival of cannibals under starvation was significantly greater. Siblings of cannibals and non-cannibals did not differ for the same variables. Non-cannibals have the benefit of leaving more siblings but suffer the cost of slightly slower development. A brief discussion of the costs and benefits of cannibalism and non-cannibalism in this study, as well as in the available literature, is included.

Key words: Benefits; Costs; Development

Introduction

Cannibalism is a very common behavior in several species and consists of killing and eating all or part of a conspecific (FOX, 1975; ELGAR; CRESPI, 1992). Cannibalism may confer direct benefits for fitness by increasing survival and/or fecundity and decreasing total development time (FOX, 1975; DUELLI, 1981; JOYNER; GOULD, 1985; VIA, 1999; ZAGO-BRAGA; ZUCOLOTO, 2004; BAYOUMY; MICHAUD, 2015). Other benefits include the removal of potential competitors (FOX, 1975; POLIS, 1981; KAKIMOTO et al., 2003), decreasing the probability of attracting predators (CHAPMAN et al., 2000) and the colonization of new environments, which are potentially facilitated by increasing rates of cannibalism (VIA, 1999). In the case of kin recognition, another benefit of cannibalism is that a cannibal could enhance the survival and reproduction of related individuals by eliminating non-related competitors, thereby increasing its inclusive fitness (PFENNIG, 1997).

The benefits of cannibalism may be reduced by the following costs (WILLIAMS; HERNÁNDEZ, 2006): (i) cannibals may be at risk of death or injury, depending on the defensive responses of conspecifics; (ii) cannibals may sometimes reduce their inclusive fitness by consuming a relative; and (iii) cannibals could increase the risk of transmission of diseases and host-specific parasites. Although it may be true that cannibalism could be a risk factor for disease transmission in some species, there are also studies that report cases of cannibalism decreasing the prevalence or spread of parasites (VAN ALLEN et al., 2017). Moreover, in some cases, cannibalism can result in developmental costs, for instance, in *Spodoptera frugiperda* (J.E. Smith, 1797)

(Lepidoptera; Noctuidae), where cannibals exhibited a slower growth rate, lower survival rate and smaller body size compared to non-cannibals (CHAPMAN et al., 1999).

As reviewed by Richardson et al. (2010), within noncarnivorous insects, the cannibals are usually juveniles and often consume eggs. Cannibals also often consume other juveniles, which usually have a smaller body size. Cannibalism is most often attributed to density-dependent factors rather than density-independent factors. However, the probability of cannibalizing can also be influenced by factors independent of density, including abiotic environmental conditions, such as high temperature, low humidity, and precipitation. Other factors that affect the probability of cannibalism include the following: asynchrony with the host plant, poor nutritional quality of the host plant (or limited availability of host plants), availability of vulnerable conspecifics (due to disease, parasitism, injury or molting), and a genetic or phenotypic predisposition to cannibalize. When nutritional factors induce cannibalism, eggs are often the target because they typically lack defenses, are rich in lipids and proteins, and are highly digestible (BAYOUMY; MICHAUD, 2015). According to Barros-Bellanda and Zucoloto (2001), cannibalistic behavior is related to chorion ingestion, which results in increasing the performance of individuals, for instance, by reducing pupation time, among other variables.

Several studies point out that cannibal caterpillars can consume eggs, larvae and, in some cases, pupae (CHAPMAN et al., 1999; BARROS-BELLANDA; ZUCOLOTO, 2001; 2005; SIGSGAARD et al., 2002; ZAGO-BRAGA; ZUCOLOTO, 2004; NAKAHARA et al., 2020, just to cite the most recent papers).

The butterfly *Heliconius erato phyllis* (Fabricius, 1775) (Nymphalidae) is not social as an adult or immature; although, adults gregariously roost at dusk. Females inspect the host plant before oviposition and lay eggs individually. The reason for this behavior is to detect and avoid other eggs or immatures on the same plant, since caterpillars can be cannibals (GILBERT, 1975; BENSON et al., 1976; BROWN JR., 1981). Another reason could be to avoid overexploitation of limited resources, which could occur with many caterpillars on a single plant. Using an experimental approach, De Nardin and Araújo (2011) showed that first instar caterpillars are prone to recognizing sibling eggs. Further, it was shown that only first instar caterpillars can cannibalize and the source of information for kin recognition is in the egg chorion (DE NARDIN et al., 2015). Moreover, Williams and Gilbert (1981) showed experimentally that structures like stipule tips in *Passiflora cyanea* (Mast., 1872) and *P. oerstedii* (Mast., 1872), which resemble eggs, induce females of *Heliconius cydno* (H.Bates, 1864) to lay fewer eggs than when these structures are absent. They also stressed that these structures evolved specifically to mimic *Heliconius* eggs because “larvae of many *Heliconius* feed on congeneric eggs and larvae” (p. 467).

Given that kin recognition has been demonstrated, and that the frequency of cannibalism is higher in the presence of only unrelated eggs compared to only sibling eggs, the objective of this study was to evaluate whether there are development differences (for each instar, pupae, and total developmental time) between cannibal and non-cannibal caterpillars.

Materials and Methods

Butterfly stocks

Females were collected from five populations around the city of Porto Alegre, the capital of the state of Rio Grande do Sul, Brazil. Two of these populations were approximately 1 km apart and separated by university buildings and a small village surrounding the campus (places called Morro Santana and Instituto de Pesquisas Hidráulicas). The other three localities were

at least 25 km apart (Estação Experimental Agronômica de Águas Belas, Águas Claras county and Lami suburb, on the outskirts of Porto Alegre), so all females were assumed to not be related. A total of 12 females were caught and were the source of the eggs used in the cannibalism tests. Each female, independent of place of origin, produced offspring with both cannibal and non-cannibal caterpillars. Details of the procedures used to collect eggs and rear immatures are in De Nardin and Araújo (2011).

The specimens were deposited in the collection of the Laboratório de Genética Ecológica, Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul (UFRGS), and were kept in a freezer. The field notebooks and research data related to this project were deposited in the same place.

Experimental procedures

Eggs were weighed using an analytic balance (BOSCH-Wägesysteme, Typ SAE 200; accuracy of 0.0001 g). Each egg was weighed three times in succession, and the average was used in all tests. Egg volume was estimated using the formula for spheroid volume (RASHIDI et al., 2008):

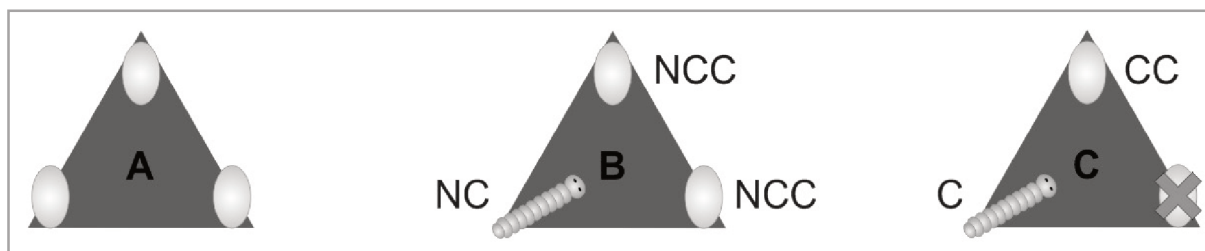
$$V_{\text{spheroid}} = \pi (LD^2)/6$$

where L is the length from the base to the apex of the egg, D is the diameter of the egg (we used the maximum diameter), and π is a constant ($\pi = 3.1416$). These measurements were made under a binocular stereoscopic microscope (Nikon, model SMZ645).

Cannibalism/non-cannibalism tests were performed according to the protocol established by De Nardin and Araújo (2011). For each test, sibling eggs were placed on the vertices of an equilateral triangle made from a green paper card with sides 0.5 cm long (Figure 1).

Only eggs from a single female were used for each test to ensure that they were full sibs (females of *Heliconius erato phyllis* are monandric – DRUMMOND III, 1984). After the first caterpillar hatched (potential cannibal or non-cannibal), each test lasted 45 minutes;

FIGURE 1: Experimental design of this study. A) Three sibling eggs were arranged on the vertices of an equilateral triangle (made from a green card, with 0.5 cm long sides). B) A non-cannibal caterpillar (NC) and its siblings (NCC = non-cannibal control). C) A cannibal caterpillar (C) and its sibling (CC = cannibal control). Egg marked with "X" means cannibalized.



cannibalism usually takes a lot less than 45 minutes, but we allowed time for the caterpillar to examine both eggs. Caterpillars were identified by a number and by the code C (cannibal) or NC (non-cannibal). Eggs that remained intact after a test were treated as controls, CC for a cannibal control or NCC for a non-cannibal control. Sometimes a cannibal caterpillar ate the two remaining eggs; in these cases, no cannibal control was available. Some of the NCC eggs died during development, leaving only one non-cannibal control.

Each caterpillar was maintained in a translucent plastic pot (8.5 cm tall x 7.5 cm diameter) with a white cap. To maintain a certain degree of moisture, a piece of wet tissue paper was placed at the bottom of the pot. All caterpillars were maintained at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and a 12h light/12h dark photophase; they were fed daily *ad libitum* with *Passiflora suberosa* (Linnaeus, 1753). The following developmental variables were recorded: egg weight and volume, duration of each instar, pupal weight, adult size (measured using the area of forewings and hindwings; for simplicity we included only the right forewing in Table 1), total growth rate (R), and mortality from hatching until adult emergence. We also recorded the longevity of first instar caterpillars under starvation.

Pupae were weighed after 24h of pupation and the growth rate (R) was calculated by the formula given in Smiley (1978):

$$R = [(\text{pupal weight}/\text{egg weight})^{1/5} - 1] / (\text{days}/5)$$

where the ratio of pupal weight to egg weight is raised to the power of 1/5 due to the number of instars. The

denominator is the number of days after egg hatching until pupation divided by 5, which again refers to the number of instars. Wing area was measured using the software ImageJ 1.37v (RASBAND, 2006).

After testing for cannibalism, some caterpillars, both cannibals and non-cannibals, were separated and isolated to test for resistance to starvation. They were put in plastic vials without leaves of the host plant to estimate how long they could survive. Whenever death occurred between 0 h and 6 a.m., the time of death was arbitrarily designated as 3 a.m.

A statistical analysis of ontogenetic variables was performed using the software SPSS v.15.0 for all parametric variables; a one-way analysis of variance (ANOVA) was performed after appropriate statistical transformations (cubic root transformation, \log_{10} transformation, and square root transformation). Our decision to use a univariate analysis of variance instead of a multivariate analysis was due to our interest in comparing each instar or stage between cannibals and non-cannibals as independent data. To test for sexual differences for cannibalism/non-cannibalism, we performed a two-way analysis of variance for the same variables as before; however, since the results were non-significant, they were not included in the paper. For some variables, such as the number of dead immatures, cannibal and non-cannibal were compared using a goodness of fit G test with Williams correction (SOKAL; ROHLF, 1995) using the software BioEstat 5.0 (AYRES et al., 2007).

The number of siblings of non-cannibals (usually two for each test) that reached adulthood was compared with survival to adulthood of the sibling of a cannibal (in these instances of cannibalism, generally one or sometimes no sibling remained). To test for the differences, a G-test with Williams correction was used. The two control groups, CC and NCC, were compared with each other for the same variables applied to cannibals and non-cannibals to determine if they have the same capacity to reach adulthood as cannibals and non-cannibals.

A question raised during the analysis above was: Is the size of the egg whose hatched caterpillar was a cannibal the same size as the cannibalized egg and remaining egg (CC)? The same question was asked about the size of a non-cannibal egg and controls (NCC). Both questions are answered in the Results. Unhatched eggs were excluded from the analysis.

Results

Eggs giving rise to cannibals were significantly larger and heavier than eggs giving rise to non-cannibals, and the resulting first instar caterpillar developed faster;

the fact that pupae from cannibal caterpillars developed faster was unexpected (Table 1).

The areas of the right and left wings were correlated (forewings: $r = 0.982$; $P < 0.01$; hindwings: $r = 0.977$, $P < 0.01$; the correlation between right forewing and hindwing, $r = 0.878$; $P < 0.01$, and between left forewing and hindwing, $r = 0.997$; $P < 0.01$); consequently, we only show the results for the right forewing.

Cannibals and non-cannibals survived to adulthood at similar frequencies (84.3 vs. 81.5%, respectively, $G = 0.176$, $P = 0.675$) and the cannibal first instars survived starvation longer than non-cannibals (44 ± 9 h vs. 39 ± 9 h, $n = 40$ in both cases, $F = 5.382$, d.f. = 1, $P = 0.023$). The egg weight and volume were used as predictors for survival under starvation in a regression, and we did not find significant results (egg weight: $b = 0.018$; d.f. = 36; $P = 0.914$; egg volume: $b = 0.70$; d.f. = 32; $P = 0.698$).

Non-cannibals left double the number of siblings as cannibals (68 vs. 34), which was expected due to our experimental design ($G = 1.073$, $P = 0.300$; d.f. = 1).

Siblings of cannibals (cannibal control – CC) and non-cannibals (non-cannibal control – NCC) did not differ significantly for any developmental parameters

TABLE 1: Mean \pm s.e. of developmental measurements in cannibal and non-cannibal *H. erato phyllis* caterpillars (untransformed values).

| Group | Egg Volume (mm ³) ¹ | Duration of each instar (days) ² | | | | | | Total ² | Growth Rate (R) | Pupal Weight (g) ¹ | Right Forewing Area (mm ²) ³ |
|--------------|--|---|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------|-------------------------------|---|
| | | L1 | L2 | L3 | L4 | L5 | Pupa | | | | |
| Cannibal | 0.630 \pm 0.009 | 3.351 \pm 0.145 | 2.054 \pm 0.082 | 2.193 \pm 0.088 | 2.649 \pm 0.081 | 4.086 \pm 0.178 | 8.559 \pm 0.153 | 22.864 \pm 0.379 | 0.940 \pm 0.020 | 0.393 \pm 0.008 | 461.996 \pm 8.973 |
| n | 59 | 57 | 56 | 57 | 57 | 58 | 59 | 59 | 58 | 59 | 42 |
| Non-Cannibal | 0.594 \pm 0.010 | 3.807 \pm 0.148 | 2.196 \pm 0.093 | 2.367 \pm 0.139 | 2.913 \pm 0.116 | 4.184 \pm 0.133 | 9.321 \pm 0.215 | 24.698 \pm 0.433 | 0.888 \pm 0.018 | 0.387 \pm 0.006 | 447.534 \pm 10.521 |
| n | 49 | 52 | 51 | 49 | 46 | 49 | 53 | 53 | 45 | 53 | 25 |
| F | 7.486 | 6.778 | 1.091 | 0.449 | 2.493 | 0.717 | 8.986 | 10.224 | 3.379 | 0.147 | 0.347 |
| P | 0.007 | 0.011 | 0.299 | 0.504 | 0.117 | 0.399 | 0.003 | 0.002 | 0.069 | 0.702 | 0.558 |
| df | 107 | 108 | 106 | 105 | 102 | 106 | 111 | 111 | 102 | 111 | 66 |

Transformed variables in statistical analyses: ¹cubic root; ²logarithmic base 10; ³square root.

and they survived to adulthood with equal probability ($G = 0.490$, $P = 0.484$; d.f. = 1) (Appendix 1).

Additional results

There is a correlation between weight and volume of the egg for the cannibal ($r = 0.686$; $P = 0.01$; $N = 58$), non-cannibal ($r = 0.499$; $P = 0.01$; $N = 45$), non-cannibal control ($r = 0.459$; $P = 0.01$; $N = 57$) and cannibal control ($r = 0.670$; $P = 0.01$; $N = 39$) groups. Due to this, we used only the volume in Table 1.

Eggs that resulted in cannibal caterpillars were no different from the cannibalized eggs in weight ($F = 0.063$; $P = 0.802$; d.f. = 1) and volume ($F = 0.838$; $P = 0.362$; d.f. = 1). The eggs of cannibals were no different in weight ($F = 0.002$; $P = 0.963$; d.f. = 1) and volume ($F = 0.108$; $P = 0.743$; d.f. = 1) from the remaining viable eggs (CC). Moreover, eggs that resulted in non-cannibal caterpillars were also no different in weight from the remaining eggs (NCC) ($F = 2.744$; $P = 0.101$; d.f. = 1), but their volume ($F = 7.814$; $P = 0.006$; d.f. = 1) was different; the eggs of the NCC group were larger in volume than those of the NC group. We have no explanation for this fact.

For cannibal, non-cannibal, cannibal control and non-cannibal control caterpillars that developed into adults, no differences in sex ratio were observed (G test with Williams correction = 1.3344, $P = 0.7210$; d.f. = 3). The sex ratio was 1:1 for all groups.

We regressed “duration of L1” on “egg volume” (both transformed) for cannibal and non-cannibal caterpillars; no significant difference was found (Cannibals: $F = 0.012$; $P = 0.914$; d.f. = 1; Non-cannibals: $F = 0.153$; $P = 0.698$; d.f. = 1).

Discussion

In nature, the opportunity for caterpillar-egg cannibalism in *H. erato phyllis* results from the fact that females often lay more than one egg close to each other when host plants are limited (DE NARDIN; ARAÚJO, personal field observations). This study showed that cannibal caterpillars have some differences in developmental variables compared to non-cannibals.

Cannibal and non-cannibal caterpillars differ in the following variables: egg volume and weight, duration of first instar and pupa phases (days), and length of development from hatching until adult emergence. The differences between cannibals and non-cannibals for the duration of the first instar are expected, since the former gain an extra meal. These differences persist in the following instars, although they are not significant. This pattern is maintained during the pupal stage, which is faster in cannibals, and in the total length of development from hatching to adulthood. The growth rate (R), a composite variable, does not seem to be affected, although the probability of accepting the null hypothesis was close to rejection. In short, cannibals develop faster than non-cannibals; from this perspective, development time is a benefit for cannibals. However, no significant differences were observed for the respective controls, even when mortality during development was estimated.

Based on the results presented here, it can be concluded that the costs for the non-cannibals occur at the beginning of development: non-cannibal caterpillars molt to the 2nd instar slower than cannibals. Similar results were obtained by Osawa (2002) and Michaud and Grant (2004) for Coleoptera. However, Tarpley et al. (1993) did not find differences when studying the development of *Diatraea grandiosella* (Dyar, 1911) (Lepidoptera); there may be an association between cannibalism and weight gain, but only smaller larvae had the propensity to be cannibals.

Eickwort (1973) also noted a reduction in time to reach the second instar in cannibals of the herbivore beetle *Labidomera clivicollis* (Kirby, 1837). Bayoumy and Michaud (2015) studied the consequences of egg cannibalism on life history variables of *Hippodamia convergens* (Guerin-Meneville, 1842) (Coleoptera: Coccinellidae) and, for neonates, cannibalism of eggs accelerated development. First instar larvae that cannibalized eggs spent more time in this instar than controls, which did not cannibalize eggs, but less time in the fourth instar. This resulted in overall faster development for the cannibals and cannibalism increased male body size. Moreover, the authors concluded that “egg cannibalism in holometabolous insects varies

with life stage, sex, and mating status, independent of extrinsic factors, such as food availability.”

Gomi et al. (2015) studied the red poplar leaf beetle, *Chrysomela populi* (Linnaeus, 1758) (Coleoptera: Chrysomelidae), and found that sibling egg cannibalism (immediately after hatching) increased the body weight of adults and the starvation tolerance of hatchlings (between 48 and 60 h after hatching) but had little effect on development time and survival from hatching to adult emergence, as well as survival of hatchlings under starvation (at 36 h after hatching). Collie et al. (2013) showed that cannibalism of a single egg by first instar larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say, 1824) (Chrysomelidae), increased larval growth rates and decreased the average time to reach the second instar (and following life stages) by one day or more.

For our results, the duration of the pupal period was also significantly shorter in cannibals. Therefore, butterflies from cannibal caterpillars emerge before non-cannibal ones; given that pupal mating occurs in *H. erato phyllis*, this could be an advantage in terms of reproduction. Under starvation, the cannibal caterpillars also survive for longer; this could be another benefit for them when food is scarce. In this context, it is worth mentioning the paper by Rodrigues and Moreira (2004), who showed that in natural conditions the seasonal variation of *Passiflora suberosa* is less intense than that of *P. misera* (19ha of an old eucalyptus plantation in the Barba Negra Forest, Barra do Ribeiro County, Rio Grande do Sul - Fig. 1b in the paper). For *P. suberosa*, the variation ranges from about 70% in April of one year to 40% in April of the next year, returning to 70% in September. For *P. misera*, this variation ranges from roughly 65% in September of one year to about 10% in May–July and April–June of the next year. In the places where we collected individuals of *H. erato phyllis* in this study, *P. suberosa* is by far the most frequent host plant used; this means that C and NC caterpillars may have no impact on their respective development, even when considering that *P. misera* has better values for “host acceptability” by ovipositing females (90% compared to 50% in *P. suberosa*) and “oviposition rate”

(2.5 for *P. misera* and 1.7 for *P. suberosa* – details in MENNA-BARRETO; ARAÚJO, 1985).

According to Michaud and Grant (2004), faster development means earlier reproductive maturity and less exposure to predation. For our results, the duration of the other instars (L2, L3, L4, L5), the rate of development, pupal weight, size of adults, and mortality during development do not suggest an increase in costs for non-cannibals. An individual that does not cannibalize may suffer the costs of renouncing a meal. However, in the long term, the non-cannibals do not appear to suffer costs, since the growth rate, weight of the pupa, and size of adults do not differ from those of cannibals. It is interesting to note that in all instars, and the pupa, the values for cannibals are always numerically smaller than for non-cannibals. Perhaps there is a general cost for non-cannibals, since they are late in all stages of development (a complement to these observations is in the next paragraph, particularly in relation to the paper by EICKWORT, 1973). Actually, since non-cannibal caterpillars have the potential to leave twice the number of siblings that reach reproductive age, and because siblings of both cannibals and non-cannibals develop the same for all variables, this suggests a true benefit for non-cannibals. However, we must consider the limitations of our experimental design.

Although it was observed that non-cannibals potentially leave twice the number of siblings, cannibalism frequently occurs in this group (the frequency of cannibalism is around 53% for sibling eggs and 83% for non-related eggs, - DE NARDIN; ARAÚJO, 2011). Although no advantage was observed for cannibals for several variables, the nutritional benefit of cannibalism does not have to be great for this behavior to evolve; in a species where generations overlap, even a small reduction in the development time of the cannibal may provide a selective advantage that is sufficient to cause the dissemination of cannibalism, even of full siblings (EICKWORT, 1973; VIA, 1999). Other studies that speculate on the evolution of cannibalism are Stevens (1989), Elgar and Crespi (1992), and Giray et al. (2001). In general, the literature on cannibalistic behavior does not highlight the selective advantages but concentrates on ecological phenomena that presumably

act to reduce cannibalism, such as habitat separation of different instars or asynchronous emergence and dispersion of siblings (BUSKIRK et al., 1984). According to Collie et al. (2013), even though reports of cannibalism benefits are common, attempts to quantify the fitness effects of these benefits are scarce. These authors used a simulation method, in the context of the kin selection theory, to estimate resultant fitness benefits of egg cannibalism by first instar larvae of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) under field conditions. Another study, involving cannibalism and selective advantages, was reported by Dobler and Kölliker (2010). These authors studied the European earwig to estimate benefits and costs of cannibal first instar nymphs. As reported by Barros-Bellanda and Zucoloto (2005), cannibalism may be due to, for instance, the low nutritional content of plant tissues and/or the influence of competition. According to Zucoloto (1993), the adaptation of individuals of the fly *Ceratitis capitata* (Wiedemann, 1824) (Tephritidae) to a diet based on animal protein probably occurred due to cannibalism during periods of competition for food. In *Atrophaneura alcinous* (Klug, 1836) (Lepidoptera: Papilionidae), egg, larva and pupa cannibalism, together with extra molting and pupal diapause, are correlated strategies that evolved during unfavorable conditions (NAKAHARA et al., 2020). Another recently published study showed that egg cannibalism in the beetle *Menochilus sexmaculatus* (Fabricius, 1781) (Coleoptera: Coccinellidae) may have effects on mate preference and reproductive output, as well as offspring development (TRIPTI YADAV; MISHR, 2021). Another interesting result of this study was that adults who were non-cannibals as larvae showed preferential mating towards non-cannibal adults; the same behavior was not present in adults from cannibal larvae. It was also found that non-cannibalistic mating pairs showed a shorter time to start mating, longer copulation duration, and greater fertility when one or both were non-cannibalistic. For other variables, such as development time and egg viability, no differences were observed.

Egg cannibalism can be an advantage for larval survival, due to the acquisition of nutrients (BARROS-BELLANDA; ZUCOLOTO, 2005). Sometimes, survival of the first instar and its foraging success may be related

to cannibalism (MICHAUD; GRANT, 2004), since this period is often critical and limiting for the larva. Survival, when there is a lack of food that might lead to the death of the caterpillar, is often cited as one of the reasons for the evolution of cannibalism (CUSHING, 1991; CLAESSEN et al., 2004). Another possibility for the advantage of cannibalism is found in the paper by Ramos et al. (2012), who studied host preference by females of *H. erato phyllis* and its correlation with larval performance under natural conditions and in an insectary. In a city park in Morro Voturuá, São Vicente (a coastal region of the state of São Paulo), *Passiflora capsularis* received about 82% of the eggs and *P. jilekii* received only 13% during the period January – June, when host plant leaves showed a higher quality for caterpillar development (another two species of *Passiflora* were also sampled, *P. alata* and *P. edulis*, with small percentages of use, 4% or less). A relevant fact related to our results is that in the period July – December, when the quality of the host plants is low, according to the authors, the use of *P. capsularis* decreased almost 10%, while that for *P. jilekii* increased more than 11% (data in Table 1). Despite this difference in female preference, their results showed that the head capsule width of fourth instar caterpillars reared on *P. capsularis* leaves was significantly greater than that of fourth instars reared on *P. jilekii* leaves; a similar effect was detected with wing length of the adults. This suggests that under similar conditions in the field, namely when there are host plants of low nutritional quality, a cannibal caterpillar could have an advantage compared to a non-cannibal one.

Moving the emphasis to non-cannibals, Lizé et al. (2006) suggested that the evolution of kin recognition in the solitary species *Aleochara bilineata* (Gyllenhal, 1810) (Coleoptera) may have been triggered by competition due to the limited dispersal capacity of its larvae.

In the present study, it was observed that cannibal caterpillars develop faster than non-cannibals in the first instar (see data in column L1 of Table 1). This could be due to the nutritional contribution supplied by the egg consumed. However, the duration of the first instar of the two control groups, which did not cannibalize, is very similar to that of the cannibals. Thus, the doubt

remains as to whether the slower development of the non-cannibals is a consequence of the fact that they did not consume an egg or a consequence of the fact that the eggs from which they hatched are smaller, which might result in smaller caterpillars that could take longer to molt to the second instar. In this study it was not possible to find the causes of this difference.

We also compared cannibal caterpillar eggs to the cannibalized eggs. It was observed that they are equal in weight and volume, indicating that cannibalism did not occur due to some deficiency in the cannibalized eggs, but probably for nutritional gain (or to eliminate potential competitors), which may be inferred from the comparison with the remaining eggs of the cannibalism tests. Another interesting observation is that the non-cannibal caterpillar eggs have a smaller volume than the remaining eggs; currently, we have no satisfactory explanation for this. However, we have preliminary experimental data suggesting that when females are exposed to an environment with fewer host plants, compared to another with plenty of host plants, they tend to lay bigger eggs that are mainly cannibals.

Both cannibals and non-cannibals persist in the population. Non-cannibals potentially leave more siblings (at least in our experimental design, although we expect that in nature any excess of non-cannibal siblings would be advantageous) and do not seem to have development costs after pupa formation. In contrast, cannibals appear to have short-term benefits, and increased starvation survival, which may be very important in situations where there is competition and little food available. As has been noted, the frequency of cannibalism is greater when unrelated eggs are nearby compared to sibling eggs, which indicates that newly hatched caterpillars can recognize related eggs (DE NARDIN; ARAÚJO, 2011). We believe that non-cannibalistic caterpillars of sibling eggs are altruistic and cannibalistic caterpillars of sibling eggs are selfish. After all, non-cannibalistic caterpillars lose a meal, which we have shown is important for their development, thereby implying costs to the altruist. Finally, we are aware that our analysis is limited to the consequences of sibling cannibalism and few generations (in this work, only one generation was studied). A more detailed comprehension

of this subject would require examining non-sibling cannibalism and the possibility of costs and benefits changing over generations.

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Appendix 1

TABLE A1: Mean \pm s.e. of developmental variables for cannibal and non-cannibal controls of *H. erato phyllis* caterpillars (untransformed values).

| Group | Egg Volume (mm ³) ¹ | Duration (days) ² | | | | | | Total ² | Growth Rate (R) | Pupal Weight (g) ¹ | Right Forewing Area (mm ²) ³ |
|----------------------|--|------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|--------------------------|-------------------------------|---|
| | | L1 | L2 | L3 | L4 | L5 | Pupa | | | | |
| Cannibal Control | 0.626 \pm 0.009 | 3.342 \pm 0.170 | 2.210 \pm 0.142 | 2.184 \pm 0.099 | 2.763 \pm 0.122 | 4.205 \pm 0.157 | 8.487 \pm 0.190 | 23.231 \pm 0.415 | 0.9000 \pm 0.020 | 0.388 \pm 0.009 | 461.861 \pm 8.870 |
| n | 39 | 38 | 38 | 38 | 38 | 39 | 39 | 39 | 39 | 39 | 34 |
| Non-Cannibal Control | 0.626 \pm 0.007 | 3.344 \pm 0.136 | 2.310 \pm 0.126 | 2.316 \pm 0.104 | 2.627 \pm 0.121 | 4.435 \pm 0.133 | 8.809 \pm 0.150 | 23.778 \pm 0.335 | 0.899 \pm 0.017 | 0.398 \pm 0.007 | 468.271 \pm 6.970 |
| n | 59 | 61 | 58 | 57 | 59 | 62 | 63 | 63 | 59 | 63 | 58 |
| P | 0.978 | 0.929 | 0.691 | 0.581 | 0.305 | 0.245 | 0.164 | 0.300 | 0.959 | 0.429 | 0.591 |
| F | 0.001 | 0.008 | 0.159 | 0.306 | 1.064 | 1.366 | 1.965 | 1.084 | 0.003 | 0.630 | 0.291 |
| df | 97 | 98 | 95 | 94 | 96 | 100 | 101 | 101 | 97 | 101 | 91 |

Transformations used in some variables: ¹cubic root; ²logarithmic base 10; ³square root.