

Density dependent variation in the life history traits of mottled emigrant butterfly, *Catopsilia pyranthe* (Linnaeus, 1758) (Lepidoptera: Pieridae)

Зависимая от плотности изменчивость признаков жизненного цикла пестрой эмигрантской бабочки *Catopsilia pyranthe* (Linnaeus, 1758) (Lepidoptera: Pieridae)

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КЛЮЧЕВЫЕ СЛОВА: внутривидовая конкуренция, время развития личинок, признаки жизненного цикла, выживаемость.

SUMMARY. An assessment of the density dependent effect on selected life history traits of the mottled emigrant butterfly, *Catopsilia pyranthe* (Linnaeus, 1758) (Lepidoptera: Pieridae) was accomplished in the laboratory. The rearing of the instar I larvae to adult was split into four density levels of 1, 2, 3 and 4 larvae per rearing containers with the fixed amount of *Cassia sophera* L leaves daily. The effects of density and sex were prominent in different life history traits. The age at pupation was delayed and the pupal weight, adult weight, the wing length and the wing breadth revealed a declining trend with reference to the density and besides, females were larger than the males in terms of size. The negative values indicated density impact on life history traits. However, further studies are required to substantiate the interactions between food and density on the life history traits to establish the interactions between food and density in shaping the body size of the butterfly *C. pyranthe*.

РЕЗЮМЕ. В лаборатории изучали влияние плотности на отдельные признаки жизненного цикла *Catopsilia pyranthe* (Linnaeus, 1758) (Lepidoptera: Pieridae). Выращивание личинок от I возраста до имаго было проведено при четырёх уровнях плотности: 1, 2, 3 и 4 личинки на контейнер с фиксиро-

ванным количеством листьев *Cassia sophera* L ежедневно. Увеличение плотности сопровождалось замедлением развития, вес куколки и взрослой особи, длина и ширина крыла имели тенденцию к снижению, кроме того, самки были крупнее самцов по размеру. Необходимы дальнейшие исследования, чтобы обосновать взаимосвязь между пищей и плотностью на признаках жизненного цикла, и установить влияние пищи и плотности на размера тела бабочки *C. pyranthe*.

Introduction

Life-history evolution theory is concerned with body size, duration of growth, rate of growth, survival and timing of adult emergence that maximizes reproductive success for life [Roff, 1992; Stearns, 1989, 1992; Abrams et al., 1996]. A large adult size can have tremendous reproductive potential and can be accomplished by faster growth or a prolonged period of growth [Gotthard, 2008]. Other aspects such as competition and the natural enemies can affect the supply of food [Applebaum, Heifetz, 1999; Gibbs et al., 2004; Gibbs, Breuker, 2005; Viswanathan et al., 2005] and ultimately affect larval development. Intraspecific competition has been shown

to effect insect growth, owing to differences in density altering the amount of food available to larvae [Putman, 1977; Applebaum, Heifetz, 1999]. Earlier studies suggested that the intraspecific competition was vigorous than interspecific competition in the insect community [Stiling, 1980]. It adversely affects the life history traits of insects [Applebaum, Heifetz, 1999] and causes longer larval developmental time, lower pupal weight, etc. [Gibbs et al. 2004]. The effects of larval feeding are reflected by the different traits of adult life history, such as wing length, ovaries, etc. [Boggs, Freeman, 2005]. Because of competition, for example, the larval growth cycle prolongs in *Aedes polynesiensis* (Diptera: Culicidae) and *Epirrita autumnata* (Lepidoptera: Geometridae) [Mercer, 1999; Tammaru et al., 2000] and shortens in *Bicyclus anynana* (Lepidoptera: Nymphalidae) [Bauerfeind et al., 2005], and larvae can affect the amount of food available by a variety of means, including social stimulation of feeding [Stamp, 1980; Nahrung et al., 2001], increased induced response from host plants [Hanson, 1983; Bernays, Chapman, 1994], or direct interaction with larvae involved in violent encounters [Gibbs et al., 2004].

The order Lepidoptera relies heavily on plants for sustenance, and the larval stages are crucial to the food chain. However, caterpillars begin their search for food as soon as they emerge from eggs whereas larvae move around only a small amount. The egg laying females play a significant role in reducing the expense of caterpillar for searching for the food. Most of the time, female lay egg on specific host plants so that the minute 0 day old larvae can get food quickly. Besides these, females often seek to sense the overall egg density or larval density on that particular individual plant before laying eggs to minimize competition [Nufio, Papaj, 2001] or food scarcity [Damman, 1991; Reisenman et al., 2013]. As a result, overcrowding of eggs has the potential to reduce larval survival [Masumoto et al., 1993]. Several factors like size, number, and distribution of larval food supplies, all influence larval density [Dethier, 1959]. The effect of competition varies differently in different species. Since the adult characteristics depend on the acquisition of larval resource, it is assumed that the adult morphology depends on the larval diets [Boggs, Freeman, 2005].

In keeping with this view, the present study was designed to measure the effect of intra-specific density-dependent competition on the larvae and the life-history trait as well as sex differences in the response of adult butterflies, using mottled emigrant, *Catopsilia pyranthe* as the model species.

C. pyranthe is a medium-sized butterfly very common in natural habitats where it gets its favorite Cassias plants. It is normally chalky white to greenish in colour and its underwing closely mottled with thin brown or green lines [Kehimkar, 2008]. The distinct red-ringed silver spots may or may not present in the middle of the underside of the hind wing [Kehimkar, 2008]. During this pilot study, it was necessary to sacrifice a large number of eggs. *C. pyranthe* is a very common butterfly in Kolkata

and its surroundings [Mukherjee et al., 2015] and its selected host plant is (*C. sophera*) easily available in Kolkata and its surroundings area. The particular butterfly and its particular host plant were selected for their easy availability which made this experiment successful.

Material and methods

Collection of eggs

During this present study, a total of twenty gravid females were collected from Jaguli, W.B. (district Nadia), India (22°92'75.99"N, 88°55'04.89"E), Kalyani, W.B., (district Nadia), India (22°97'51"N, 88°43'45"E) and were brought back to the laboratory with proper care and minimum trouble. In the laboratory, the collected females were transferred in a single transparent plastic cage (1x1x1 m; Perspex sheet) for oviposition at a stable temperature (28.38°C ± 0.68) and relative humidity (84.68% ± 2.15). The females were placed with *C. sophera* plants, which served as an oviposition site, and larval food resource in this Perspex chamber and fresh flowering plants and sugar solution for food for females. The females laid egg singly on leaf of the host plant. The leaf with freshly laid eggs was collected and put in Petri dishes (10 cm x 1.5 cm depth; glass, Borosil®) with moist cotton to prevent the leaf from drying and provide sufficient humidity conditions [Rao et al., 2016]. Before inserting the eggs in the Petri plates, the microbial was washed both inside and outside the platter walls with detergent water and additionally with absolute alcohol.

Experimental design

The experiment was conducted from February 2016 to June 2016 in laboratory. The 0-day old larvae were split into four different treatments and the experiment was carried out with thirty replicates per treatment. The treatments were divided at the density of one, two, three and four larvae per Petri dish and an equal amount of food was given (32 mg *C. sophera* fresh leaves per day, [before starting this experiment, it was measured that 32 mg food / day was *ad libitum* food for a single larva]) in each treatment. Larvae were observed at 24-hour intervals and frass and residual food was removed and fresh food was given daily. The larvae that survived from each treatment gradually transformed in to pupae and after emergence, each adult individual was transferred into a small container mentioning its treatment (Tarson® specimen container). The lid of the containers was perforated to breathe to adults and no food was provided to the adult butterflies and in this situation adults live by metabolizing the reserve food materials obtained during larval life [Briegel, 1990]. Details of the butterfly parameters considered in the analysis have been collected as individual replicates. The traits such as age at pupation (AP, in days), pupal weight (PW, in mg), adult weight (AW, in mg), adult length (AL, in mm), forewing length (FWL, in mm), forewing breadth (FWB, in mm), hindwing length (HWL, in mm), and hindwing breadth (HWB, in mm) of

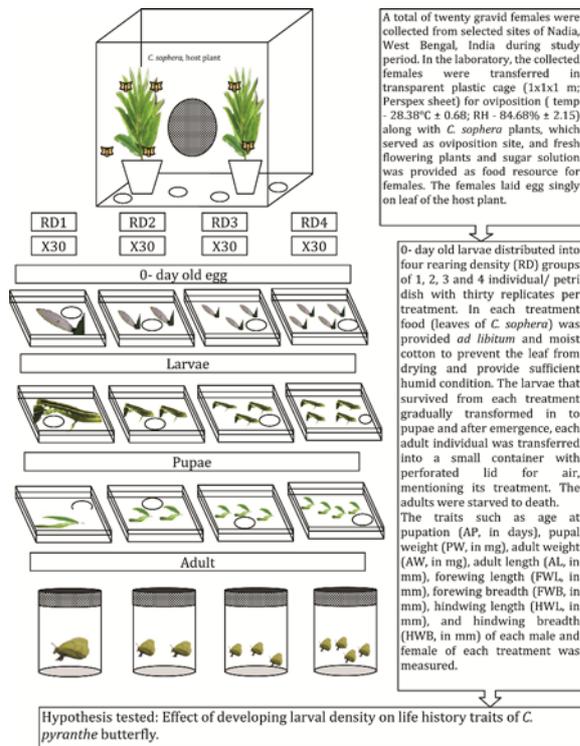


Fig. 1. The outline of the experimental design followed for the evaluation of the density dependent effects on the life history traits of the butterfly *C. pyranthe*.

Рис. 1. План эксперимента, использованный для оценки влияния плотности на особенности жизненного цикла бабочки *C. pyranthe*.

each male and female of each treatment was measured to know the effect of density and competition for food during larval development. Adult traits were assessed after the natural death of adults. The experimental design was outlined in Fig. 1.

Data analysis

The data of any larva that failed to attain to the pupal stage was expelled from data analysis. The study was carried out on the basis of age at pupation (AP, in days), pupal weight (PW, in mg), adult weight (AW, in mg), adult length (AL, in mm), forewing length (FWL, in mm), forewing breadth (FWB, in mm), hindwing length (HWL, in mm), and hindwing breadth (HWB, in mm) of each male and female butterfly individual. In order to comment on the variability with respect to the different parameters, data on larval density (density 2, 3, 4) was subjected to 2-way analysis (ANOVA) with density (1, 2, 3, 4 individuals/ tray) and sex as explanatory variables for the variations in the eight life history traits. The values of life-history features of butterfly developed from the treatments (2, 3, 4 larvae per rearing petri dish) were compared with the value of life-history features of butterfly developed from single larva treatment considered as a control set to measure the density impact [Sharmila Bharathi *et al.*, 2004; Banerjee *et al.*, 2017] using following formula:

$$\text{Density Impact (DI)} = \frac{(A-B)}{[(A+B)/2]}$$

where A = value of any trait of an individual reared under particular density (i.e 2/3/4 larvae per petridish) and B = value of any trait of an individual reared under density one (i.e 1 larva per petridish). Following Zar [1999] the statistical analysis was conducted using the XL STAT program [Addinsoft, 2009].

Results

In the present study, it was found that there was an effect of density dependent competition on life history traits as well as the proportion of survivorship of butterfly *C. pyranthe*. Besides this, sex specific differences in the life history traits were also observed. The proportion of survivorship was also affected by rearing density ($\chi^2 = 7.815$, d.f. = 3, $P < 0.001$) and it was highest in density one followed by density two, density three, density four (Fig. 2).

The mortality rate was the highest at the larval

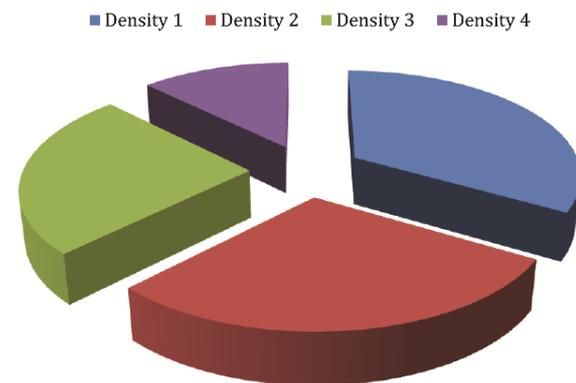


Fig. 2. The proportion of survivorship of *C. pyranthe* in different initial larval rearing density (ranging from 1 through 4).

Рис. 2. Доля выживших *C. pyranthe* при разной начальной плотности выращивания личинок (от 1 до 4).

condition and for this, the sex specific difference of survivorship was not measured in this present study. The age at pupation was lengthened and the pupal weight, adult weight, the wing length and the wing breadth exhibited a declining trend concerning the density. The mean value of age of pupation of a female was (8.81 ± 0.18 days) higher than age of pupation of male (8.25 ± 0.25 days) at density one. Similarly, the mean value of pupal weight (569.93 ± 22.41 mg), adult weight (55.36 ± 2.01 mg), adult length (20.06 ± 0.26 mm), forewing length (30.75 ± 0.23 mm), forewing breadth (19.43 ± 0.25 mm), hindwing length (25.56 ± 0.34 mm), hindwing breadth (23 ± 0.27 mm) of a female was higher than the mean value of pupal weight (406.01 ± 17.06 mg), adult weight (40.5 ± 1.70 mg), adult length (19.12 ± 0.39 mm), forewing length (29.5 ± 0.46 mm), forewing breadth (18.37 ± 0.46 mm), hindwing length (24.12 ± 0.58 mm), hindwing breadth (21.5 ± 0.46 mm) of a male at density one respectively. Likewise, in other treatments, the age of pupation of females was longer than males, and females were larger in terms of pupal weight, adult weight, wing length, and wing breadth

than those males (Fig. 3). The results of the 2-way ANOVA reflected that the life history traits varied significantly with the sampling density and sex (Table 1). The variations (Mean \pm SE) of density impact (DI) on different life history traits under intraspecific competitions were showed in Fig. 4.

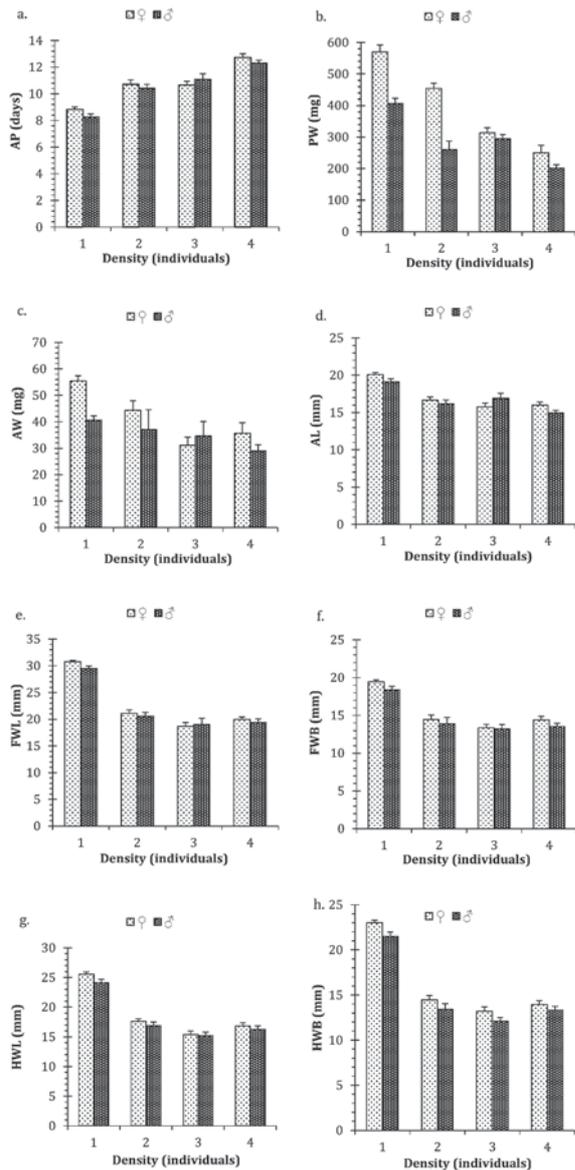


Fig. 3. The differences in the life history traits of *C. pyranthe* considering male and female separately under intraspecific competitions in minimalist form of density of the individuals. The life history traits considered are (a) age at pupation (AP, in days), (b) pupal weight (PW, in mg), (c) mass of adult individual (AW, in mg), (d) length of adult individual (AL, in mm), (e) forewing length (FWL, in mm), (f) forewing breadth (FWB, in mm), (g) hindwing length (HWL, in mm), and (h) hindwing breadth (HWB, in mm).

Рис. 3. Различия признаков жизненного цикла *C. pyranthe* с учетом самцов и самок отдельно при внутривидовой конкуренции в минималистской форме плотности особей. (b) масса куколки (PW, в мг), (c) масса взрослой особи (AW, в мг), (d) длина взрослой особи (AL, в мм), (e) длина переднего крыла (FWL, в мм), (f) ширина переднего крыла (FWB, в мм), (g) длина заднего крыла (HWL, в мм) и (h) ширина заднего крыла (HWB, в мм).

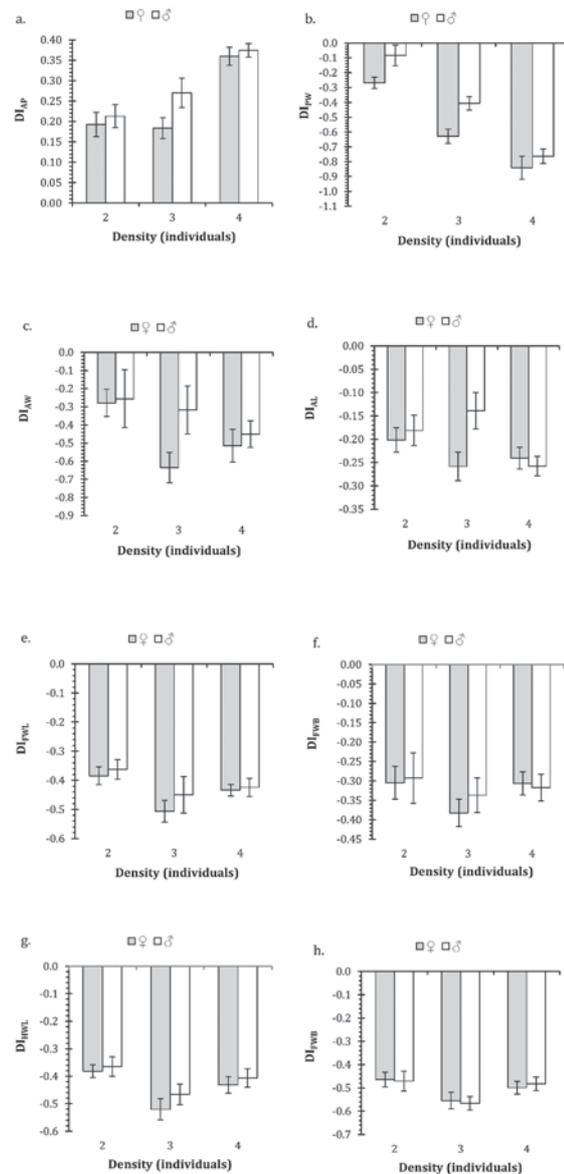


Fig. 4. The variations (Mean \pm SE) of density impact (DI) on different life history traits of *C. pyranthe* butterfly species under intraspecific competitions. Shaded bars represent ♀ while non-shaded bars represent ♂. The life history traits are shown in sequence as (a) age at pupation (AP, in days), (b) pupal weight (PW, in mg), (c) adult weight (AW, in mg), (d) adult length (AL, in mm), (e) forewing length (FWL, in mm), (f) forewing breadth (FWB, in mm), (g) hindwing length (HWL, in mm), and (h) hindwing breadth (HWB, in mm). Values less than zero indicates density impact.

Рис. 4. Вариации (среднее \pm SE) влияния плотности (DI) на различные признаки жизненного цикла бабочек *C. pyranthe* в условиях внутривидовой конкуренции. Заштрихованные столбцы представляют ♀, а незаштрихованные столбцы представляют ♂. Параметры жизненного цикла показаны в следующей последовательности: (a) возраст окукливания (AP, в днях), (b) вес куколки (PW, в мг), (c) вес взрослой особи (AW, в мг), (d) длина взрослой особи (AL, в мм), (e) длина переднего крыла (FWL, в мм), (f) ширина переднего крыла (FWB, в мм), (g) длина заднего крыла (HWL, в мм) и (h) ширина заднего крыла (HWB, в мм). Значения меньше нуля указывают на влияние плотности.

Discussion

The amount of food during larval conditions influences the traits of life history of the mottled emigrant

butterfly *Catopsilia pyranthe* [Mukherjee *et al.*, 2020]. In the present study, the density of larvae affected size, larval duration and survivorship in *C. pyranthe*. These effects reflected in adult traits prominently and similar

Table 1. The results two way ANOVA with initial rearing density (1, 2, 3, and 4 individuals/ tray) and sex as explanatory variables against the eight life history traits of *C. pyranthe* butterfly as response variables.

Таблица 1. Результаты двухфакторного дисперсионного анализа с исходной плотностью выращивания (1, 2, 3 и 4 особи) и полом в качестве факторов, влияющих на восемь признаков жизненного цикла бабочки *C. pyranthe*.

a.					b.				
Source	DF	SS	MS	F	Source	DF	SS	MS	F
Age at pupation (AP, in days)					Pupal weight (PW, in mg),				
DENSITY	3	210.45	70.15	68.12	DENSITY	3	1095484.25	365161.42	78.79
SEX	1	0.96	0.96	0.93	SEX	1	106467.71	106467.71	22.97
DENSITY*SEX	3	3.81	1.27	1.23	DENSITY*SEX	3	70661.86	23553.95	5.08
Error	96	98.86	1.03		Error	96	444910.67	4634.49	
Total	103	316.71			Total	103	2034376.41		
c.					d.				
Source	DF	SS	MS	F	Source	DF	SS	MS	F
Adult weight (AW, in mg),					Adult length (AL, in mm)				
DENSITY	3	3950.60	1316.87	8.29	DENSITY	3	234.87	78.29	31.30
SEX	1	925.22	925.22	5.83	SEX	1	2.60	2.60	1.04
DENSITY*SEX	3	997.83	332.61	2.10	DENSITY*SEX	3	20.01	6.67	2.67
Error	96	15241.15	158.76		Error	96	240.12	2.50	
Total	103	23193.80			Total	103	553.35		
e.					f.				
Source	DF	SS	MS	F	Source	DF	SS	MS	F
Forewing length (FWL, in mm)					Forewing breadth (FWB, in mm)				
DENSITY	3	1866.89	622.30	110.98	DENSITY	3	449.20	149.73	43.89
SEX	1	5.84	5.84	1.04	SEX	1	10.56	10.56	3.10
DENSITY*SEX	3	7.66	2.55	0.46	DENSITY*SEX	3	2.97	0.99	0.29
Error	96	538.31	5.61		Error	96	327.48	3.41	
Total	103	2711.84			Total	103	874.99		
g.					h.				
Source	DF	SS	MS	F	Source	DF	SS	MS	F
Hindwing length (HWL, in mm)					Hindwing breadth (HWB, in mm).				
DENSITY	3.00	1268.32	422.77	101.00	DENSITY	3.00	1337.62	445.87	167.89
SEX	1.00	12.23	12.23	2.92	SEX	1.00	25.75	25.75	9.70
DENSITY*SEX	3.00	5.00	1.67	0.40	DENSITY*SEX	3.00	2.52	0.84	0.32
Error	96.00	401.85	4.19		Error	96.00	254.95	2.66	
Total	103.00	1898.15			Total	103.00	1825.99		

The F-values marked in bold are significant at $P < 0.001$ level. Here the explanatory variables are density levels and sex of the species and the response variables are — (a) age at pupation (AP, in days), (b) pupal weight (PW, in mg), (c) adult weight (AW, in mg), (d) adult length (AL, in mm), (e) forewing length (FWL, in mm), (f) forewing breadth (FWB, in mm), (g) hindwing length (HWL, in mm), and (h) hindwing breadth (HWB, in mm).

Значения F, выделенные жирным шрифтом, значимы на уровне $P < 0,001$. Здесь объясняющими переменными являются уровни плотности и пол вида, а переменными реакции являются — (а) возраст при окукливании (AP, в днях), (b) вес куколки (PW, в мг), (c) вес взрослой особи (AW, в мг), (d) длина взрослой особи (AL, в мм), (e) длина переднего крыла (FWL, в мм), (f) ширина переднего крыла (FWB, в мм), (g) длина заднего крыла (HWL, в мм) и (h) ширина заднего крыла (HWB, в мм).

to the previous results like in mosquitoes [Agnew et al., 2000; Banerjee et al., 2017], chironomid midges [Hooper et al., 2003], stonefly [Lieske, Zwick, 2008], European grapevine moth [Thiéry et al., 2014]. The competitive effects are pronounced as a manifestation of resource-based competition, with increased rearing density at fixed amount of food. The higher the density the more pronounced the competitive effects. The larval development correspondence with pupal weight, adult weight, adult body length, wing length (both forewing and hindwing) suggests that the development process has affected the subsequent manifestation of the adult characteristics. Larval development is a crucial process of life history that defines the characteristics of adults and their subsequent reproductive success in holometabolous insects [Ureña et al., 2016]. The resource acquisition by the larva in the course of the developmental process defines the adult size, longevity and reproductive success in butterflies and many other insects [Gibbs et al., 2004; Gibbs, Breuker, 2005]. In the present case, the pupation age trade-offs and the adult body size is made in the perspective of the caterpillar rearing rate. The resource acquisition should be maximized to ensure that a caterpillar reaches a suitable adult stage. Higher densities during the growth period led to a decrease of the pupal size similar to the result found in case of *Pieris napi* (Lepidoptera: Pieridae) [Kivelä, Välimäki, 2008]. The pupa resulting from the four, three, two rearing density were smaller in size and the age of pupation compared with the one individual rearing rate later. Earlier studies have already illustrated that whatever the competition process, the individuals with inadequate nutrition will face high mortality and that survivors will be likely to exhibit reduced size at maturity and delayed growth [Roff, 1992; Stearns, 1992; Agnew et al., 2002; Cameron et al., 2007; Välimäki, Kaitala, 2007; Kivelä, Välimäki, 2008]. The findings from this research also confirmed the predicted results, i.e. high mortality and longer larval period and size reduction in adults. The interspecific competition during larval growth diminishes fitness of adult reducing body size [Gotthard et al., 1994; Nylin, Gotthard, 1998].

Besides, the effect of density on size and the larval length was found to be sex-dependent. The developmental time of the females in each group was longer than males and females displayed greater body weight and longer wing length and breadth than males. (Fig. 3 and Table 1). As observed in other species of butterflies [Gibbs et al., 2004; Gotthard, 2008; Kivelä, Välimäki, 2008], density dependent effects on the adult butterflies are common due to the constraints in the available resources often determined by the environmental conditions. Density impact (DI) on different traits like pupal weight, adult weight, adult length, wing length and wing breadth was prominent in the present study (Fig 4). Similar to this study density impact was also recorded in *Aedes aegypti* and *Aedes albopictus* [Banerjee et al., 2017], *Drosophila melanogaster* [Roy et al., 2018]. The present study is a preliminary effort to use the mottled emigrant as a model species to elucidate the density

dependent effects in the butterflies. Further studies using other butterfly species will provide insight into the generality of the density-dependent effects in the butterflies covarying with resource quality and quantity.

Conclusion

For each of the traits of life history, the density effects were prominent, with the correlations remaining either positive or negative concerning each other. The age at pupation, pupal weight, adult weight, adult body length, forewing length, and hind wing length showed differences in the larva's rearing density. Like earlier researches the present result suggests that the relative density of larvae on the host plant may have a significant impact on the life history traits and, consequently, the fitness of the individual mottled emigrant butterfly. More studies, however, are required to substantiate the interactions between food and density on the individual's life history traits to create the interactions between food and density in shaping the body size of the mottled emigrant butterfly *C. pyranthe*.

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Competing Interests. As authors of this article we declare no competing interest.

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