EARLY DETECTION OF *TRICHOGRAMMA CHILONIS* SEXES USING THE EGG COLOR AND SIZE OF ITS FACTITIOUS HOST, *CORCYRA CEPHALONICA*

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ABSTRACT

Early Detection of **Trichogramma chilonis** *sexes using the egg color and size of its factitious host* **Corcyra cephalonica**. This study was aimed to detect the sexes of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) prior to their emergence using the egg color and size of their factitious host, *Corcyra cephalonica* (Lepidoptera: Pyralidae). A gray scale of 1 – 7 was used to rank the egg color while a micrometer was used to observe the egg size under a dissecting microscope. Ten of newly laid *C. cephalonica* eggs were glued in two rows (0.5 cm x 0.4 cm distance) on a piece of oviposition paper. Five replications of such batches were exposed in a test tube to a gravid *T. chilonis* female for 24 hours under 100 watts of illumination. Following the withdrawal from the oviposition arena the eggs were each observed daily for their color and size and the sex of the emerged *T. chilonis* progeny was documented. Binomial Z-test at 0.05 level of significance was used to validate the predicted proportion of *T. chilonis* sexes from five batches of 50 parasitized *C. cephalonica* eggs. The results showed as predicted that more *T. chilonis* female progeny emerged from light-colored *C. cephalonica* eggs (scale 2 – 5) following oviposition by parent parasitoid or from larger *C. cephalonica* eggs ($\geq 500\,\mu m$).

Key words: sex, *Trichogramma*, early detection, *Corcyra* egg

INTRODUCTION

Trichogramma chilonis Ishii (Hymenoptera: Trichogrammatidae) has been used as a biological control agent against various insect pests attacking crops worldwide, including corn borers, sugarcane borers, and cotton bollworm (Li, 1994). The egg parasitoid that has been used to control sugarcane borers in Lampung, Indonesia was introduced from Java and formerly known (or misidentified) as *T. australicum* Girault. The parasitoid has been mass-reared in the laboratory and periodically released at the sugarcane field at Terusan Nunyai area, Central Lampung (owned by PT Gunung Madu Plantations) since 1976 (Sunaryo, 1988; Putri, 2006). In the laboratory the mass-rearing of *T. chilonis* is facilitated using eggs of the rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) as the factitious host.

The parasitization *T. chilonis* in the field population of sugarcane stemborer varies from 10% to 40% (Dewi, 2007). In some occasions the parasitoid performance could even be lower than 10%. Variations in the field conditions during the parasitoid release including weather and population dynamics of the host pest might have contributed to the parasitoid performance. In addition, the performance might also depend on the biological condition of the parasitoid itself, including its sex ratio. The parasitoid sex ratio which favors females (female-biased) would potentially better perform than that which favors males because only the females that actually sting, oviposit, and thus parasitize the host pest. It is therefore important that the sex ratio of *T. chilonis* be first considered before their release as the indicator of their potential performance in the field. However, the parasitoid sex is normally manifested after their emergence from the host eggs. The research question is thus as follows: Cand (or how can) *T. chilonis* sex be detected prior to emergence from their host eggs?

During the mass-rearing of *T. chilonis* in the laboratory, it has been observed that *C. cephalonica* eggs change in color and vary in size (Sunaryo, 1988). The parasitized eggs turn darker than unparasitized ones. Furthermore, variation in the dark coloration is also occurred within the parasitized egg group. This lead to further questions as follows: (1) Could the color variation in the parasitized host eggs be indicative of the sex of *T. chilonis* progeny developed therein? (2) Would the size of the host eggs exert some kind of preference of parental *T. chilonis* to lay eggs therein which developed into progeny of certain sex? The objective of this study was to detect the sex of *T. chilonis* prior to their emergence using the color and size of parasitized *C. cephalonica* eggs.

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MATERIALS AND METHODS

The study was conducted at Research & Development Laboratory, PT Gunung Madu Plantations, Terusan Nunyai, Central Lampung during Mei-August 2006. Two sets of experiment were set, i.e. characterization experiment and validation experiment. In the first experiment, ten of newly laid *C. cephalonica* eggs were placed with a povinal glue and arranged in two rows (0.5 cm x 0.4 cm distance) on a piece of exposure arena (a *Padalarang* paper) of 2 cm x 1 cm size. Five of such systems (containing a total of 50 *C. cephalonica* eggs) were each exposed to a gravid female *T. chilonis* of the 6th generation for 24 hours under 100 watts of illumination in an oviposition test tube. Following the withdrawal from the oviposition tube the eggs were each observed daily for their color and size and the sex of the emerged *T. chilonis* progeny was documented. The colors were scored using a grayscale from 1 to 7, where 1 = creamy white, 2 = gray, 3 = gray with black dots, 4 = gray with black spots, 5 = blackish gray, 6 = grayish black, and 7 = black (Figure 1). The egg size was measured using Olympus micrometers (a 10 x eyepiece Model NCWHK and 1/100 objective Model OB-M). The longest axis in the cross-section of a *C. cephalonica* egg was taken to be the size of the egg (in µm). The sex of emerging *T. chilonis* progeny was determined using antennal segmentation and ornamentation as the delimiting character (Pinto & Stouthamer, 1994). Individuals whose antennae each consisting of a single, elongate flagellar segment fringed with long and robust hairs were taken to be males while those whose antennae each consisting of three flagellar segments (two funicular + one claval) ornamented with delicate, short, and less hairs to be females. The frequencies of either sex were plotted against the color score and size of *C. cephalonica* egg from where they emerged. The results of characterization experiment (prediction) were then tested in the validation experiment.

The validation experiment was set by exposing similar *C. cephalonica* eggs to gravid *T. chilonis* females in the oviposition tube as above. However, in this experiment the total number of the exposed eggs was 250 which were placed randomly in groups of 50 on exposure arenas of 7 cm x 1 cm each. As were in the first experiment, the eggs color and size in this experiment were observed daily after exposure to gravid female *T. chilonis* for 24 hours and under 100 watts illumination. But the egg color score and size

data in this experiment were used to predict the proportion of any sex of *T. chilonis* progeny prior to their emergence. The proportion of female *T. chilonis* progeny was predicted as p_1 and that of male as q_1 where $q_1 = 1 - p_1$. The proportion of females is the ratio between the number of female individuals and the total number individuals (i.e. n_1 = females + females). Meanwhile, it was predicted that females of *T. chilonis* progeny emerged from parasitized *C. cephalonica* eggs color-scored 2 – 5 or sized ≥ 500 µm and that males of *T. chilonis* progeny emerged from those eggs color-scored 6 – 7 or sized < 500 µm. Thus,

$$p_1 = \frac{X_1}{X_1 + Y_1}$$

where X_1 = number of parasitized *C. cephalonica* eggs color-scored 2 – 5 or sized ≥ 500 µm and Y_1 = number of parasitized *C. cephalonica* eggs color-scored 6 – 7 or sized < 500 µm. Then, following their emergence, each *T. chilonis* progeny of either sex was tallied as such that the real (observed) proportion of females (p_2) were documented as follow:

$$p_2 = \frac{X_2}{X_2 + Y_2}$$

where X_2 = actual number of *T. chilonis* females and Y_2 = actual number of *T. chilonis* males emerged from parasitized *C. cephalonica* eggs.

Analysis was done in two steps. First, a two-tailed Z-test with critical regions of < -1.96 and > 1.96 was used to test whether the prediction about *p* held, i.e. whether the observed proportion of *T. chilonis* females (p_2) was true as predicted (p_1). The $p_1 - p_2$ difference was tested at 0.05 level of significance using the following Z-statistic (Walpole, 1982):

$$\text{Z-statistic} = \frac{p_1 - p_2}{\sqrt{(p_1 q_1 / n_1) + (p_2 q_2 / n_2)}}$$

where $n_1 = n_2 = n$ (number of parasitized *T. chilonis* eggs) $= X_1 + Y_1 = X_2 + Y_2$. Second, a one-tailed Z-test with a critical region of > 1.645 was used to see whether the values of p_2 were greater than 0.5, i.e. whether population of (parasitized) *C. cephalonica*
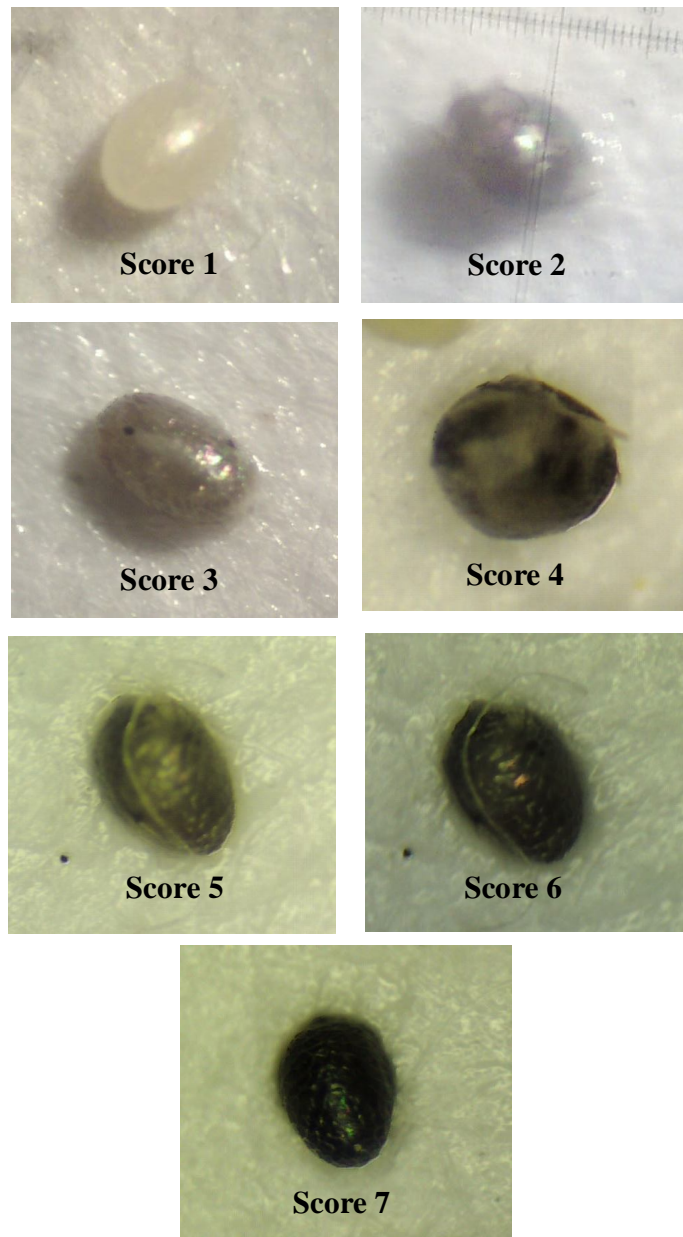
Figure 1. The colors of *Corcyra cephalonica* eggs scored in a gray scale from 1 to 7

eggs consisting of larger proportion of those color-scored 2 – 5 (light) or sized $\geq$ 500 µm (large) would indeed facilitate the emergence of more *T. chilonis* females than males (with sex ratio > 0.5). The test was done at 0.05 level of significance using the following Z-statistic (Walpole, 1982):

$$\text{Z-statistic} = \frac{p_2 - 0.5}{\sqrt{p_2 q_2 / \text{n}}}$$

where p_2, q_2, and n are as above.

RESULTS AND DISCUSSION

Result from characterization experiment showed that either sex of *T. chilonis* progeny emerged from parasitized *C. cephalonica* eggs of any color score or size. The actual parasitization was high (92%). The parasitized *C. cephalonica* eggs facilitated the emergence of more females of *T. chilonis* progeny (56%) than males (44%), giving a female-biased sex ratio. Within the female population, a bigger proportion (88%) emerged from the light colored, 2 – 5 scored eggs. In contrast, most males of *T. chilonis* (75%) emerged from the darker scored *C. cephalonica* eggs (6 – 7) (Figure 2). In term of size, smaller *C. cephalonica* eggs (< 500 µm) facilitated the emergence of more males (79%) than females of *T. chilonis* whereas more females (76%) emerged from relatively larger host eggs ($\geq$ 500 µm) (Figure 3). There was no absolute cut-offs for either sex manifestation of *T. chilonis* in terms of *C. cephalonica* egg color score or size. It was even true that both sexes of *T. chilonis* progeny also emerged from a small portion (4%) of parasitized *C. cephalonica* eggs (color score 2 – 4 and size 550 – 575 µm). However, there were two apparent cut-off indicators for emergence of *T. chilonis* sex, i.e. *C. cephalonica* egg color score of 5 (blackish gray) and egg size of 500 µm. The test results showed that light-colored (scored $\leq$ 5, from gray to blackish gray) or larger (sized $\geq$ 500 µm) *C. cephalonica* eggs facilitated the emergence of more *T. chilonis* females. It was thus predicted that larger proportion of females in the *T. chilonis* progeny population (female-biased sex ratio) should be resulted from the population of parasitized *C. cephalonica* eggs with higher proportion of those color-scored 1 – 5 (first prediction) or sized $\geq$ 500 µm (second prediction).
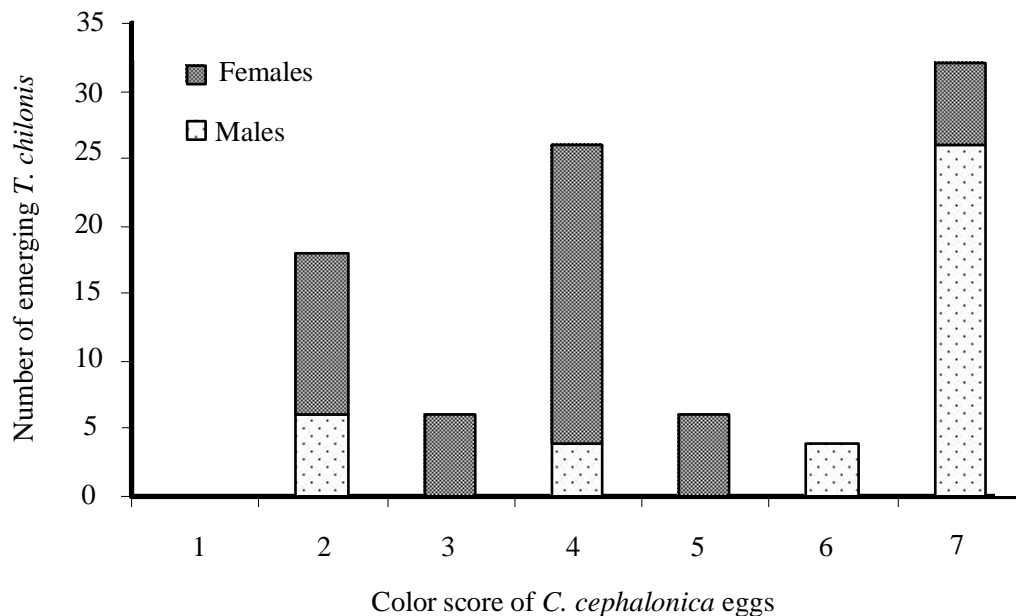

Figure 2. Number of emerging *T. chilonis* of either sex from the parasitized *C. cephalonica* eggs of various color scores
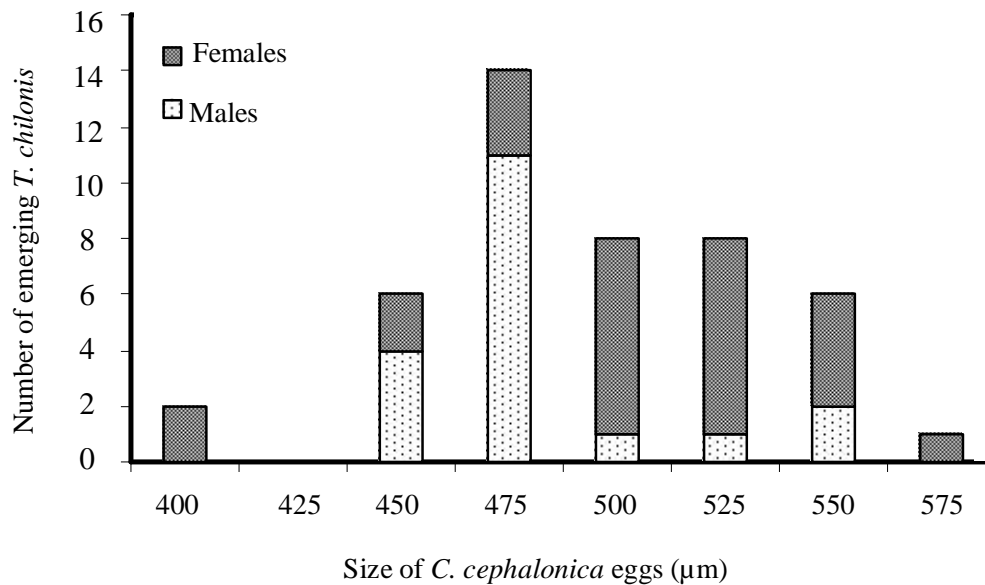
Figure 3. Number of emerging *T. chilonis* of either sex from the parasitized *C. cephalonica* eggs of various sizes

The results of validation experiment showed that the first and second prediction of the characterization experiment held (Figure 4 and Figure 5). As depicted in both figures, the observed female proportions (p_2) in general were true as predicted (p_1). Figure 4 showed that batches consisting of more parasitized *C. cephalonica* eggs scored 2 – 5 ($0.60 \leq p_1 \leq 0.94$) indeed produced more females ($0.70 \leq p_2 \leq 0.78$) than males of *T. chilonis*. Figure 5 also showed that more females ($0.64 \leq p_2 \leq 0.83$) than males of *T. chilonis* were produced from those batches consisting of *C. cephalonica* eggs sized 500 µm or larger ($0.58 \leq p_1 \leq 0.79$). All observed proportions of the female cases (p_2) were not different from those hypothesized (p_1) at 0.05 level of significance, except in two cases which were predicted using *C. cephalonica* egg color at 4 days prior to emergence (Figure 4). All observed female proportion cases predicted using egg color were significantly greater than 0.5 (female-biased sex ratio) (Table 1) and all cases predicted using egg size were also significantly greater than 0.5 (Table 2). As an implication from the prediction on the sex proportion, the results shows that higher number of *T. chilonis* females than males indeed emerged from

lighter-colored (Figure 6) or larger-sized *C. cephalonica* egg populations (Figure 7).

The color or size of *C. cephalonica* eggs could relate to the body color or size of *T. chilonis*. It was no surprise when darker parasitized host eggs produced more males while the lighter ones produced more female parasitoids because *T. chilonis* male body color *is* indeed darker than that of female. In other words, the parasitoid body color is manifested in the parasitized host egg color. Similar principle could work for the size: larger host eggs produces larger (i.e. female) parasitoids. In this study the predictions about proportion of female parasitoid using host egg color and size were met. In other words, color and size of the host eggs are good indicators for the parasitoid sex ratio. But other factor may affect the female-biased sex ratio of *T. chilonis* in this study and other (for instance in Putri, 2006). It has been well established that most, if not all, members of Hymenoptera (ants, bees, wasps) are arrhenotokous, i.e. their unfertilized eggs develop into males while fertilized eggs develop into females, and *T. chilonis* as a wasp does that. A successful egg fertilization would produce females, while a failure would end up with males. One of the adaptive strategies to couple with this reproductive
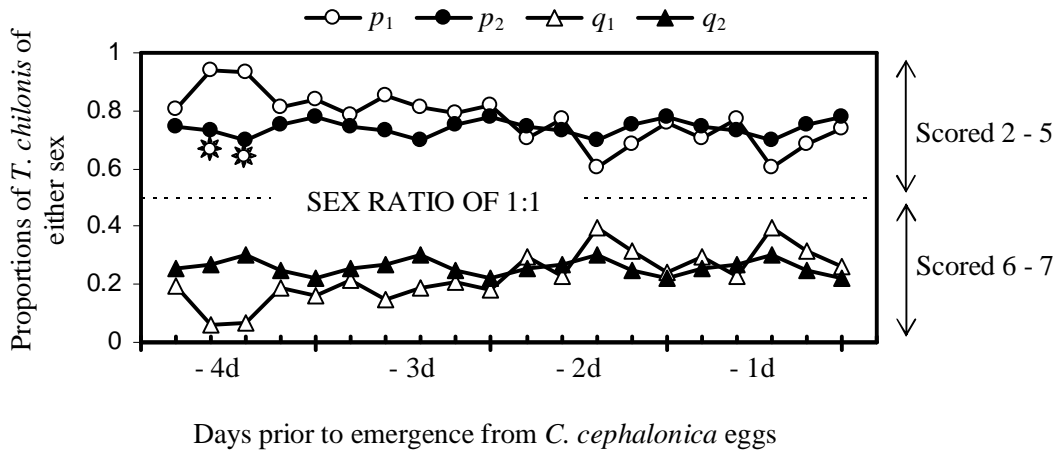
Figure 4. Predicted and observed proportions of *T. chilonis* of either sex that emerged from more *C. cephalonica* eggs scored light (2 – 5) than dark (6 – 7) (p_1 = predicted female proportion, q_1 = predicted male proportion, p_2 = observed female proportion, q_2 = observed male proportion, sun signs = p_1 significantly different from p_2 at 0.05 level)
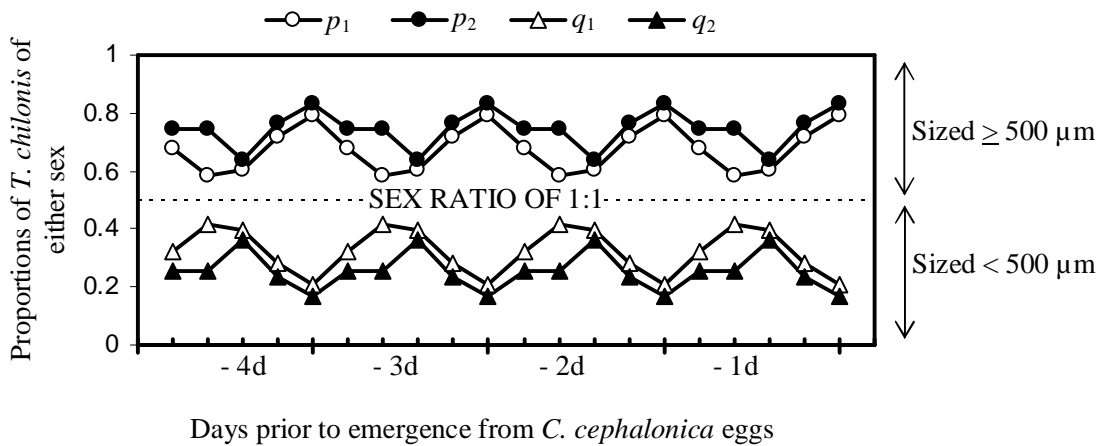

Figure 5. Predicted and observed proportions of *T. chilonis* of either sex that emerged from more *C. cephalonica* eggs sized large ($\geq$ 500 µm) than small (< 500 µm) (p_1 = predicted female proportion, q_1 = predicted male proportion, p_2 = observed female proportion, q_2 = observed male proportion, all p_1 are not significantly different from p_2 at 0.05 level)
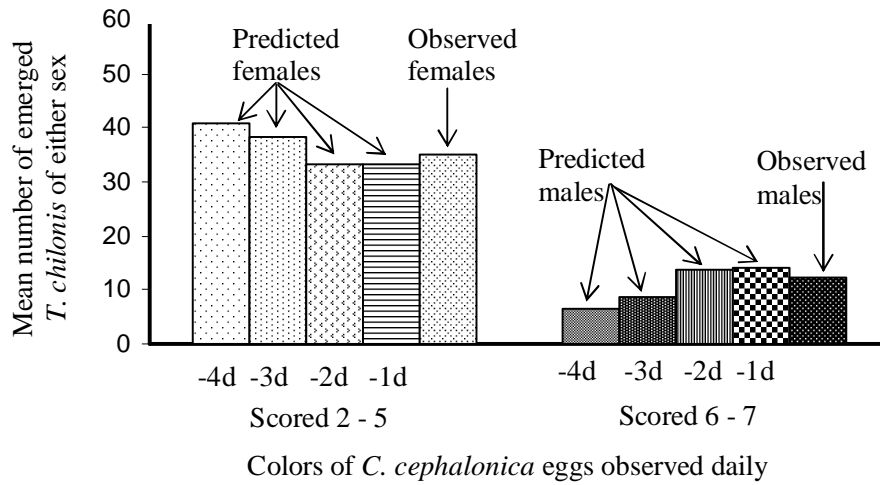
Figure 6. Predicted and observed number of *T. chilonis* females and males emerged from lighter and darker-colored *C. cephalonica* eggs
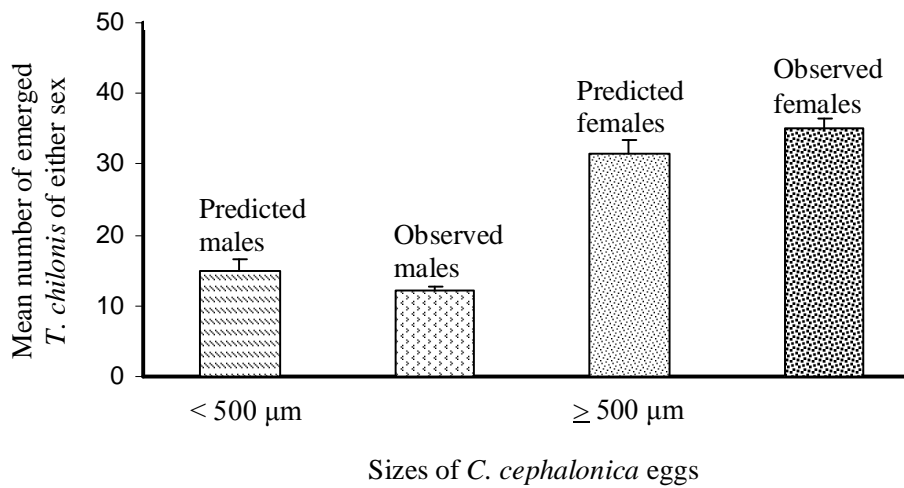

Figure 7. Predicted and observed number of *T. chilonis* females and males emerged from smaller and larger *C. cephalonica* eggs

system is the inbreeding behavior, where brothers mate with sisters (Thornhill & Alcock, 1983). To be effective, this behavior necessitates a brother to brother competition for access of mating with sisters (Hamilton, 1967; Alexander & Sherman, 1977). The pressure of the competition in turn facilitates the development of a polygynous-monandrous trait where one male can fertilize many females while one female is receptive to only one male. In such a situation the female parent should produce more female than male progeny because producing additional males would not only be unnecessary but also be of wasted parental investment, as may be the case in this study.

The outcome of this study is that *T. chilonis* sex could be early-detected using the egg color or size of their factitious host, *C. cephalonica*. Detection using the color of parasitized host eggs could be done as early as four days before the parasitoid emergence. This could provide additional information about how effective (or ineffective) are the batches of parasitized host eggs before being applied to the field. A batch dominated by darker-colored host eggs would be inferior in terms of efficacy because they would produce a parasitoid population with a male-biased parasitoid sex ratio, consisting of more false parasitoids, i.e. males of *T. chilonis* that could not parasitize. For field inundation purposes, it would be beneficial to select the parasitized batches dominated by lighter-colored *C. cephalonica* eggs because those would produce *T. chilonis* population with female-biased sex ratio consisting of more females (true parasitoids).

The host egg size, could give more profound contribution. This study shows that the host egg size could be used as the predictor for the parasitoid sex even before the egg being parasitized. This could lead to a very important technological implication for the mass-production of more effective *T. chilonis* in the laboratory. Methods or equipment could be developed to screen and select *C. cephalonica* eggs as such that only their larger eggs ($\geq$ 500 µm) to be prepared to produce larger number of true parasitoids (*T. chilonis* females) being ready for the field release.

CONCLUSIONS

The first part of this study predicted that *T. chilonis* male progeny tended to emerge from *C. cephalonica* eggs that turned darker following oviposition by parent parasitoid while larger *C. cephalonica* eggs tended to facilitate emergence of *T.*

chilonis females. The second part of this study showed in general that the predictions held. Batches of *C. cephalonica* eggs turning not as dark (gray scale of 2 – 5) following parasitization produced more females (proportion > 0.5) than males of *T. chilonis*. Parasitization on larger-sized host eggs ($\geq$ 500 µm) also produced more *T. chilonis* females. The latter opened opportunities to enhancement of the parasitoid mass-rearing technology in the laboratory.

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