Egyptian Journal of Biological Pest Control

RESEARCH

Open Access

How long-term mass rearing affects the quality of the *Trichogramma embryophagum* (Hartig) (Hymenoptera: Trichogrammatidae) reared on *Sitotroga cerealella* (Olivier) eggs

Farzaneh Sadat[1], Alireza Nazari[2*], Shahriar Jafari[3] and Zahra Rafiei Karahroudi[1]

Abstract

Background: Maintaining the quality and efficacy of biological control agents during long-term mass rearing plays a crucial role in the success of a biological control program. In this study, the biological traits of a local population colony of *Trichogramma embryophagum* (Hartig) with rearing on *Sitotroga cerealella* (Olivier) eggs for over 40 generations (G) were assessed.

Results: Female adult longevity was significantly different among generations, ranging from 7.98 d in G5 to 5.19 d in G40. The reared wasps showed highest fecundity (60.50 eggs/female) in G5 compared to the other generations. The female sex ratio varied from 63.16% in G5 to 49.31% in G40. Significant differences were observed in population growth parameters and the highest gross reproductive rate (*GRR*) (40.96 eggs/individual) and net reproductive rate (R_0) (38.21 eggs/individual) were found in G5. However, a non-significant difference was found in the intrinsic rate of natural increase (*r*) until the 10th generation, but its values significantly declined with increasing the generation numbers. The finite parasitism rate (ω) ranged from 0.468 host/parasitoid/day in G5 to 0.274 host/parasitoid/day in G40.

Conclusions: The results showed that the quality of *T. embryophagum* reared under continuous laboratory conditions declined after 10 generations, and for use of them in biological control programs under field conditions, the reared population should be refreshed by adding wild individuals from time to time.

Keywords: *Trichogramma embryophagum*, Mass rearing, *Sitotroga cerealella*, Quality control

Background

Among different recommended methods to control the pests in integrated pest management (IPM), biological control with the use of natural enemies has special importance due to its safety, selectivity and as a result producing healthy products. Trichogrammatid' wasps are one of the main groups of the insect's egg parasitoids that are widely used all over the world. The different species of genus *Trichogramma* has been used successfully as egg parasitoids to control more than 30 key pest species in inundative or augmentative biological control programs (Vinson et al. 2015). Mass rearing and release of these wasps leads to a significant decrease in damage caused by the larval stage of the pest.

Augmentative or inundative biological control programs rely on mass production and releases of natural enemies and in continuous rearing the quality of the produced individuals has much importance (van Lenteren 2003). The process of *Trichogramma* mass rearing often continues for a long time with many generations; therefore the quality of reared wasps in this prolonged process was also affected by reared conditions (Smith 1996). One

*Correspondence: a-nazari@iau-arak.ac.ir
[2] Department of Sustainable Agriculture, Food Security Research Institute, Arak branch, Islamic Azad University, Arak, Iran
Full list of author information is available at the end of the article

of the concerns in the mass rearing process is that they are produced under ambient conditions and in limited space may adapt to laboratory conditions and lose some characters during long-term maintenance and experience inbreeding depression, resulting in poor performance of the released wasp in the field (Badran et al. 2020). Therefore, they may be unable to find and parasite their hosts under variable field conditions; therefore it is very important to study the changes that may occur during mass rearing of natural enemies.

Quality control in mass rearing of the parasitoids is one of the important measures that was used to prevent defeats in biological control programs (van Lenteren 2003). In quality control process some traits including parasitism rate, sex ratio, fecundity, longevity, body size and weight and flight activity or host-searching ability have to be evaluated (Lu et al. 2017). Reduction in the fecundity, survival and adult longevity of some trichogrammatid' wasps reared for several generations have been reported (Ghaemmaghami et al. 2021a,b).

Trichogramma brassicae (Bezdenko), *T. embryophagum* (Hartig) and *T. pintoi* Voegele are the most widespread species of the Trichogrammatidae family in Iran (Ebrahimi et al. 1998). *T. embryophagum* has a wide host range. This species is annually reared over more than 15–20 generations mainly to control the *Ectomyelois ceratoniae* Zeller in some regions of Iran.

Although several studies were accomplished on different aspects of *T. embryophagum*, no information was available on its quality during long-term mass rearing. Therefore, the effects of long-term mass rearing on the quality of *T. embryophagum* reared on *Sitotroga cerealella* (Olivier) over 40 generations were assessed.

Methods

Insect host rearing

This study was conducted during the years of 2018 to 2020. In order to establish the colony of *S. cerealella*, its eggs were obtained from an insectary, in the Agriculture and Natural Resources Research Institute of Hamadan Province, Hamadan, Iran. The stock culture of *S. cerealella* was initiated under constant temperature of 26 ± 1 °C in the Entomological laboratory of the Agriculture and Natural Resources Research Institute of Hamadan Province, Hamadan, Iran. The prepared *S. cerealella* eggs were reared in cylindrical containers (19 cm diameter and 8 cm height) on barley grains until the end of pupal stage. The newly emerged adults were transferred to funnels (diameter 150 mm) covered with a fine mesh net at top and kept up-side-down. After mating, the moth eggs were collected on pieces of paper placed as egg laying substrate to ease egg collection under the wider side of the funnel.

Parasitoid colony

To obtain the initial colony of *T. embryophagum*, the egg traps [*S. cerealella* eggs placed on pieces of white papers (200 × 100 mm)] were placed at a height of about 2 m above the ground on the leaves of pomegranate trees, in Tange-Siab, Kuhdasht County, Lorestan Province, Iran, in May 2018. The traps were collected after 24 h and transferred to the laboratory. The parasitized eggs were reared in a growth chamber set at 26 ± 1 °C, 60% ± 5% RH and a 16: 8 h (L: D) photoperiod until the emergence of *T. embryophagum* adults, which were then reared in rectangular plastic containers (300 × 200 × 150 mm) on *S. cerealella* eggs under the same conditions as mentioned above. The collection process was repeated several times in a limited time to supply a sufficient amount of parasitoids.

Experimental design

After emergence of the adult wasps and oviposition, more than 100 newly parasitized eggs of hosts (less than one day old) were selected from the colony and kept in a glass container (100 mm in diameter, 60 mm in height) until the adult wasps emerged. After the emergence of the adults, each pair of male and female wasps was placed in a glass cylinder (100 × 16 mm) containing about 120–150 eggs (less than one day old host glued on a piece of paper 10 × 50 mm). The parasitoid wasps were regularly supplied by diluted honey on cotton rolls after emergence. Dead males during the experiments were replaced by newly emerged ones (< 24 h old) from the stock colony. These individuals were excluded from the statistical analyses. Exposed egg papers were collected after 24 h and replaced daily with refresh ones until the death of the last females. The exposed eggs were separately maintained in a growth chamber at 26 ± 1 °C, RH of 65 ± 5% and a photoperiod of 16L: 8D hrs. The time interval between egg exposure and the time of emergence of adults was considered as the duration of the immature stages development. The glass cylinders were monitored and longevity, mortality and survivorship of adult females and males were recorded by visual examination every 12 h until the death of the last individual. Fecundity of each parasitoid wasp was also calculated by counting and recording the parasitized eggs of the host daily (as evidenced by blackening) over its life span. Sex ratio was estimated as a percentage of females. Total life span of females was calculated for those wasps that died as female adults and presented as a total duration of immature stages and adult longevity for females. These procedures were repeated at the generations of 5, 10, 15, 20, 25, 28, 30, 32, 34, 37 and 40 of the *T. embryophagum* colony. The

counts of the studied generations were 5 generations until the 25th one. By increasing the number of generations and the effects of long-term rearing on their characteristics, the counts among the studied generations decreased to 2 or 3 generations. All experiments were carried out in an incubator set at 26 ± 1 °C, 60% ± 5% RH and a photoperiod of 16: 8 h (L: D).

Statistical analysis

The life history data were analyzed using age-stage, two-sex life table theory (Chi 1988). The age-stage survival rate (s_{xj}) (the probability that a newly laid egg will survive to age x and stage j), age-stage-specific fecundity of female (f_{xj}) (the number of offspring produced by female adult of age x and stage j), the age-specific survivorship (l_x) (the probability that a newly laid egg would survive to age x), age-specific fecundity (m_x) (the mean number of eggs produced per individual at age x) and also the age-stage life expectancy (E_{xj}), as well as the population parameters, include the intrinsic rate of natural increase (r), the finite rate of increase (λ), the gross reproductive rate (GRR), the net reproductive rate (R_0) and the mean generation time (T) were calculated by TWOSEX-MSChart program (Chi 2019b). Bootstrap procedure was used to estimate the variances and the standard errors of population growth parameters (Huang and Chi 2013). To obtain the stable estimates, 10,000 bootstrap samples were used. Bootstrap values of different generations of *T. embryophagum* were then compared with paired-bootstrap procedure (Bahirai et al. 2019).

Daily parasitism rates of the cohort were used to estimate the parasitism parameters for each generation. The age-specific parasitism rate (k_x) is the mean number of host parasitoid at age x that was calculated by the following equation (Chi and Yang 2003):

$$k_x = \frac{\sum_{j=1}^{\beta} S_{xj} C_{xj}}{\sum_{j=1}^{\beta} S_{xj}}$$

where β is the number of stages. The net parasitism rate (c_0) shows the mean number of hosts parasitized by an individual parasitoid during its entire life span, and was calculated as (Chi and Yang 2003):

$$C_0 = \sum_{x=0}^{\infty} \sum_{j=1}^{\beta} S_{xj} C_{xj} = \sum_{x=0}^{\infty} l_x k_x,$$

The transformation rate from host population to parasitoid offspring (Q_p) that shows the number of hosts that is needed for a parasitoid to produce an offspring (Chi et al. 2011) is calculated as:

$$Q_p = \frac{C_0}{R_0}$$

The stable parasitism rate (ψ) is the total parasitism capacity of a stable population which total size is unity (Chi et al. 2011), and is calculated as:

$$\psi = \sum_{x=0}^{\infty} \sum_{j=1}^{\beta} \alpha_{xj} c_{xj}$$

a_{xj} is the proportion of individuals belonging to age x and stage j in stable age-stage distribution (SASD). The finite parasitism rate (ω) shows the parasitism potential of a parasitoid population by combining its finite rate of increase (λ), age-stage parasitism rate (c_{xj}) and stable age-stage structure (a_{xj}) (Chi et al. 2011; Yu et al. 2013), and is calculated as:

$$\omega = \lambda\varphi = \lambda \sum_{x=0}^{\infty} \sum_{j=1}^{\beta} \alpha_{xj} c_{xj}$$

Parasitism rate data were analyzed using the computer program CONSUME-MSChart (Chi 2019a). The bootstrap resampling method (10,000 bootstraps) was used to estimate the variances and the standard errors of the parasitism parameters. Comparison of parasitism parameters was done based on paired-bootstrap test using TWOSEX-MSChart program (Ghaemmaghami et al. 2021a, b). The parasitism capacity was compared among the generations 5, 10, 15, 20, 25, 28, 30, 32, 34, 37 and 40 of *T. embryophagum*. Microsoft Excel 2013 was used to draw the graphs.

Results

Adult longevity, reproductive parameters and survival

Although the duration of the immature stages of *T. embryophagum* varied slightly over successive generations, it was non-significantly affected by the increase in generation numbers. It was between 10 and 11 days. Unlike, significant differences in female and male longevity were observed among generations. The longest female longevity (7.98 ± 0.36 d) was estimated in G5, while in G40 showed the shortest female longevity (5.19 ± 0.40 d). The longest male longevity was estimated in G5 (6.54 ± 0.40 d), while the shortest was in G32 (4.22 ± 0.39 d) and G40 (4.22 ± 0.33 d). The longest total life span of females was counted in G5 (18.40 ± 0.38 d) then decreased over the generations. The shortest female total life span (15.35 ± 0.42 d) was in G40 (Table 1). However, a non-significant difference was observed in total life span until the 15th-generation. Ovipositional days differed significantly among generations, ranging from 5.83 ± 0.19 d in G5

Table 1 Duration of adult longevity and total life span (d), sex ratio (female ratio) and fecundity (eggs) of sequential generations of *Trichogramma embryophagum* reared on *Sitotroga cerealella* eggs

No. generation	Number of individuals	Adult longevity (Female)	Adult longevity (Male)	Total life span (Female)	Oviposition days	Sex ratio % (Female/total)	Fecundity
5	76	7.98 ± 0.36^{a}	6.54 ± 0.40^{a}	18.40 ± 0.38^{a}	5.83 ± 0.19^{a}	63.16	60.50 ± 2.35^{a}
10	69	7.32 ± 0.42^{ab}	6.07 ± 0.48^{ab}	18.22 ± 0.38^{a}	5.30 ± 0.28^{ab}	59.42	52.71 ± 2.81^{b}
15	64	7.08 ± 0.37^{abc}	6.04 ± 0.51^{ab}	17.83 ± 0.40^{ab}	4.89 ± 0.25^{bc}	56.25	43.97 ± 1.99^{c}
20	71	6.54 ± 0.38^{bc}	5.31 ± 0.46^{bc}	16.95 ± 0.41^{bc}	4.51 ± 0.21^{c}	54.93	35.72 ± 2.02^{d}
25	74	6.22 ± 0.31^{cd}	5.24 ± 0.31^{bc}	16.68 ± 0.41^{cde}	4.36 ± 0.19^{c}	55.41	29.66 ± 1.59^{e}
28	67	5.45 ± 0.31^{de}	4.45 ± 0.42^{c}	16.13 ± 0.32^{cde}	3.78 ± 0.17^{d}	56.71	27.76 ± 1.59^{ef}
30	72	5.68 ± 0.21^{de}	4.66 ± 0.37^{c}	15.92 ± 0.26^{de}	3.74 ± 0.09^{d}	51.39	26.81 ± 1.21^{ef}
32	75	5.55 ± 0.23^{de}	4.22 ± 0.39^{c}	15.82 ± 0.24^{e}	3.55 ± 0.13^{d}	50.67	24.89 ± 1.02^{fg}
34	76	5.50 ± 0.31^{de}	4.42 ± 0.32^{c}	15.74 ± 0.24^{e}	3.75 ± 0.18^{d}	50.00	22.66 ± 1.27^{gh}
37	71	5.43 ± 0.28^{de}	4.38 ± 0.32^{c}	15.51 ± 0.25^{e}	3.69 ± 0.16^{d}	52.11	20.43 ± 1.30^{h}
40	73	5.19 ± 0.40^{e}	4.22 ± 0.33^{c}	15.35 ± 0.42^{e}	3.53 ± 0.16^{d}	49.31	19.24 ± 1.88^{h}

The means marked by the same letters within the same column are not significantly different (Paired-bootstrap test, $P < 0.05$)

to 3.53 ± 0.16 d in G40 (Table 1). Non-significant differences were observed between the ovipositional days of the 5th and 10th generations. Fecundity varied from 60.50 ± 2.35 eggs/female in G5 to 19.24 ± 1.88 eggs/female in G40. As shown in Table 1, the fecundity reduced to less than half after the 25th generation. Also, different generations showed significant variations in sex ratio, ranging from 63.16% females in G5 to 49.31% females in G40. According to the results after G30, the sex ratio of females was less than 55% (Table 1).

The highest values of the age-stage-specific survival rate (s_{xj}) for females occurred in days of 12–15, 12, 13–15, 12–15, 12, 12, 12–14, 12, 12, 11 and 11 in G5, G10, G15, G20, G25, G28, G30, G32, G34, G37 and G40, respectively (Fig. 1). Females survived longer than males in all tested generations. The age-stage-life expectancy (E_{xj}) of sequential generations is shown in Fig. 2. The highest female value of E_{xj} was 9.17, 8.14, 8.28, 6.73, 6.82, 6.75, 6.01, 6.64, 6.70, 6.14 and 6.16 days for 5, 10, 15, 20, 25, 28, 30, 32, 34, 37 and 40th generations, respectively (Fig. 2).

The age-specific survivorship (l_x), age-stage-specific fecundity of females (f_{xj}) and age-specific fecundity (m_x) of sequential generations of *T. embryophagum* are shown in Fig. 3. As indicated the highest egg laying period occurred in the first 3 days after emergence of females and then declined suddenly (Fig. 3). Peak value of m_x for *T. embryophagum* in G5, G10, G15, G20, G25, G28, G30, G32, G34, G37 and G40 occurred at the ages of 10 d (10.08 eggs), 12 d (9.11 eggs), 12 d (7.01 eggs), 11 d (6.74 eggs) 11 d (5.29 eggs), 11 d (5.54 eggs), 11 d (4.28 eggs), 11 d (4.19 eggs), 10 d (3.43 eggs), 11 d (3.38 eggs) and 11 d (3.98 eggs), respectively.

Population growth parameters

As shown in Table 2, all population growth parameters of *T. embryophagum* on *S. cerealella* were affected by sequential generations. The highest values of *GRR* (40.96 ± 3.71 eggs/individual), R_0 (38.21 ± 3.65 eggs/individual), r (0.296 ± 0.008 d^{-1}) and λ (1.345 ± 0.011 d^{-1}) were found in G5. By increasing generation numbers from G5 to G40, the values of *GRR*, R_0, r and λ decreased and their lowest values were obtained in G40. However, non-significant differences were observed among the values of *GRR*, R_0, r and λ in the 5th and 10th generations. The longest and shortest values of T were recorded in G10 with 12.63 ± 0.13d and 11.63 ± 0.15 d in G37, respectively.

Parasitism capacity

The age-stage-specific parasitism rate (c_{xj}) of *T. embryophagum* females on *S. cerealella* eggs over 40 generations is shown in Fig. 4. The highest values of c_{xj} in G5, G10, G15, G20, G25, G28, G30, G32, G34, G37 and G40 were 24.39, 25.11, 23.50, 16.04, 13.91, 13.18, 11.46, 13, 13, 11 and 12.75 hosts/parasitoid, respectively (Fig. 4). As per analysis of parasitism parameters, G5 had the highest value of net predation rate (c_0) (38.21 ± 3.65 hosts/parasitoid), stable parasitism rate (ψ) (0.348 ± 0.016 hosts/parasitoid) and finite parasitism rate (ω) (0.468 ± 0.26 hosts/parasitoid/day). However, the lowest values for c_0, ψ and ω were observed in G40 with 9.75 ± 1.48 hosts/parasitoid, 0.226 ± 0.018 hosts/parasitoid and 0.274 ± 0.026 hosts/parasitoid/day, respectively (Table 3). The transition rate values (Q_p) for all generations were close to 1 ($R_0 \approx c_0$), because

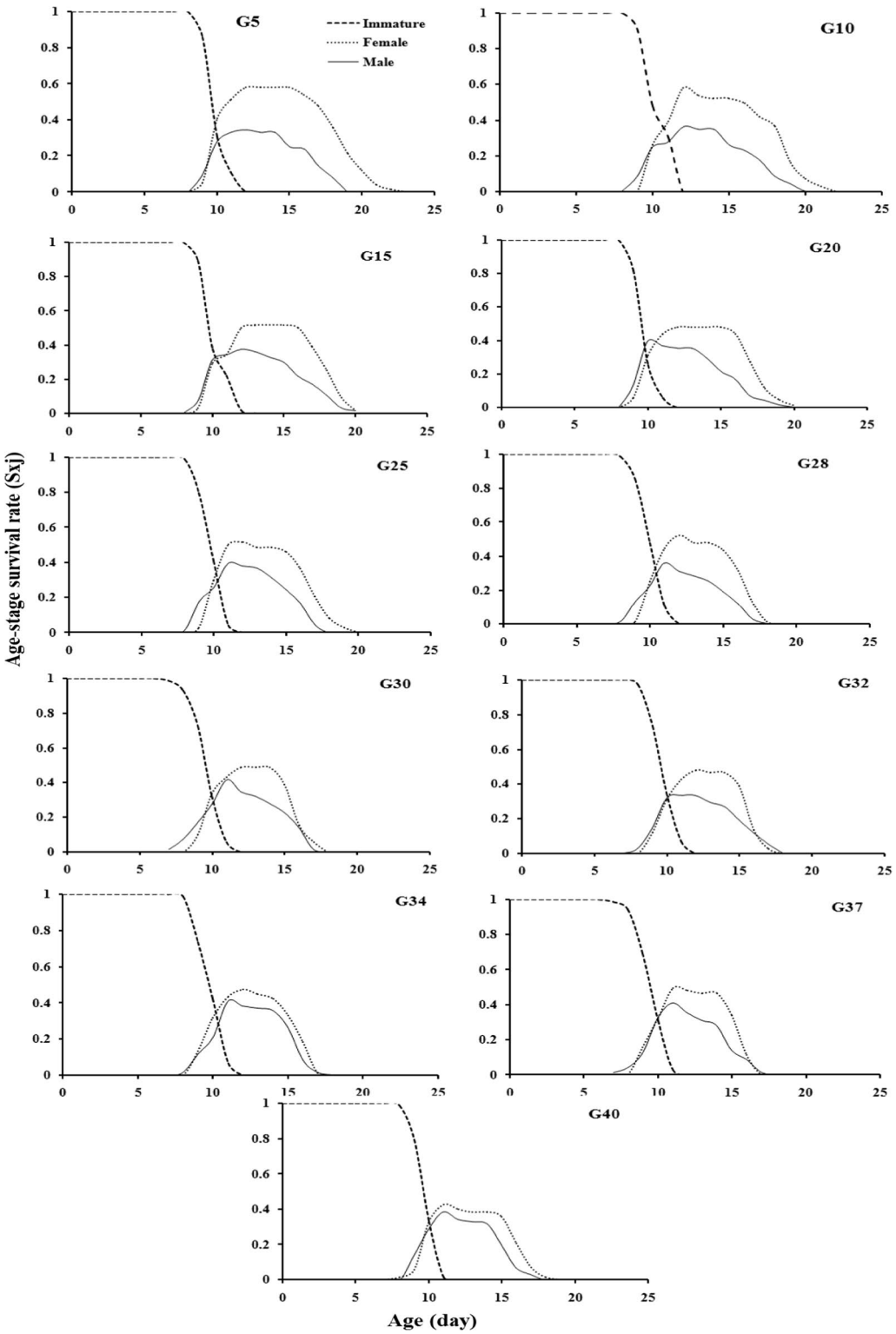

Fig. 1 The age-stage survival rate (s_{xj}) of sequential generations (G) of *T. embryophagum* reared on *S. cerealella* eggs

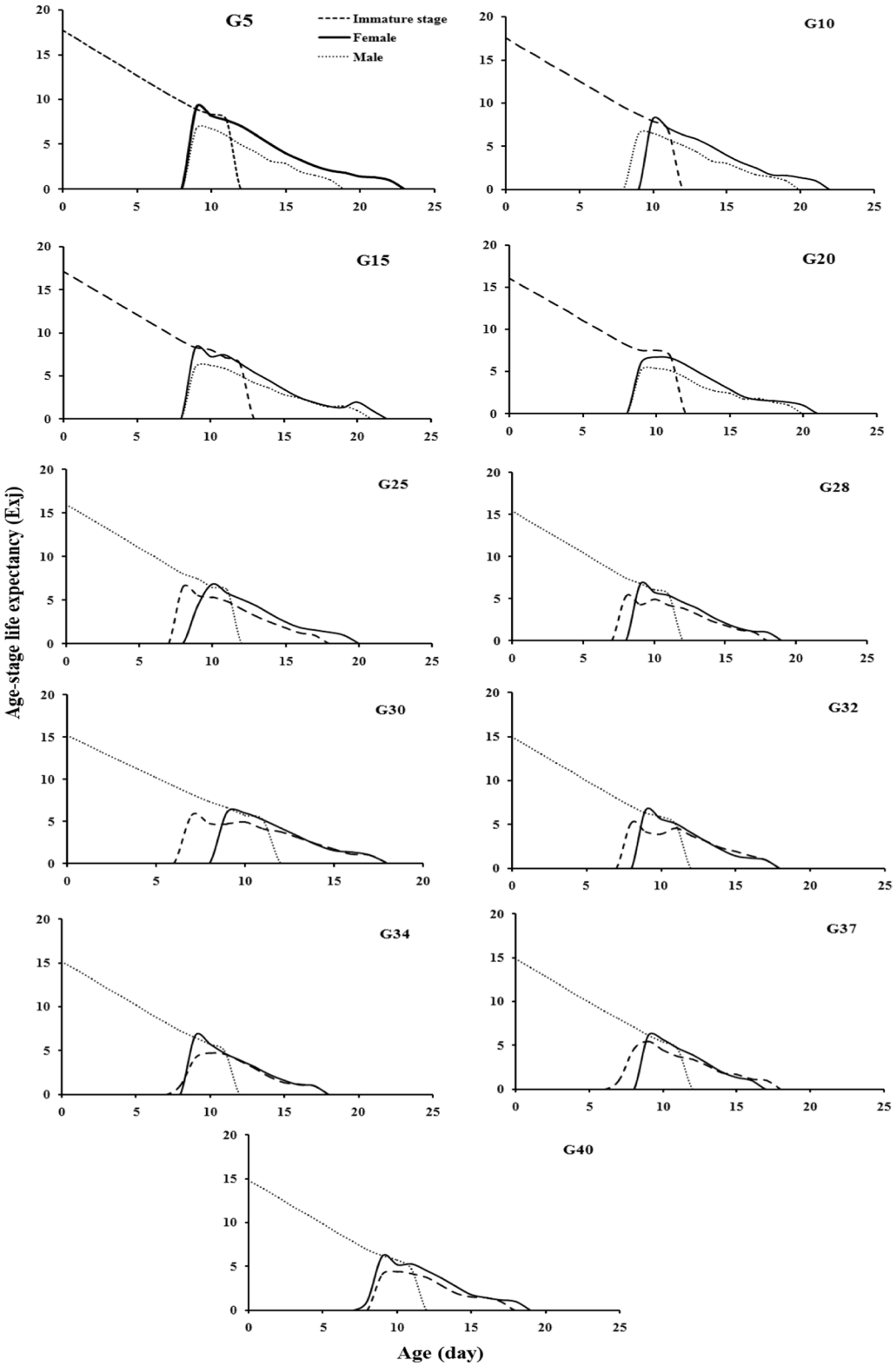

Fig. 2 The age-stage-life expectancy (E_{xj}) of sequential generations (G) of *T. embryophagum* reared on *S. cerealella* eggs

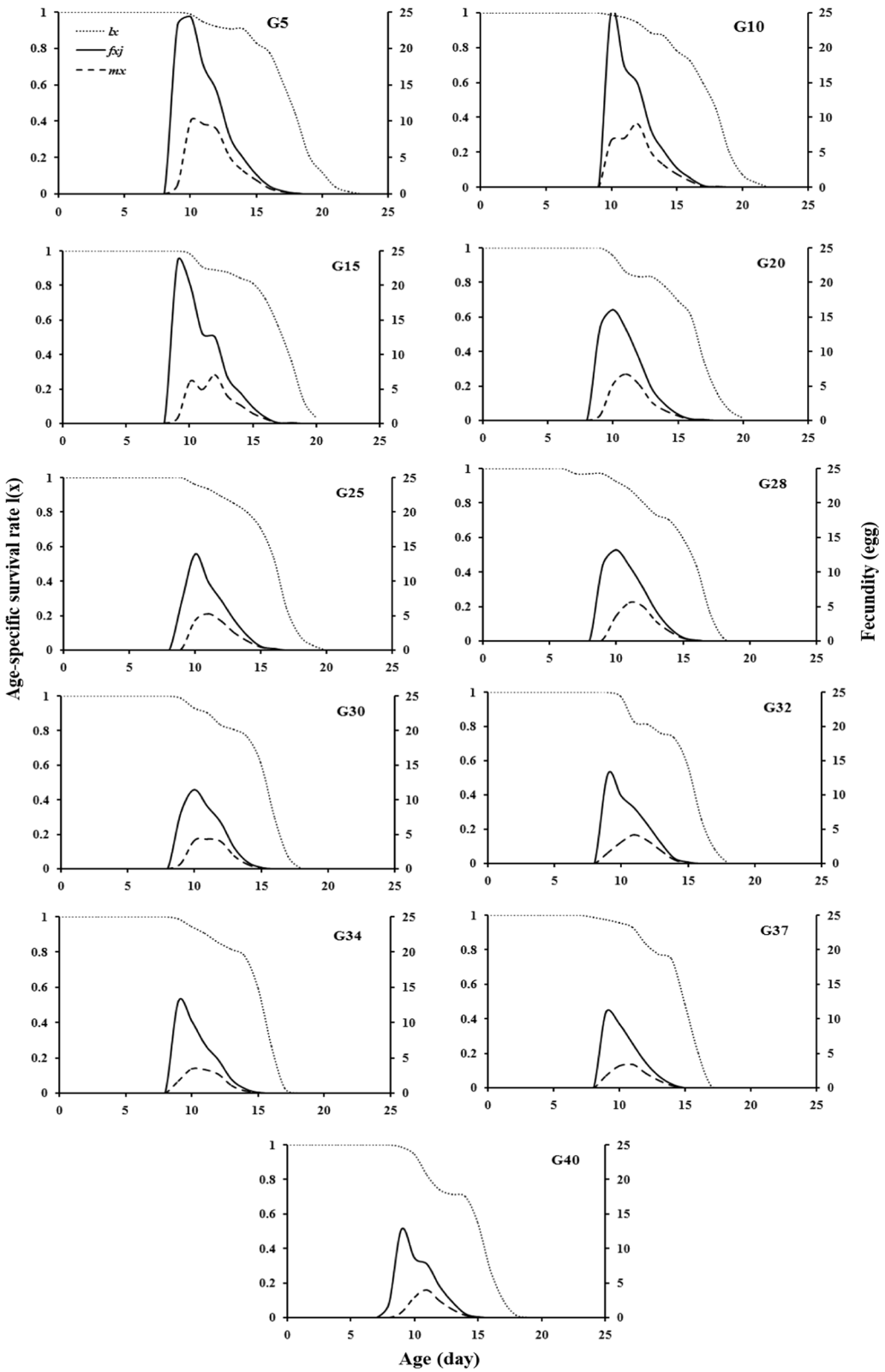

Fig. 3 The age-specific survivorship (l_x), age-stage-specific fecundity of female (f_{xj}) (eggs), and age-specific fecundity (m_x) of sequential generations of *T. embryophagum* reared on *S. cerealella* eggs

Table 2 Population growth parameters of sequential generations of *Trichogramma embryophagum* reared on *Sitotroga cerealella* eggs

No. generation	Gross reproductive rate (*GRR*) (eggs/individual)	Net reproductive rate (R_0) (eggs/individual)	Intrinsic rate of natural increase (r) (d^{-1})	Finite rate of increase (λ) (d^{-1})	Mean generation time (T) (d)
5	40.96 ± 3.71^{a}	38.21 ± 3.65^{a}	0.296 ± 0.008^{a}	1.345 ± 0.011^{a}	12.29 ± 0.13^{bc}
10	33.89 ± 3.60^{ab}	31.32 ± 3.53^{ab}	0.273 ± 0.010^{ab}	1.314 ± 0.013^{ab}	12.63 ± 0.13^{a}
15	27.48 ± 3.12^{bc}	24.73 ± 2.96^{bc}	0.257 ± 0.011^{bc}	1.293 ± 0.014^{bc}	12.48 ± 0.17^{ab}
20	22.72 ± 2.51^{cd}	19.62 ± 2.35^{cd}	0.244 ± 0.011^{cd}	1.277 ± 0.013^{cd}	12.18 ± 0.14^{bcd}
25	18.22 ± 2.00^{de}	16.43 ± 1.93^{de}	0.228 ± 0.010^{de}	1.256 ± 0.012^{de}	12.30 ± 0.11^{bc}
28	18.36 ± 2.01^{de}	15.75 ± 1.90^{def}	0.223 ± 0.010^{def}	1.250 ± 0.012^{def}	12.37 ± 0.14^{abc}
30	15.69 ± 1.76^{ef}	13.78 ± 1.68^{efg}	0.218 ± 0.011^{def}	1.244 ± 0.013^{def}	12.02 ± 0.13^{cde}
32	14.67 ± 1.60^{ef}	12.61 ± 1.53^{efg}	0.213 ± 0.011^{ef}	1.238 ± 0.014^{ef}	11.88 ± 0.12^{def}
34	12.50 ± 1.52^{f}	11.33 ± 1.45^{fg}	0.208 ± 0.012^{ef}	1.231 ± 0.014^{ef}	11.68 ± 0.14^{ef}
37	11.74 ± 1.45^{f}	10.65 ± 1.40^{g}	0.203 ± 0.012^{ef}	1.225 ± 0.015^{ef}	11.63 ± 0.15^{f}
40	11.74 ± 1.64^{f}	9.75 ± 1.48^{g}	0.192 ± 0.013^{f}	1.212 ± 0.016^{f}	11.87 ± 0.14^{def}

The means marked by the same letters within the same column are not significantly different (Paired-bootstrap test, $P < 0.05$)

according to the observation, *T. embryophagum* usually lays only one egg in each host egg.

Discussion

Maintaining the quality of natural enemies that reared for many generations is one of the most important problems which should be considered, because continuous mass rearing in insectaries with stable conditions may decrease their performance under field conditions (Bertin et al. 2017). Although based on the information obtained from this study, *T. embryophagum* was able to complete its life cycle on *S. cerealella* eggs over 40 generations, but its life table parameters and parasitism performance significantly affected over generations.

As a result of this work, the immature developmental time of *T. embryophagum* was not influenced by increasing the generation number and varied from 10 to 11 days. In line with these results, the duration of immature stage of *T. brassicae* reared on *S. cerealella* eggs over 45 generations was not affected by increasing generation numbers (Ghaemmaghami et al. 2021a). The developmental times of *T. embryophagum* on *Plutella xylostella* (Akbari et al. 2012) and on *E. ceratoniae* eggs (Mohseni et al. 2016) were 10 d, which are close to the present findings.

Different generations of *T. embryophagum* showed significant variations in adult longevity. Obtained results were in agreement with those of Ghaemmaghami et al. (2021a, b), who reported a significant decrease in female's adult longevity of *T. brassicae* reared on *S. cerealella* and *E. kuehniella* Zeller over 45 generations. Also, similar results reported for *Trichogramma dendrolimi* Matsumura over 30 generations (Lu et al. 2017). Ranjbar Aghdam and Attaran (2015) reported 7.15 d for adult longevity of *T. embryophagum* on *S. cerealella*, close to the obtained in G5 (7.98 d). But Haghani (2001) reported 12.37 and 11.29 d as adult longevity for *T. embryophagum* on *E. kuehniella* and *S. cerealella*, respectively, which are greater than our data.

In present study, a significant difference in ovipositional days was observed among sequential generations and the longest ovipositional days were observed in G5 with 5.83 d and with increasing the generation number, its value declined reaching 3.53 d in G40. However, a non-significant difference was observed between the ovipositional days in the 5th and 10th generations. On the contrary, Ghaemmaghami et al. (2021a) reported that the ovipositional days of *T. brassicae* reared on *S. cerallella* eggs were non-significantly different among generations until G35. Mohseni et al. (2016) reported 8.20 d for ovipositional days of *T. embryophagum* on *E. ceratoniae* eggs which was longer than those obtained in this study. These differences can be due to the different host species.

Fecundity is a very important criterion for assessing the quality of reared parasitoids, because it determines the cost of mass production and can be influenced during long-term mass rearing. In this work, the fecundity of *T. embryophagum* decreased over 40 generations, which is similar to those reported for *T. brassicae* reared on *S. cerealella* and *E. kuehniella* (Ghaemmaghami et al. 2021a,b). The values of fecundity obtained in the different generations in this study were less than those obtained by Mohseni et al. (2016) for this parasitoid on *E. ceratoniae* (74.40 eggs/female). Different factors may contribute to such a discrepancy such as the used population of parasitoids, host species and some reared conditions.

The sex ratio in mass rearing and inundative release of parasitoid wasps is an important factor (Ebrahimi 1996) that influencing the economic profitability of

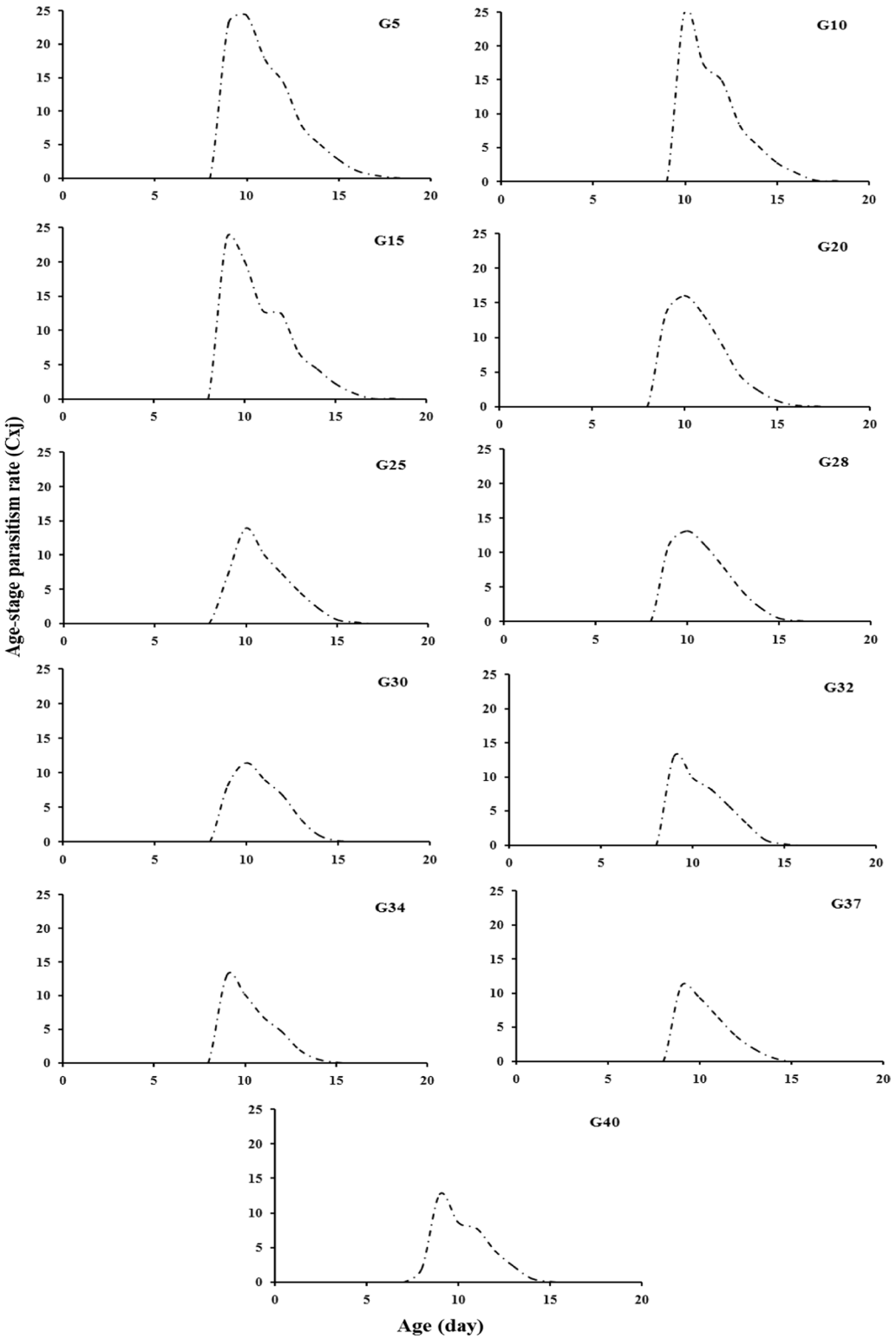

Fig. 4 The age-stage parasitism rate (c_{xj}) of sequential generations of *T. embryophagum* reared on *S. cerealella* eggs

Table 3 Parasitism parameters of sequential generations of *Trichogramma embryophagum* reared on *Sitotroga cerealella* eggs

No. generation	Net parasitism rate (c_0) (hosts/parasitoid)	Transition rate values (Q_p)	Stable parasitism rate (ψ) (host/parasitoid)	Finite parasitism rate (ω) (host/parasitoid)
5	38.21 ± 3.65^{a}	1	0.348 ± 0.016^{a}	0.468 ± 0.026^{a}
10	31.32 ± 3.53^{ab}	1	0.317 ± 0.017^{ab}	0.417 ± 0.027^{ab}
15	24.73 ± 2.96^{bc}	1	0.298 ± 0.018^{bc}	0.386 ± 0.028^{bc}
20	19.62 ± 2.36^{cd}	1	0.284 ± 0.017^{bcd}	0.363 ± 0.026^{bcd}
25	16.43 ± 1.93^{de}	1	0.267 ± 0.016^{cde}	0.335 ± 0.023^{cde}
28	15.75 ± 1.90^{def}	1	0.261 ± 0.016^{cde}	0.326 ± 0.023^{cde}
30	13.78 ± 1.68^{efg}	1	0.254 ± 0.017^{cde}	0.316 ± 0.024^{cde}
32	12.61 ± 1.53^{efg}	1	0.249 ± 0.017^{de}	0.309 ± 0.024^{de}
34	11.33 ± 1.45^{fg}	1	0.243 ± 0.018^{de}	0.299 ± 0.025^{de}
37	10.65 ± 1.37^{g}	1	0.238 ± 0.017^{de}	0.292 ± 0.024^{e}
40	9.75 ± 1.48^{g}	1	0.226 ± 0.018^{e}	0.274 ± 0.026^{e}

The means marked by the same letters within the same column are not significantly different (Paired-bootstrap test, $P < 0.05$)

mass rearing (Badran et al. 2020). In accordance with IOBC guidelines, that sex ratio of *Trichogramma* species in mass production programs should be higher than 50% (van Lenteren et al. 2002). In the present study, the ratio of females was greater than 50% female in all generations, except in G34 and G40. But contrary to those reported for some species of *Trichogramma* (Nordlund et al. 1997; Pratissoli et al. 2004; Ghaemmaghami et al. 2021a, b), current study shows that sex ratio percentage of *T. embryophagum* is affected by rearing generations.

Intrinsic rate of natural increase (r) along with net reproductive rate (R_0) is a key parameter in life table studies (Bahirai et al. 2019). R_0 is defined as the average number of female offspring that would be born by each female during its life span. The value of R_0 decreased from 38.21 eggs/individual in G5 to 9.75 eggs/individual in G40. R_0 in this study in G5 (38.21 eggs/individual) was close to those reported for the parasitoid on *S. cerealella* by Haghani and Fathipour (2003) (37.63 eggs/individual) and on *E. cerotoniae* (37.20 eggs/individual) by Mohseni et al. (2016).

Also the mean generation time (T) is defined as the time required for a newborn female to replace herself by R_0-folds at the stable age-stage distribution. Although T values in the present study differed among generations, they did not show a regular trend. Accordingly, each female required 12.63 d to be replaced by 31.32 females in G10, but in G37 each female was replaced by 10.65 females in 11.63 d. According to these results, the profitability of producers was greatly reduced due to the role of females in reproduction in recent generations.

Haghani and Fathipour (2003) reported 16.49 and 16.37 d for mean generation time (T) of *T. embryophagum* on *S. cerealella* and *E. kuehniella*, respectively, that are higher than those obtained in our study. According to Mohseni et al. (2016), the mean generation time (T) of this parasitoid on *E. cerotoniae* was 11.64 d that is close to the obtained finding.

Intrinsic rate of natural increase (r) combined the effects of mortality, duration of life stage and fecundity; therefore, it is influenced by preimaginal survival, developmental time, longevity of females, fecundity value and sex ratio, which are all affected in long-term mass rearing. In the present study, the intrinsic rate of natural increase (r) of *T. embryophagum* decreased from 0.296 d^{-1} in G5 to 0.192 d^{-1} in G40. Also, in accordance with those reported for *T. brassicae* reared on *S. cerealella,* we found no significant difference between the r values in the 5th and 10th generations. Similar to the present study, Ghaemmaghami et al. (2021a, b) showed the r value of *T. brassicae* reared on *S. cerealella* and *E. kuehniella* eggs decreased from 0.250 d^{-1} and 0.242 d^{-1} in G5 to 0.167 d^{-1} and 0.174 d^{-1} in G45. Haghani and Fathipour (2003) reported 0.218 d^{-1} as r for this parasitoid reared on *S. cerealella*. Also Mohseni et al. (2016) reported that the value of intrinsic rate of natural increase was 0.311 d^{-1} that is higher than the obtained results.

Finite rate of increase (λ) also varied among successive generations. The results showed that the population of *T. embryophagum* in the 5th generation increased 1.345 times daily than the previous day, while in the 40th generation, its population increased by 1.212 times every day than the previous day. Haghani (2001) reported that the finite rate of increase for this species on *S. cerealella* was 1.244 (day^{-1}) which is close to our finding in 30th generation.

In the present study, numbers of hosts parasitized by each female (c_0) decreased with increasing generations,

ranging from 38.21 (hosts/parasitoid) in G5 to 9.75 (hosts/parasitoid) in G40. Although the finite parasitism rate of *T. embryophagum* was influenced by generations, non-significant differences in finite parasitism rate (ω) until the 10th generation were found.

Conclusions

In the present study, the life table and parasitism parameters of *T. embryophagum* on *S. cerealella* eggs over 40 sequential generations were studied. The values of more estimated parameters were affected by generations. These findings suggested that after 10 generations, the reared population should be refreshed regularly by adding wild individuals collected from the field to strengthen the culture.

Abbreviations

G: Generation; *GRR*: Gross reproductive rate; R_0: Net reproductive rate; r: Intrinsic rate of natural increase; ω: Finite parasitism rate; s_{xj}: Age-stage survival rate; f_{xj}: Age-stage-specific fecundity of female; l_x: Age-specific survivorship; m_x: Age-specific fecundity; E_{xj}: Age-stage life expectancy; λ: Finite rate of increase; T: Mean generation time; k_x: Age-specific parasitism rate; c_0: Net parasitism rate; Q_p: Transition rate values; ψ: Stable parasitism rate; c_{xj}: Age-stage parasitism rate; a_{xj}: Stable age-stage structure; SASD: Stable age-stage distribution; d: Day.

Acknowledgements

This research is a part of the Ph.D dissertation of the first author and was supported by Islamic Azad University, Arak branch, which is greatly appreciated.

Authors' contributions

All authors conceived, performed and designed the study. Material preparation, data collection and analysis were mainly performed by FS, AN and ShJ. The first draft of the manuscript was written by FS and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Compliance with ethical approval.

Consent for publication

Not applicable.

Competing interests

Not applicable.

Author details

[1]Department of Entomology, Arak Branch, Islamic Azad University, Arak, Iran. [2]Department of Sustainable Agriculture, Food Security Research Institute, Arak branch, Islamic Azad University, Arak, Iran. [3]Department of Plant Protection, Faculty of Agriculture, Lorestan University, Khorramabad, Iran.

Received: 11 May 2021 Accepted: 7 August 2021
Published online: 30 August 2021

References

Akbari F, Askarianzadeh A, Zamani AA, Hosseinpour MH (2012) Biological characteristics of three *Trichogramma* species on the eggs of diamondback moth (*Plutella xylostella* L.). Arch Phytopathol Pflanzenschutz 1:1–5

Badran F, Fathipour Y, Bagheri A, Attaran M, Reddy GVP (2020) Effects of prolonged mass rearing on life history traits of *Habrobracon hebetor* (Hymenoptera: Braconidae). Int J Pest Mang. https://doi.org/10.1080/09670874.2020.1830198

Bahirai F, Jafari Sh, Lotfollahi P, Shakarami J (2019) Effect of temperature on life table parameters of *Rhyncaphytoptus ficifoliae* Keifer (Trombidiformes; Diptilomiopidae). Syst Appl Acarol 24(8):1394–1405

Bertin A, Pavinato VAC, Parra GRP (2017) Fitness-related changes in laboratory populations of the egg parasitoid *Trichogramma galloi* and the implications of rearing on factitious hosts. Biol Control 62(4):435–444

Chi H (1988) Life-table analysis incorporating both sexes and variable development rates among individuals. Environ Entomol 17:26

Chi H (2019a) CONSUME-MSChart: computer program for consumption rate analysis based on the age stage, two-sex life table. 2019.02.01 ed. Available in: http://140.120.197.173/Ecology/Download/CONSUMSChart.zip. Accessed on 01 Feb 2019

Chi H (2019b) TWOSEX-MSChart: a computer program for the age-stage, two-sex life table analysis. 2019.01.09 ed. Available in: http://140.120.197.173/Ecology/Download/TWOSEX-MSChart.rar. Accessed on 09 Jan 2019

Chi H, Yang TC (2003) Two-sex life table and predation rate of *Propylaea japonica* Thunberg (Coleoptera: Coccinellidae) fed on *Myzus persicae* (Sulzer) (Homoptera: Aphididae). Environ Entomol 32:327–333

Chi H, Mou DF, Allahyari H, Yu JZ, Huang YB, Yang TC, Farhadi R, Gholizadeh M (2011) Finite predation rate: a novel parameter for the quantitative measurement of predation potential of predator at population level. Nat Preced. https://doi.org/10.1038/npre.2011.6651.1

Cônsoli F, Parra JR, Zucchi R (2010) Egg Parasitoids in agroecosystems with emphasis on *Trichogramma*. Springer, Netherlands, Dordrecht

Ebrahimi E (1996) The species of *Trichogramma* Westwood in Iran. In: Proceedings of the twentieth international congress of entomology; Firenze, Italy

Ebrahimi E, Pintureau B, Shojai M (1998) Morphological and enzymatic study of the genus *Trichogramma* in Iran (Hym. Trichogrammatidae). Appl Entomol Phytopathol 66(1/2): 39–42

Ghaemmaghami E, Fathipour Y, Bagheri A, Talebi AA, Reddy GVP (2021a) Quality control of the parasitoid wasp *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) over 45 generations of rearing on *Sitotroga cerealella*. Insect Sci 28: 180–190

Ghaemmaghami E, Fathipour Y, Bagheri A, Talebi AA, Reddy GVP (2021b) Continuous rearing on *Ephestia kuehniella* reshaped quality of the parasitoid wasp *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae). J Asia-Pacific Entomol. https://doi.org/10.1016/j.aspen.2021.02.013

Haghani M (2001) Investigation on demography and behavior of the *Trichogramma embryophagum* (Hym.: Trichogrammatidae) on laboratory hosts [MS thesis]. Tarbiat Modares University, Tehran, p 97

Haghani M, Fathipour Y (2003) The effect of the type of laboratory host on the population growth parameters of *Trichogramma embryophagum* Hartig (Hym: Trichogrammatidae). J Agric Sci Natur Resour 2(2):117–124

Huang YB, Chi H (2013) Life tables of *Bactrocera cucurbitae* (Diptera: Tephritidae): with an invalidation of the jackknife technique. J Appl Entomol 137:327–339

Lu X, Han S, Li Z, Li L (2017) Biological characters of *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) reared *in vitro* versus *in vivo* for thirty generations. Sci Rep 7:17928

Minitab (2017) Minitab 18 statistical software. Minitab Inc., State College, Pennsylvania, USA

Mohseni E, Abbasipour H, Attaran MR, Askarianzadeh A (2016) Evaluation of the life table characteristics of three species of the genus *Trichogramma* on the carob moth, *Ectomyelois ceratoniae* under laboratory conditions. Biocontrol Plant Protect 3(2):49–58

Nordlund DA, Wu ZX, Greenberg SM (1997) *In vitro* rearing of *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae) for ten generations, with quality assessment comparisons of *in vitro* and *in vivo* reared adults. Biol Control 9:201–207

Pomari-Fernandes A, Buen AF, Queiroz AP, De Bortoli SA (2015) Biological parameters and parasitism capacity of *Telenomus remus* Nixon (Hymenoptera: Platygastridae) reared on natural and factitious hosts for successive generations. Afr J Agric Res 10(33):3225–3233

Pratissoli D, Oliveira H, Roberto Gonçalves J, Zanuncio J (2004) Changes in biological characteristics of *Trichogramma pretiosum* (Hym.: Trichogrammatidae) reared on eggs of *Anagasta kuehniella* (Lep.: Pyralidae) for 23 generations. Biocontrol Sci Tech 14:313–319

Ranjbar Aghdam H, Attaran M (2015) Collecting, identifying and selecting a native strain of egg parasitoid wasps, *Trichogramma* spp. for biological control of codling moth, *Cydia pomonella* in Damavand region. Biol Control Pests Plant Dis 46 (1): 1–8

Smith SM (1996) Biological control with *Trichogramma*: advances, successes and potential for their use. Ann Rev Entomol 41:375–406

van Lenteren J, Hale A, Klapwijk JN, Schelt Steinberg S (2002) Guidelines for quality control of commercially produced natural enemies. In: van Lenteren J (ed) Quality control and production of biological control agents: theory and testing procedures. CAB International, Wallingford, pp 265–303

van Lenteren JC (2003) Quality control and production of biological control agents: theory and testing procedures, p. 327. CABI, Wallingford, Oxon, UK

Vinson SB, Greenberg SM, Liu TX, Rao A, Volosciuc LF (2015) Biological control of pests using Trichogramma: current status and perspectives. Northwest A & F University Press, Yangling

Yu JZ, Chi H, Huei-Chen B (2013) Comparison of the life table and predation rate of *Harmonia dimidiate* (F.) (Coleoptera: Coccinellidae) fed on *Aphis gossypii* Glover (Hemiptera: Aphididae) at different temperatures. Biol Control 64:1–9

Publisher's Note