Climatically Isolated Populations of *Habrobracon hebetor* Say (Hymenoptera: Braconidae) Demonstrate Striking Differences in Life History Traits

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**ABSTRACT**

Augmentative release of native natural enemies is a prominent strategy for suppression of crop pests. Intrinsic differences among populations of natural enemies may affect their efficiency in pest management programs. We characterized life history traits of 13 climatically and geographically isolated populations of *Habrobracon hebetor* Say (Hymenoptera: Braconidae) from different regions of Iran to assess their suitability for biological control of noctuid moths. All experiments were performed at 25±1°C, 65±5 RH and 16:8 (L:D) hours photoperiod regime. *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) was used as a laboratory host. Our results revealed significant variation in female longevity, paralysis and parasitism rate, sex ratio, reproductive rate and host allocation among different *H. hebetor* populations tested. The highest number of offspring (124.2) and parasitized larvae (160.1) were observed in Bandar Lengeh and the lowest ones was observed in Urmia and Jiroft, respectively (3.4 and 9.3 for Urmia and 3.1 and 10.1 for Jiroft). Also, the wasps of Bandar Lengeh paralyzed more than 95% of the introduced host larvae. The longest female longevity and male production (21.3 days and 80, respectively) were in Bandar Abbas and Bandar Lengeh (21.3 days and 80, respectively), while the lowest ones were in Dehloran (12.9 days and 40.2, respectively). Gorgan population deployed the highest number of *Ephestia kuehniella* larvae in foraging behavior test. These results show considerable variation in the life history traits of various populations of *H. hebetor*, which may affect performance of these populations under field condition.

**Keywords:** Biological control, Ectoparasitoid, *Ephestia kuehniella*, Host allocation, Population differentiation.

**INTRODUCTION**

Life history theory, and particularly the concept of trade-offs, provides an important tool for understanding the evolutionary processes (Burton *et al.*, 2010, Aktipis *et al.*, 2013, Flatt and Heyland, 2011). Life history traits include traits that affect fitness directly, such as resource acquisition and allocation, foraging behavior, stress tolerance, resisting parasite or predator attack, and intraspecific competitiveness (Boggs, 2009, Wajnberg *et al.*, 2012). Therefore, life history considers organisms from both ecological and evolutionary perspectives (Mopper and Strauss, 2013, Wong *et al.*, 2013). There is a lot of information concerning the effect of

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population differentiation on the life history traits of natural enemies. Liu et al. (2001) showed that geographic populations of *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae), a pupal parasitoid of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) were different in survival and female progeny at the same temperature. Difference in the life history traits among geographically distinct populations of natural enemies has also been revealed regarding *Asobara tabida* Nees (Hymenoptera: Braconidae) on *Drosophila melanogaster* meigen (Diptera: Drosophilidae) (Kraaijeveld and van Alphen, 1994), *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) on *Sarcophaga bullata* Parker (Diptera: Sarcophagidae) (Parker and Orzack, 1985; Orzack and Parker, 1990), *Leptopilina boulardi* (Barbotin et al., 1979) (Hymenoptera: Figitidae) on *D. melanogaster* (Moiroux et al., 2010), and *Microtonus aethiopoides* Loan (Hymenoptera: Braconidae) on *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) (Sundaralingam et al., 2001).

Parasitoid wasps are amongst the most intensively studied insects in terms of life history dynamics due to their top-down role in host population dynamics, which makes them economically as well as ecologically important (Roux et al., 2010). Differences in the spatial distribution of host patches, the length of seasons, host availability and suitability, and competition between parasitoids, all contribute to the selection and diversification in life history among populations of a parasitoid insect (Ellers and van Alphen, 1997).

Parasitoids are frequently employed as biological control agents. Biological control is a sustainable pest control approach that has been successful in controlling several destructive insect pests (Huffaker, 2012). However, not all instances in which biological control has been attempted, have been successful. Low performance of a particular biological agent would discourage wider application of biological control (Hance et al., 2007). Some attempts have failed to suppress insect pests due to lack of sufficient information on which population of natural enemies would be most effective (Denoth et al., 2002). Geographically and climatically different populations of a parasitoid species can be highly diverged in life history traits (Liefing et al., 2009). Therefore, basic studies on intra-species, population-level variation in life history traits may provide crucial information when deciding which natural enemies to employ, hence, quantifying life history traits variation in climatically or geographically diverged populations of parasitoid wasps is a necessary step when designing a biological control program.

*Habrobracon hebetor* has been widely involved in various host-parasitoid interaction studies due to its high reproductive rate, short generation time and considerable host-species range (Casas, 1989; Kidd and Jervis, 1989; Brower and Press, 1990; Akinkurolere et al., 2009; Askari Seyahooei, 2010). In the current study we compare life history traits of different populations of *Habrobracon hebetor* Say (Hymenoptera: Braconidae) to evaluate the potential of locally adapted populations as candidates for involvement in biological control programs. This parasitoid is widely distributed across Iran and forms geographically and climatically isolated populations (Farahbakhsh, 1961; Forouzan et al., 2008; Askari Seyahooei et al., 2018 a, b). Hormozgan province is located in south of Iran along the Persian Gulf and its climate is characterized by short temperate winters and relatively long, dry and hot summers in which hosts for activity of *H. hebetor* are usually available. Western and Northwestern populations from Dehloran and Urmia experience a much colder climate that restricts activity of *H. hebetor* to a short period in a year.

**MATERIALS AND METHODS**

**Host Rearing**

The Mediterranean flour moth, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae), was used as laboratory host for rearing the
parasitoid, *H. hebetor*. *E. kuehniella* eggs were prepared from the second generation of a laboratory-reared stock in Agricultural Research Center of Hormozgan (Iran), originally established from a wild-type collected from naturally infested flour. Rearing was performed using plastic basin containers (40x18 cm) filled with 1,000 g of a 2:1 mixture of wheat flour and rough wheat bran. The food was decontaminated at 60°C for 2 days and then 0.2 g of flour moth eggs were dispersed on top of the substrate. The containers were then covered with black sterile cotton cloth. All culturing and experiments described below were performed at 25±1°C, 65±5 RH and 16:8 (L:D) photoperiod regime (Chen et al., 2011).

Populations of *H. hebetor* were sampled from 13 climatically and geographically different locations across Iran (Figure 1). To perform trapping, 10 fourth and fifth instar larvae *E. kuehniella* were enclosed between two layers of netting, put on the open side of plastic cages (3x7x6 cm) and placed in the field for 24 hours. The parasitized larvae were incubated in a growth chamber under 25±1°C, 65±5 RH and 16:8 (L:D) photoperiod until emergence of the *H. hebetor* adults. The wasps were held individually inside ventilated clear plastic cages covered with white muslin net under the same conditions prior to experiment. The wasps were supplied with a saturated sugar solution on cotton rolls as a carbohydrate source during the oviposition period. This experiment was conducted with 13 treatments, each replicated five times. Each replication consisted of three plastic cages.

**Longevity**

To study longevity in various populations of *H. hebetor*, two newly emerged females (< 24 hours) and one male were housed together for 24 hours. The females were then introduced together into a plastic cage containing 10 fifth-instar larvae of *E. kuehniella*. The wasps were transferred daily into new plastic cages containing 10 fresh fifth-instar host larvae. All wasps were provided with a saturated sugar solution for their entire life. Survival of the wasps was recorded daily until the death of the last wasp in each tube. The experiment was replicated three times for each population, with 10 wasps in each replicate (total of 30

Figure 1. Map of the Islamic Republic of Iran. ID codes on the map refer to the sampling points of the *Habrobracon hebetor*. BA= Bandar Abbas; BL= Bandar Lengeh; DL= Dehloran; GR= Gorgan; HA= Haji Abad; HD= Hamadan; IF= Isfahan; JI= Jiroft; MN= Minab; RD= Rudan; SH= Shiraz; SZ= Sarpol-e-Zahab, UR= Urmia.
female wasps for each population).

**Paralysis and Parasitism Rate Experiments**

To study host paralysis and parasitism rates in the different populations of *H. hebetor*, newly emerged wasps were provided with larvae of *E. kuehniella*. To do so, two mated female wasps were released into a ventilated clear plastic cage (9.5×7.5×5.5 cm) containing 10 fifth instar *E. kuehniella* larvae. After 24 hours, the wasps were transferred to new cages with 10 fresh fifth instar host larvae using an aspirator. This was repeated until the death of the last wasp for each population. The number of paralyzed larvae was counted daily by stimulating the larvae with a smooth painting brush and checking their movement. The numbers of emerged wasps and moths were recorded to calculate parasitism rate.

**Sex Ratio and Fecundity**

Total fecundity for each population was obtained by calculating the total number of progeny from the longevity and paralysis/parasitism rate experiments. Sex ratio was also extracted from the same data by determining the sex of the emerged wasps in both experiments.

**Host Finding**

A handmade, four-channel glass olfactometer (arm length 15 cm, arm diameter 2.5 cm and the central cavity diameter 5 cm) was used to study host-finding behavior of *H. hebetor* in the different populations. To do so, two arms of the olfactometer were supplied with 10 fifth instar host larvae and the other two arms were left empty. Host-searching behavior of *H. hebetor* in various populations was investigated by releasing 10 female wasps for each replicate in the central cavity of the olfactometer and recording their movement for 2 hours. We recorded the number of wasps entering channels with or without host larvae or remaining in the central cavity at 5 min intervals during 2 hours.

**Data Analysis**

The normality of data was checked by kurtosis and skewness tests in SPSS prior to analysis of the data. One-way Analysis Of Variance (ANOVA) was used to test for significant differences between populations. Means were compared by Least Significant Difference (LSD) tests when significant differences were found in the measured parameters. Statistical analysis was performed in SAS version 9.1.3.

**RESULTS**

**Longevity**

Female longevity differed significantly among *H. hebetor* populations from different climatic zones (*F*= 2.65; df= 12, 182; *P*< 0.01). The longevity was the longest in Bandar Abbas and Bandar Lengeh (both 21.3 days) and the shortest in Dehloran (12.9 days; Table 1).

**Progeny**

The number of progeny per female differed profoundly between the different populations of *H. hebetor* (*F*= 80.94; df= 12, 182; *P*< 0.01). The population from Bandar Lengeh produced the highest number of progeny (124.2 offspring) followed by Bandar Abbas (86.5 offspring), while the population from Urmia only produced 3.4 progeny (Table 1).
### Table 1. Mean (±SE) values of the life history trait studied in the different populations of *Habrobracon hebetor.* *a*

<table>
<thead>
<tr>
<th>Population</th>
<th>No of the paralyzed larvae</th>
<th>No of progeny</th>
<th>No of the parasitized larvae</th>
<th>Female longevity</th>
<th>Sex ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA</td>
<td>199.9 ± 11.77a</td>
<td>86.5 ± 7.03b</td>
<td>110.4 ± 8.17b</td>
<td>21.3 ± 1.40a</td>
<td>80.1 ± 3.26a</td>
</tr>
<tr>
<td>DL</td>
<td>119.7 ± 11.04b</td>
<td>7.1 ± 1.33</td>
<td>21.2 ± 3.53ef</td>
<td>12.9 ± 1.23c</td>
<td>40.2 ± 6.57c</td>
</tr>
<tr>
<td>IF</td>
<td>169.8 ± 19.28ab</td>
<td>16.5 ± 2.99efgh</td>
<td>42.7 ± 6.06cde</td>
<td>18.2 ± 2.13abc</td>
<td>57.3 ± 6.71abc</td>
</tr>
<tr>
<td>GR</td>
<td>149.1 ± 15.31ab</td>
<td>21.1 ± 5.18defg</td>
<td>41.4 ± 6.60cde</td>
<td>15.0 ± 1.54bc</td>
<td>56.9 ± 5.95abc</td>
</tr>
<tr>
<td>HA</td>
<td>194.3 ± 15.31a</td>
<td>69.7 ± 6.35c</td>
<td>96.9 ± 8.35b</td>
<td>20.3 ± 1.63abc</td>
<td>68.5 ± 2.89ab</td>
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<tr>
<td>HD</td>
<td>150.7 ± 16.16ab</td>
<td>13.3 ± 1.98efgh</td>
<td>33.9 ± 3.82cde</td>
<td>16.4 ± 1.77abc</td>
<td>54.1 ± 5.12bc</td>
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<tr>
<td>JJ</td>
<td>135.0 ± 9.42b</td>
<td>3.1 ± 1.04f</td>
<td>10.1 ± 3.28f</td>
<td>14.5 ± 1.02bc</td>
<td>52.4 ± 11.94bc</td>
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<tr>
<td>BL</td>
<td>203.1 ± 15.24a</td>
<td>124.2 ± 6.23a</td>
<td>160.1 ± 7.13a</td>
<td>21.3 ± 1.63a</td>
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<td>MN</td>
<td>174.6 ± 21.39ab</td>
<td>28.2 ± 3.21de</td>
<td>52.7 ± 5.96c</td>
<td>18.0 ± 2.14abc</td>
<td>61.5 ± 6.91abc</td>
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<tr>
<td>RD</td>
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<td>32.3 ± 4.39d</td>
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<tr>
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<td>155.5 ± 13.26ab</td>
<td>8.5 ± 2.07fgh</td>
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<td>17.1 ± 1.60abc</td>
<td>49.1 ± 7.80bc</td>
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<tr>
<td>SI</td>
<td>174.7 ± 20.07ab</td>
<td>22.7 ± 3.85dfe</td>
<td>48.2 ± 5.83cde</td>
<td>17.8 ± 2.14abc</td>
<td>70.9 ± 3.53ab</td>
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<tr>
<td>UR</td>
<td>134.2 ± 9.09b</td>
<td>3.4 ± 1.03h</td>
<td>9.3 ± 2.96f</td>
<td>14.3 ± 0.89bc</td>
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<td>57.23</td>
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<td>12/182</td>
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<td>12/182</td>
</tr>
<tr>
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<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.0027</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

*a ID codes in the table refer to the sampling points of *Habrobracon hebetor* populations (see Figure 1). Means in each column with the same letters are not significantly different according to LSD (P< 0.01). b Standard Error of Mean.*
Sex Ratio

The populations exhibited a male biased trend in their reproduction, although significant differences were found among populations in sex ratio (F= 3.04; df= 12, 182; P< 0.01). The populations from Bandar Abbas and Bandar Lengeh produced the strongest male-biased sex ratio (80.1 and 80.0% males, respectively). The population from Dehloran produced a female-biased sex ratio (40.2% males; Table 1).

Host Paralysis

Different populations of *H. hebetor* displayed significant differences in the number of paralyzed host larvae (F= 2.86; df= 12/182; P< 0.01). The highest numbers of paralyzed larvae per wasp occurred in Bandar Lengeh, Bandar Abbas, and Haji Abad populations (203.1, 199.9, and 194.3, respectively). Dehloran, Urmia and Jiroft showed the smallest number of paralyzed larvae per wasp (119.7, 134.2 and 135; Table 1).

Parasitism

Various populations of *H. hebetor* also differed in the number of parasitized host larvae (F= 57.23; df= 12, 182; P< 0.01). The highest number of parasitized host larvae per individual wasp occurred in Bandar Lengeh (160.1) followed by Bandar Abbas and Haji Abad (110.4 and 96.9, respectively). The lowest number of parasitized host larvae per wasp were observed in Urmia and Jiroft populations (9.3 and 10.1, respectively; Table 1).

Host Allocation

Significant variation in host allocation was observed among populations of *H. hebetor* (F= 1.13; df= 12/26; P< 0.01). The Rudan population showed significantly lower allocated hosts than the other populations (Figure 2).

DISCUSSION

In this study, we assessed life history traits in geographically and climatically
isolated populations of *H. hebetor*. Our results revealed striking differences between different populations in all life history traits investigated.

We found profound variation in parasitism and paralysis rates among populations of *H. hebetor*. The highest parasitism and paralysis rates were observed in Hormozgan populations (Bandar Lengeh, Bandar Abbas, and Haji Abad). The high aggressive behavior of Hormozgan *H. hebetor* populations to paralyze and parasitize *E. kuehniella* larvae may apply to other hosts as well. For example, Bagheri *et al.* (2019) showed a high efficiency of a Bandar Abbas *H. hebetor* population in controlling the cotton bollworm, *Helicoverpa armigera* (Hübner). (Lepidoptera: Noctuidae).

The efficacy of *H. hebetor* and *Trichogramma evanescence* Westwood (Hymenoptera: Trichogrammatidae) in this bio-control program was comparable to that of chemical control. High contrast in parasitism rate by releasing *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) against Asian citrus psyllid *Diaphorina citri* (Hemiptera: Liviidae) was reported by Pluke *et al.* (2008). Association of *Wolbachia* endosymbiont with the aggressive population of *T. radiata* was the main reason mentioned for this discrepancy in results. Similarly, variation in aggressive behavior of *H. hebetor* populations may be related to difference in bacterial endosymbiont fauna of the studied populations, which needs to be studied more.

We suggest that the mild winters in Hormozgan provide a long cycle for this parasitoid, which may allow *H. hebetor* to encounter more hosts and experience more parasitism behavior. By contrast, hosts are only available for a short period of the year in the northern sites, presumably selecting for reduced paralysis and parasitism rates.

Long lifespan enables parasitoids to encounter more hosts and produce more offspring. We found the longest female longevity and highest number of offspring in the southern populations from Bandar Lengeh and Bandar Abbas. This may be explained by the higher parasitism rates in these populations. Energy reserves obtained from hosts is expected to be an important factor to prolong longevity in parasitoids. It has been shown that higher amounts of protein gained in larval stages allows parasitoids to produce more offspring during the adult stage (Broadway and Duffey, 1986). Host-mediated differences in energy reserve of *H. hebetor* were demonstrated by Akinkurolere *et al.* (2009).

Sex ratio also showed substantial variation among the studied populations of *H. hebetor*. Half of the populations showed male-biased sex ratios (Bandar Abbas, Bandar Lengeh, Haji Abad, Rudan, Minab and Shiraz), while sex ratio in the other populations was close to 50:50. Nearly all tropical and subtropical populations (except for Jiroft) showed a male-biased sex ratio. These populations usually experience higher temperatures, which have been shown to affect sex ratio in insects (Hoelscher and Vinson, 1971). Askari-Seyahooie *et al.* (2018a) showed that sex ratio was not an important factor in augmentative releasing program compared with other life history traits like paralysis.

We found no differences in host allocation in all populations, except Rudan. Apparently, the different populations do not experience different selection pressures on host allocation. Alternatively, evolution of this trait may be constrained. However, it is possible that host allocation is affected by changing host or temperature (Casas, 1989; Stamp and Bowers, 1990; Amat *et al.*, 2006). This idea warrants further investigation. Although there are some important questions left to be answered in future studies, this study provided a basic information regarding populations’ ability that can be applied successfully in the future augmentative releasing programs. However, determining the Thermal thresholds (T0 and Tmax) and optimal Temperature (Topt) are important issues, which should be clarified in populations attempted to be involved in
augmentative programs. In addition, developmental threshold in both *H. hebetor* population and the host should be close to each other to reach a hopeful biocontrol result.

Nowadays, displacement of biological agents is a common worldwide trade, in which biological agents like parasitoid wasps are imported and released to control different pests in greenhouses or even in open fields. One of the most important worries regarding this practice is the threat of gene flow between locally adapted populations and imported exotic populations, which may disrupt the local adaptation. This challenge can be faded by focusing on selection of locally adapted populations.

**CONCLUSIONS**

This study demonstrated considerable variation in life-history traits of *H. hebetor* as a well-known parasitoid model studied in the laboratory condition. Variation in life-history traits influences performance of parasitoids in the field, which can be translated to the efficacy when these agents have been used in bio-control programs. From these results, we can conclude that local adaptation is the main platform to choose the right population of parasitoid in bio-control programs. Evolution in life-history traits may provide specific criteria to improve performance of a population in a particular climate in comparison with other populations from a different climate.

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Life History Traits in Populations of H. hebetor