Are the reproductive traits of two populations of *Propylaea quatuordecimpunctata* affected by the geographic conditions and the prey species?

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ABSTRACT

Life history parameters of the ladybird populations, *Propylea quatuordecimpunctata* (L.) (Coleoptera: Coccinellidae), collected at two locations in Iran (Karaj and Sari) were recorded on two prey species, *Aphis fabae* Scopoli, 1763 and *Aphis gossypii* Glover, 1877 (Homoptera: Aphididae). The duration of most developmental stages (egg, first, second and third instar larvae) of two local populations of *P. quatuordecimpunctata* differed significantly, except for the fourth instar and pupal periods. A comparison of life table parameters between the two populations with different preys, showed that the net reproductive rate (R_0) was not significantly different (P > 0.05). The intrinsic rate of increase (r), the mean generation time (T) and the finite rate of increase (λ) of the population from Sari on *A. gossypii*, differed significantly from the same population fed on *A. fabae* (P < 0.05). Our results demonstrated that reproductive traits of two populations of *P. quatuordecimpunctata* were affected by the geographic conditions and the prey species.

Keywords: *Aphis fabae*; *Aphis gossypii*; Coccinellidae; Intrinsic rate of increase; Population

Introduction

Biological pest control represents an important alternative for chemical pest control and has the advantage that it is permanent, economical, and environmentally friendly (1). However, before installing a biological control program, it is important to gather as much ecological information as possible on both players of the interaction. Part of this is useful to determine the ecological conditions most suitable for

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oviposition and other life table parameters of the control agent; therefore, such data are important to effectively use biological pest control (2). Hence, generating life history data for economically important species at different trophic levels is a basic and important task for pest management (3).

One way to describe the life history of an organism is the life table (e.g. 4, 5). A life table can provide important growth and developmental parameters, which is especially important as many organisms used for biological control not only have predatory stages (i.e larvae, adults), but also non-predatory stages (i.e. eggs and pupal stages) (3). Therefore, when selecting organisms as biological control agents, it is necessary to determine population characteristics, including growth rate, optimal growth conditions, stage differentiation and fecundity.

One of the most important life table parameters is the intrinsic rate of increase (r) for comparing the growth potential of different species under specific climatic and nutritional conditions and thus for selecting species as biological control agents (6). The intrinsic rate of increase as well as other life history traits may be affected by temperature (7, 8), food quality and quantity (3, 9, 10, 11, 12), specific physio-chemical parameters (13, 14); and also, biotic factors (e.g. competition, mutualisms) (15).

One of the most important insect pests of various crops in Iran is the cotton aphid, *Aphis gossypii* Glover, 1877 (Homoptera: Aphididae), a polyphagous and cosmopolitan species (16). It has been recorded on over 320 different plant species worldwide (17). Another major pest of several cultivated crops in the world is the black bean aphid, *Aphis fabae* Scopoli, 1763 (Homoptera: Aphididae), which also has a wide host range with more than 200 host plant species, 50 of which are found in Iran (18, 19, 20). One of the common predators of both species, as well as other aphids, is the 14-spotted ladybird beetle, *Propylaea quatuordecimpunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae). The species is widely distributed in both the Nearctic and the Palearctic regions of the world (21). Due to its broad range of prey species, *P. quatuordecimpunctata* may represent an optimal control agent for a variety of aphids. However, it has

been shown that life table parameters of predators may strongly differ depending on the prey species (3, 9-12). Hence, in order to optimize pest control, the performance of the predator should be tested for a specific prey species against which it may be used. Kalushkov & Hodek (5) studied the life table para-meters of *P. quatuordecimpunctata* when reared on a variety of aphids species, including *Acyrthosiphon pisum* (Harris, 1776), *Aphis (Aphis) craccivora* Koch, 1854, *Eucallipterus tiliae* (Linnaeus, 1758), *Euceraphis betulae* (Koch, 1855), *Phorodon (Phorodon) humuli* (Schrank, 1801) and *Myzus (Nectarosiphon) persicae* (Sulzer, 1776) (Homoptera: Aphididae) and found differences in performance related to the prey species. Females of *P. quatuordecimpunctata* fed on *M. persicae* laid slightly more eggs than those fed on *A. craccivora* (10). Further, different predators may perform differently on the same prey; a study by Kontodimas et al. (6) compared the performance of different predators on the same prey species and found that *P. quatuordecimpunctata* fed on *A. fabae* had a lower fecundity compared to *Coccinella septempunctata* Linnaeus, 1758, and *Ceratomegilla undecimnotata* (Schneider, 1792).

In addition to different nutritional sources, the local environmental conditions may affect life table para-meters. The abiotic and biotic environments exper-ienced by organisms vary in space and time (22), and natural selection produces adaptations to these different environments, i.e., variation in fitness-related traits. This often results in local adaptation of populations (23, 24). Phoofolo & Obrycki (14) performed a detailed analysis of phenotypic variation in reproductive traits of several Nearctic and Palaearctic populations of *C. septempunctata* and *P. quatuordecimpunctata* and found intra-specific differences depending on the origin of the populations.

In the present study, life history data of *P. quatuordecimpunctata* populations, fed on different prey species, were collected. We analyzed an age-stage, two-sex life table that considers the variation in development among individuals and between the sexes. The effects of two prey species and of the geographic origin of the predator as well as their interactions on the

development, longevity, and female fecundity were assayed. The results may be used as indicators for the suitability of this beetle species for the control of the two aphid species, and also provide information about differences between two local populations of *P. quatuordecimpunctata* collected from Iran.

Materials and methods

Plants

Cucumber plants (*Cucumis sativus* L.) were reared in plastic pots (13 cm diameter, 10 cm height) in a climate-controlled greenhouse (25±5°C, 50–70% RH, 16L:8D). One-month old plants with five leaves were used to rear *A. gossypii*. Broad bean plants (*Vicia faba* L.) were grown under the same conditions. Plants with a height of approximately 8 cm were used to rear *A. fabae*.

Insect colonies

Two species of aphids were collected from an alfalfa (*Medicago sativa* L.) farm in Karaj (Alborz Province, Iran; 35° 48' 04.6" N, 50° 57' 39.6" E, elevation: 1315 m). *Aphis fabae* and *A. gossypii* were cultured on *Vicia faba* and *Cumumis sativus*, respectively, in the laboratory of the College of Science, University of Tehran for 6 months. Colonies were kept in an incubator (Fater Electronic, Iran) at 23±2°C, 55±5% RH and 16L:8D photoperiod. We sampled two populations of the fourteen spotted ladybird beetles in two different climatical regions of the Alborz Mountains: one from alfalfa in Karaj (Alborz Province) and another from Sari (Mazandaran Province, 36° 39' 17.7" N, 53° 10' 21.5" E, elevation: 0 m). The colonies were reared in boxes (18×15×5 cm), tightly covered with a fine mesh net under standardized conditions in the lab. Two populations were cultured on both aphid colonies for one week before using their eggs in the life table experiments.

Life tables

Before initiating the life table study, 20 pairs of *P. quatuordecimpunctata* from each population were prepared and kept in plastic breeding containers (18×15 cm, 5 cm height) to collect the eggs. Females laid eggs in clusters on lids and walls of breeding containers. One

hundred eggs laid within a 24 h period were moved to Petri dishes (8 cm diam.) using a fine paint brush; each individual egg was kept in a single Petri dish and then incubated in an incubator. The 100 eggs of each population (Karaj and Sari) were divided into two equal groups: for one group, mixed instar stages of *A. fabae* on broad bean leaves, *Vicia faba*, were supplied every day. For the other group, mixed instars of *A. gossypii* were supplied on cucumber leaves, *Cucumis stativus*, every day. This setup resulted in four experimental groups: Sari population fed on *A. fabae* (SAF), Sari population fed on *A. gossypii* (SAG), Karaj population fed on *A. fabae* (KAF) and Karaj population fed on *A. gossypii* (KAG).

Hatched larvae of each population were moved to new Petri dishes (8 cm diam.). The presence of exuviae was used as evidence of molting and longevity. One hundred cotton aphids of mixed ages were supplied every day as prey to the larvae on cucumber leaves and one hundred black bean aphids of mixed ages were supplied daily to the larvae on broad bean leaves. When the adults emerged, females and males were paired and kept together in Petri dishes (8 cm diameter). More than 150 aphids were counted and provided to each pair daily. Female fecundity and survival were determined daily and the number of laid eggs was recorded; larvae were reared to adulthood to determine the sex ratio. Observations were made daily until the last adult died.

Life table analysis

Developmental times of all individuals, raw data on the survivorship, longevity, and female daily fecundity were analyzed according to the 'age-stage, two-sex life table' (27, 28) using the computer program TWOSEX-MSChart (29). The effects of different prey and geographic origin as well as their interactions (prey × geographic origin) on the duration of the different development stages of the two predator populations were performed using two-way ANOVA (factorial) in SPSS v. 22.

Following Chi and Liu (27), the age-stage specific survival rate (sxj) (where x=age and j=stage) and the population parameters r (intrinsic rate of increase), λ (finite rate of increase), R_0 (net reproductive rate) and T (mean generation time) were calculated. The net

reproductive rate (R_0) was calculated as follows:

$$R_0 = \sum_{x=0}^{\infty} L_x m_x$$

(1)

The intrinsic rate of increase (r) was estimated by using the iterative bisection method and the Euler-Lotka equation with the age indexed from 0 (30):

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

(2)

The finite rate of increase (λ) and the mean generation time (T) were calculated as follows:

$$\lambda = e^r$$

(3)

$$T = \frac{lnR_0}{r_m}$$

(4)

To estimate the means, variances and standard errors of the population parameters, bootstrap method was used (31). To generate less variable results, 100,000 iterations were performed (7). We used the Tukey-Kramer test (32) to compare the differences among treatments.

Results

Age-stage, two-sex life table

Of the 50 eggs analyzed for each population, 50 KAF, 50 KAG, 46 SAF, 47 SAG hatched successfully, and, of these eggs, 42 KAF, 36 KAG, 37 SAF, 38 SAG developed into imagos. The means of the developmental periods of each pre-adult stage of *P. quatuordecimpunctata* are given in Table 1. The longest pre-adult developmental period was 17.23 days for beetles from Sari feeding on *A. fabae*, while the shortest was 15.93 days for beetles from Karaj feeding on *A. gossypii* (Table 1).

The duration of different life stages of *P. quatuordecimpuncta* are provided in Table 1. Our results showed significant differences in the developmental times of the 1st instar (F=4.95, d.f.=1,

P=0.027), 2nd instar (F=37.58, df=1, P<0.0005) and 3rd instar (F=9.78, df=1, P<0.002) larvae on different prey species (Table 2). The developmental times of the two local populations differed significantly for the egg stage (F=15.43, df=1, P<0.0005), the 1st instar (F=10.54, df=1, P=0.001), 2nd instar (F=10.25, df=1, P=0.002) and 3rd instar (F=8.31, df=1, P=0.004) larvae. Developmental times of the 2nd instar larvae (F=9.39, df=1, P=0.003) also showed an interaction of prey item and geographic origin (Table 2).

All life table parameters are shown in Table 3. Beetles from Sari feeding on *A. gossypii* had the highest intrinsic rate of increase (r) (0.222±0.008), the highest net reproductive rate (R0) (359.9±69.403) and the highest finite rate of increase (λ) (1.249±0.010) compared to the other treatments (Table 3). The lowest r (0.196±0.010) and R0 (235.6±56.735) were recorded for beetles from Sari feeding on *A. fabae*. Significant differences were found in the intrinsic rate of increase (r), mean generation time (T) and finite rate of increase (λ) (P<0.05) for the Sari population fed on the different aphid species. However, the net reproductive rate did not differ significantly between the two local populations (P>0.05).

The highest fecundity of *P. quatuordecimpunctata* was observed in beetles from Sari feeding on *A. gossypii* (999.72±45.07 eggs) and females of *P. quatuordecimpunctata* were more fecund in the Sari population compared to the Karaj population (Table 4). Comparisons of the fecundity, pre-oviposition and oviposition times of *P. quatuordecimpunctata* among populations and prey species and their interaction are presented in Table 5. The differences in the total fecundity were also significant on different prey species and between the two local populations (P=0.009 and P<0.0005, respectively). Preovipositional and ovipositional periods varied significantly for both populations (Table 5).

Table 1. Population parameters, developmental times and adult longevities of *P. quatuordecimpuncta* between populations and prey species.

Parameters		Populations						
		Karaj fed on *A. fabae*		**Karaj fed on *A. gossypii***		**Sari fed on *A. fabae***		**Sari fed on *A. gossypii***
		N	mean±SE	N	mean±SE	N	mean±SE	N	mean±SE
Developmental time(d)									
Egg		50	3.5±0.049ba	50	3.4±0.07 b	46	3.6±0.078 a	47	3.6±0.047 a
1st instar		45	2.6±0.093ba	46	2.8±0.073 a	42	2.3±0.096 c	42	2.5±0.066 bc
2nd instar		44	1.5±0.068b	43	1.3±0.063 c	42	2.0±0.097 a	40	1.3±0.068 c
3rd instar		44	1.7±0.057ba	43	1.3±0.085 c	42	2.0±0.076 a	39	1.5±0.082 b
4th instar		44	2.5±0.069a	41	2.5±0.105 a	40	2.5±0.103 a	39	2.4±0.08 a
Pupa		42	4.9±0.036a	36	4.8±0.087 a	37	4.9±0.04 a	38	4.9±0.067 a
Pre-adult		42	16.8±0.111a	36	15.9±0.265b	37	17.2±0.123 a	38	16.3±0.171 b
longevity	F	17	105.5±0.88a	16	101.5±0.79v	13	102.5±0.82b	18	102.6±0.53b
	M	25	93.3±1.14a	20	88.7±2.78b	24	93.9±0.81a	20	93.7±1.01a

Note: Means (±SE) followed by the different letter within columns are significantly different based on F-LSD range tests at P<0.05.

Table 2. Comparison of the effects of two preys, two populations of predator and their interactions (predator×prey) on developmental times and adult longevities of *P. quatuordecimpuncta*.

	Prey		Predator		Prey*Predator	
	F	**P**	**F**	**P**	**F**	**P**
Developmental time(d)						
Egg	1.51	0.22	15.43	<0.0005	0.169	0.682
1st instar	4.95	0.027	10.54	0.001	0.009	0.927
2nd instar	37.58	<0.0005	10.25	0.002	9.39	0.003
3rd instar	9.78	0.002	8.31	0.004	0.054	0.816
4th instar	0.633	0.427	0.251	0.617	2.99	0.086
Pupa	2.15	0.144	0.192	0.661	1	0.319
Adult	0.127	0.722	0.358	0.550	3.71	0.056
Pre-adult	28.77	<0.0005	4.48	0.036	0.031	0.860
longevity	1.07	0.30	0.174	0.677	1.85	0.174

Table 3. Population parameters (r - intrinsic rate of increase, λ - finite rate of increase, R_0 - net reproductive rate, T - mean generation time) and their standard errors for *P. quatuordecimpuncta* fed on different prey species, estimated by using all individuals and the bootstrap technique.

Parameters	Populations			
	Karaj fed on *A. fabae*	**Karaj fed on *A. gossypii***	**Sari fed on *A. fabae***	**Sari fed on *A. gossypii***
	mean±SE	mean±SE	mean±SE	mean±SE
r (d^{-1})	0.197±0.008 b	0.208±0.010 b	0.196±0.010 c	0.222±0.008 a
λ (d^{-1})	1.127±0.010 b	1.232±0.012 b	1.127±0.012 b	1.249±0.010 a
R_0 (offspring)	240.26±50.444	273.62±58.199	235. 6±56.735	359.9±69.403
T (d)	27.848±0.295a	26.88±0.475ab	27.844±0.242a	26.468±0.266 b

Note: Means (±SE) followed by the different letter within columns are significantly different based on F-LSD range tests at P<0.05.

Table 4. Fecundity, pre-oviposition and oviposition of *P. quatuordecimpuncta* between populations and prey species.

	Karaj fed on *A. fabae*	**Karaj fed on *A. gossypii***	**Sari fed on *A. fabae***	**Sari fed on *A. gossypii***
	mean±SE	mean±SE	mean±SE	mean±SE
Fecundity (egg)	706.65±51.85c	855.06±41.07b	906.15±31.77ba	999.72±45.07a
pre-oviposition (day)	3.18±0.14a	2.97±0.17ba	2.58±0.11bc	2.33±0.11c
Oviposition (day)	21.08±0.42c	22.62±0.30b	24.19±0.49a	24.08±0.37a

Note: Means (±SE) followed by the different letter within columns are significantly different based on F-LSD range tests at P<0.05.

Table 5. Comparison of the effects of two preys, two populations of predator and their interactions (predator×prey) on the fecundity, pre-oviposition and oviposition of *P. quatuordecimpuncta*.

	Prey		Predator		Prey*Predator	
	F	P	F	P	F	P
Fecundity (egg)	7.22	0.009	14.62	<0.0005	0.371	0.545
pre-oviposition (day)	0.886	0.350	13.14	0.001	0.529	0.470
Oviposition (day)	3.53	0.065	32.79	<0.0005	3.94	0.051

Discussion

The northern and southern boundaries of the Alborz Mountains differ in temperature, humidity, altitude and climate (26). Different kinds of prey and temperature have been shown to affect the developmental rate of coccinellid beetles (11). Similar to other findings, we observed the development of the two pre-adult populations of *P. quatuordecimpuncta* varied depending on their geographic origin and the species of prey presented to them (Table 2).

The duration of the second instar stage was significantly different in two prey species (P<0.0005), two populations (P=0.002) and their interactions (P=0.003). Several non-exclusive factors may contribute to these differences: local adaptation, the physiological status of the host plant, food requirements, enzyme equipment and nutritional budgets required for the development and reproduction of the ladybirds may all explain the observed differences (33-34). However, a previous study suggested an important effect of the prey species: Both populations (Karaj and Sari) preferred feeding on *A. gossypii* in the presence of *A. fabae* (P<0.0001) (35). Hence, the predator may be adapted to this species and gain the highest net energy feeding on it (36).

Our results further showed a significant difference between the pre-oviposition periods in the two populations (P=0.001). Beetles from Karaj feeding on *A. fabae* had the longest (3.18±0.14) and beetles from Sari feeding on *A. gossypii* (2.33±0.11) had the shortest pre-oviposition period. It has previously been shown that an increase in the quality of food decreases the pre-oviposition periods (37). The shorter pre-oviposition period and development times (second, third, and fourth instars and pupae) on *A. gossypii* relative to *A. fabae* again suggest that *A. gossypii* has a higher nutritional value and is a more suitable prey for the ladybird (38).

The fecundity of two populations had significant differences when we examined the effect of different prey species and two populations. Females from Sari were more fecund compared to the Karaj population when fed on *A. gossypii* (999.72±45.07 eggs).

The quality of food is very important because influences directly on the biological aspects of Coccinellidae (39). When the ingested food has a poor quality, the reproductive rates, i.e. oviposition and fecundity decrease (40). Our fecundity data suggest higher reproduction compared to those reported previously (6). This provides further evidence that the actual fecundity of a species is not constant and some factors such as food resources and environmental conditions may affect on development and reproduction of the ladybirds.

The intrinsic rate of natural increase (r) is the most important parameter to describe the growth potential of a population under given climatic and food conditions, because 'r' reflects an overall effect on development, reproduction and survival (41). In our study, the intrinsic rate of increase (r), the mean generation time (T) and the finite rate of increase (λ) of the Sari population differed depending on the prey species. The intrinsic rate of increase (r) was higher for beetles feeding on *A. gossypii* than for beetles feeding on *A. fabae*. This further supports the above findings of a higher food quality of *A. gossypii* for the ladybird.

The values of 'r' for *P. quatuordecimpuncta* are preying on *A. fabae* are 0.166, 0.197 and 0.196 females/female/day according to Kontodimas & Stathas (11) and our results.

Variation in the intrinsic rate of population increase (r) could be related to three main factors: development rate, fecundity and longevity (42). The reason for the higher intrinsic rate of increase in this study for beetles from Sari feeding on *A. gossypii* was a higher fecundity (999.72±45.07 eggs). Hence, our study suggests that different prey species and different populations lead to

changes in fecundity, which can be applied when choosing the best natural enemy for a prey species.

Conclusion

Our study provides further evidence for the effects of different prey species and the geographic conditions on the life table parameters and reproductive traits of two populations of *P. quatuordecimpunctata*. Our findings show that the ladybird is a more effective predator for *A. gossypii* than for *A. fabae*.

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Editorial Note

Volume 7, issue 2 of Progress in Biological Sciences was initialy scheduled to be published in December 31, 2017. However, some administrative changes leaded to a major delay in processing of the manuscripts. This issue is actually published in May 1, 2020. Editor-in-chief apologizes deeply for any inconvenience caused especially to the authors.