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Testing Genetic Build Response of the Predator, *Coccinella septempunctata* (L.) to Genetic Improvement by Random Allogamy Technique

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ABSTRACT

Coccinella septempunctata L. was reared under laboratory conditions using allogamy technique aiming to convert the natural local progeny to laboratory progeny for the purpose of continuing the predator production, as well to sustain numerical values of its biological and morphological parameters. The research was conducted on local *C. septempunctata* strains collected from agro-ecosystems in the Syrian coast. The results proved the stability of the genetic structure of such progenies through three generations as all statistical differences were non-significant at 5% significance level. Also, the results confirmed the response of these progenies to the abovementioned method where fecundity raised (7.5 %) from 98.2±20.8 in parent generation (Po) to 105.6± 20.3 in 2[nd] generation (F2). Predation rate of larvae raised (6.8 %) from 88.8±2.87 in Po to 94.8±12.31 in F2. As well were the values of all other biological parameters (developmental period, reproductively, survival rates and female body length).

Key Words: *Coccinella septempunctata,* Biological parameters, allogamy, Syria.

INTRODUCTION

Biological control is a main factor in Integrated Pest Management (IPM) programs because of its inter- and intra-reactions among living organisms which help in suppressing pest populations under economic threshold levels by the action of its elements; parasitoids, predators and/or pathogens (Arnetr, 1967).

Aphids are one of the serious groups of insect pests that attack green plants; therefore, their natural enemies have received high attention through studying, mass rearing and releasing (Mondor and Warren, 2000).

Predators belonging to family Coccinellidae, comprise a group of the most active predatory species, that feed on different sap sucking pests including aphids, whiteflies, jassids, scale insects and mites as well other small insects. This family gained a considerable attention as important group of predators in the biological control of insect pests attacking different crop plants. Several coccinellid species were recorded on different field, vegetable and fruit crops in several parts of the world. Aphids constitute the essential food for the majority of Coccinellidae (Saharaoui *et. al.*, 2001).

The species *Coccinella septempunctata* L. has a particular condition in the pest control programs against the aphids which have become resistant to many insecticides due to their short life-cycle, consequently their high reproductivity and number of generations per year (Ewent and Chiaug, 1966 and Mathur, 1983).

The present study was carried out for the first time in Syria on Syrian strains of the predatory species, *C. septempunctata* using allagomy technique for the rearing to evaluate its reactions on the local adapted strain to the Syrian agro-ecosystems. Also, the study focused on the following aspects reported by White *et al.*, 1970, Wllkes 1972, Hoy 1981 and Uygum and Sekeroglu, 1987:

1- Rearing for multiplication of the local strains of *C. septempunctata* under laboratory conditions, using allogamy method to transfer the natural strain to a laboratory strain to be mass produced, then released and evaluated under field conditions.

2- Determining morphological parameters in both natural and laboratory strains for several generations.

3- Estimating the effect of allogamy on the genetic structure of the obtained strains and evaluating its response for artificial genetic improvement process using Students' Statistical Analysis and experimental designs (Kassem, 1982 and Dospekhov, 1985).

MATERIALS AND METHODES

The study was carried out during 2003 and 2004 at the Insect Laboratory of the Faculty of Agriculture, University of Damascus and Lattakia Center for Rearing Natural Enemies, Lattakia rearing and mass production of the predators, particularly *C. septempunctata* have not reached yet the level of laboratory practical applications in the country, therefore implementation of the three phases of basic rearing techniques of predators; breeding the host plant, rearing the prey(s) and rearing the traget predatory species was followed (WIlkes, 1972).

1- Breeding of the host plant:

Vicia faba was the host plant chosen for rearing the aphids as it is easy to grow under the laboratory conditions throughout the year and is a perfect host for the black bean aphid, *Aphis fabae*. The bean plants were grown on sterilized sawdust in plastic pots under room temperature and placed close to glass windows for natural light (Hussain *et al.*, 1999).

2- Rearing of the prey:

As the bean seedlings grew up, those were transferred to the aphid rearing room. Pots were placed into a large wooden cage (100 x 50 x 55 cm) (10 pots per cage) covered with transparent plastic provided with holes (covered with fine muslin) for ventilation. Plants infested with *A. fabae* (free of natural enemies) were collected from the fields and placed directly paired on the grown bean seedlings inside the cages for 15 – 20 days at 18±2 °C and 65 % R.H. and 18h L/Day. (Gaspar *et al.*, 2000).

3- Rearing of *C. septempunctata*:

The predator was reared in similar cages of the aphid. Aphid infested pots were placed in the predatory cages. 20 ♀ and 20 ♂ *C. septempunctata* adults were released in each cage (one couple/pot). The predator was reared under the abovementioned laboratory conditions. The forty individual predators were collected from the fields to represent natural strain and to become a basic laboratory strain or founding generation (P) (Kumar *et al.*, 1996 and Kindlmann *et al.*, 2000). The predator was reared for 3 generations. As the predator reached its pupal stage, the pupae were separated into small cages, at 20 °C until emergence of *C. septempunctata* adults which were used for studying some biological parameters (Hodek and Ceryngier, 2000).

Experimental design:

The experiment was designed according to Student's method (Dospekhov, 1985). Five replicates (represnted by 5 small cages (50 x 30 x 35 cm) were used / generation, each was covered by transparent plastic and provided with holes for ventelation). Each cage had 3 aphids' infested pots. After collecting the mother source of the predator from the large cages (3rd stage), 5 ♂ and 5 ♀ were isolated (one couple/ small cage) to establish the first founding generation (P) (this was the transmission process from natural strain to the laboratory strain) (Uygun and Sekeroglu, 1987). By hatching the predator eggs, mother male and female adults were removed from the 5 cages. Each cage was considered a replicate. Larvae were fed to develop to adults and collected from the cages to establish the parent generation (Po) (1st investigated generation), from which each couple was placed into a cage. The biological parameters studied were: fertility and fecundity of mother females, preying potential of the 4th larval instar, developmental period, reproduction capacity along mother female longevity (Ferragut *et al.*, 1987, Dixon *et al.*, 2000, Evans, 2000 and Evans *et al.*, 2000). As the adult emerged in the cages, they were distributed in new cages to establish the 1st crossing generation (F1). The crossing was made as follows:

- 1st cage: ♀ from 1st cage X ♂ from 2nd cage.
- 2nd cage: ♀ from 2nd cage X ♂ from 1st cage.
- 3rd cage: ♀ from 3rd cage X ♂ from 4th cage.
- 4th cage: ♀ from 4th cage X ♂ from 3rd cage.
- 5th cage: ♀ from 5th cage X ♂ from mother cage.

By the end of studying the biological parameters, all parents were removed from the cages before occurrence of the progeny to avoid mixing. By emergence of the adults inside the cages, they were collected to establish 2nd crossing generation (F2) using the same methods and measurements of F1.

Statistical analysis:

Data were statistically analyzed using the computer software package SPSS10

RESULTS AND DISCUSSION

1- Biological and morphological parameters of *C. septempunctata*

Data of biological and morphological parameters of *C. septempunctata* were studied for three generations and summarized in table (1). As shown in the table, almost all averages recorded in Po (Parent generation) increased gradually towards F2 through F1, except that of the fertility. Obvious increases (about 3 folds) were recorded among the three generations in both the fecundity and the survival rates of the predator. Statistical analysis showed significant differences among the

Table (1): Total averages of biological and morphological parameters of *Coccinella septempunctata* L. for three generations under laboratory conditions.

Parameter	Parent generation (Po)	1st crossing generation (F1)	2nd crossing generation (F2)
Fertility (No. of eggs/♀/month)	98.2 ± 20.8 a	93.6 ± 19.3 a	105.6 ±20.3 a
Preying potential of 4th larval instar (aphid nymphs/larva/ day)	88.8 ± 2.87 a	90.2 ± 7.78 a	94.8 ± 12.3 a
Preying potential of adults (aphid nymphs/adult/day)	64.1 ± 2.11 a	65.2 ± 4.08 a	67.3 ± 6.57 a
Developmental period (days) (larva – adult)	27.4 ± 0.6 a	28.1 ± 1.1 a	28.7 ± 1.0 a
Fecundity (No. of progeny/ female)	13.0 ± 3.1 a	38.1 ± 10.9 a	40.4 ± 11.1 a
% Survival rate (fecundity/fertility x 100)	12.0 ± 1.5 a	32.8 ± 6.9 a	35.0 ± 3.8 a
Female length (mm)	9.02 ± 0.1 a	9.1 ± 0.3 a	9.4 ± 0.4 a
Sex ratio (F : M)	1.2 : 1	1.06 : 1	1.17 : 1

N = 5

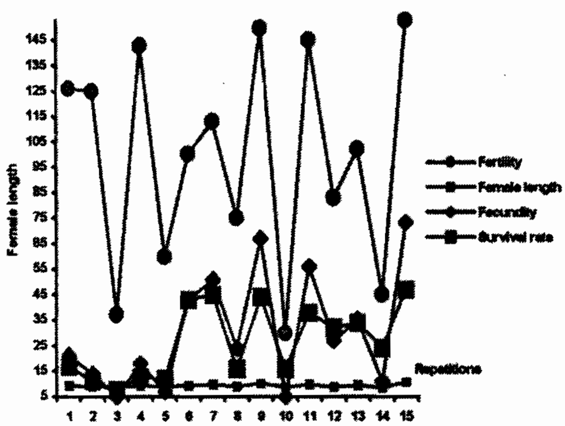

Fig. (1): Dynamic changes in genetic biological parameters of *C. septempunctata* through genetic relations prevailing among them

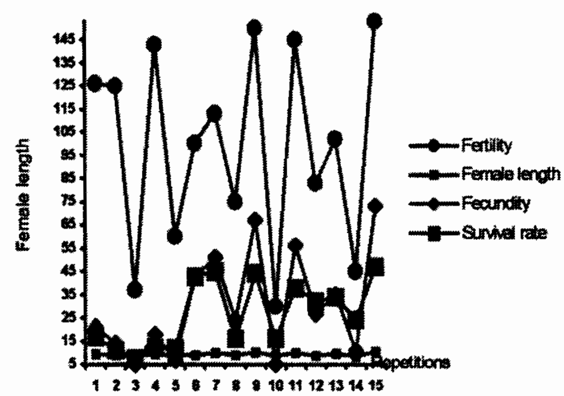

Fig. (2): Morphological dynamic changes of biological parameters through genetic association relationships prevailing among them

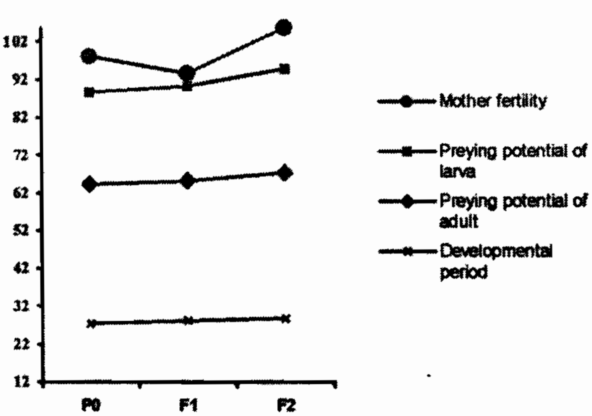

Fig. (3): Dynamic changes of the fertility with the preying potential of insects from one side and developmental period from the other side for three generations

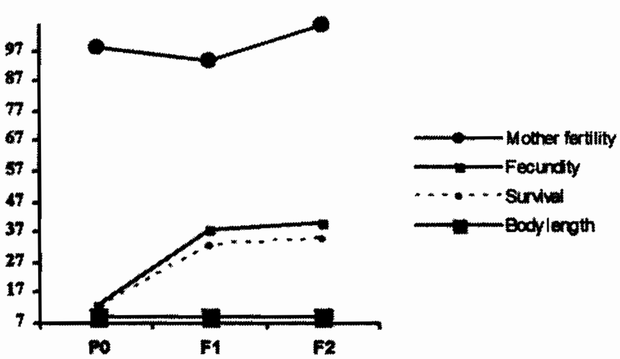

Fig. (4): Dynamic change of the mother fertility and production, longevity and the length of the body generations in fecundity and survival rates (Table 1). Therefore, mass rearing necessarily requires cross mating method (allogamy) for better knowledge of genetic improvement through selection of properties since this multi-gene is responsible for occurrence of more genes that may be carried on one chromosomes or more (Ayal *et al.,* 1987).

In order to achieve the best possible way for genetic improvement, it must link the study of genetic relations prevailing among the biological and morphological genetic parameters and their mechanism of changes through the three generations in addition to the total and integrated analysis of the dynamic of changes among other parameters at the end of the second cross mating generation.

2- Genetic relations prevailing among biological and morphological parameters

Studying the genetic correlations among the studied parameters required considering the three generations, with their fifteen replicates as one statistical group since no significant difference was found among them. Correlation coefficient values were positively correlated with survival (+ 0.5) and reproduction (+ 0.7) which indicated that the increase of the genetic trait of the female fertility of the predator *C. septempunctata* led to increase of survival and reproduction rates but decreased developmental period and preying potential of adults because of the negative genetic correlation (- 0.2) found with each parameter. The impact of high fertility on the reduction of preying potential of the predatory 4^{th} larval instar was obvious and negative (- 0.7). On the other hand, genetic correlations of the other biological parameters showed positive weak correlations between preying potential of each of *C. septempunctata* adults (+ 0.2) and 4^{th} larval instar (+ 0.2) and developmental periods as well between preying potential of both adults and 4^{th} larval instar. The previous findings of the coefficient values of the most important biological parameters showed that the former index of fertility plays the major role in inter-genetic changes of the predator's biology as it was positivly corroleated with survival, reproduction and negativly with preying potential and developmental periods. Neverthless, fertility has no absolute domenance since it has no strong correlations; positively or negatively with all biological parameters.

As shown in fig. (1), fertility rates were adversely represented to that of the preying potential of 4^{th} larval instar and were fluctuated largely to indicate the negative correlation between them. Preying potential of adults coincided with developmental periods in parallel stable positive correlation.

As concluded from previous results that any process of improving the genetic value to raise the fertility rate independent in rearing will lead to substantial decline in preying potential rates of larvae and adults and developmental periods. Therefore, to avoid the decline and deterioration of a property while improving genetically another property correlated negatively with it confirmed the importance of rearing the predators according to the laws of artificial selection and genetic improvement based on the numerical value of selection evidence that leads to lifting of the values of all biological parameters included in its offset as the case of rearing civil large livestock and poultry, bees and silk worms. The most important point in the study of genetic correlation between the above factors was the results led to the inclusion of morphological index of the length of the female when studying the dynamic and changing values of the rest of the parameters through genetic linkages.

As shown in fig. (2), the female length parameter was correlated positively with fertility (correlation coefficient between them (+ 0.8)). The index marched in parallel with the index of fertility in its impact on the values of other parameters through the linkages which were weak and negative with adults' preying potential (r = - 0.3), being similar to that between female length and developmental periods (- 0.3), while the correlation of the morphological parameter was moderately negative with preying potential of the 4^{th} larval instar (- 0.7) and positive with the fecundaty and survival rates which showed similar impact with the fertilty and reflecting a strong positive correlation between both of them. The length of body linked strongly positive with the fecundaty, (r = + 0.9). As well, was its correlation with survival parameter which was moderately positive with coefficient value (+ 0.7) (Fig. 2).

It could be explained, that the long female body is more ready to give more eggs and the possibility of its progeny to adapt, adjust and survive regardless of the number of preys that devoured. This result encourages the adoption of morphological selection, which aims to recruit longer and larger females for rearing to improve the biological specifications that leads to higher fertility index based on their strong positive link. This matter has a practical practice in the biological control laboratories due to the easy selection of lengths on one hand and the speed in the selection of parents carrying this apparent capacity on the other hand, compared with the procedures and measurements needed for parents selection based on the status of fertility. Therefore, any action that aims to improve body length will lead necessarily to improve and increase fertility rate and other parameters with the same machinery behavior and reflecting the mutual influence within the structure of the genetic kind. Finally, it should be noted the strong positive correlation between parameters of fecundity and survival as the value of their genetic correlation coefficients was + 0.9. The previous findings of the values of the genetic link between morphological parameter/ (body length0 and other biological parameters confirm the big role plays by fertility parameter in the total genetic changes in the predator's biology.

3- Study of change dynamics of total parameters at the end of 2nd crossing generation

As the different character appear depending upon particular order of the responsible genetic factors within the genetic structure of the type, the cross mating did not show new to improve and raise the level of values of these properties to a significant level. This type of rearing preserved the values of attributes from decline and improved slightly their numerical values. The fertility increased from 93.6 in the mothers of the 1st cross mating generation to 105.6 in the mothers of the 2nd cross mating generation (which represents the 1st cross mating generation of this parameter). This means that the predator remained conservative on its genetic structure of this character through three generations which opens a lot of scope in the genetic improvement of the predator. The same trend was observed in case of feeding capacity of the 4th larval instar as well of the adults. Also, there has been little apparent improvement on the selected morphological character (length of female) which measured 9.02 mm in the generation of parents and increased to 9.40 mm at the end of the 2nd cross mating generation.

As shown in fig. (3), the values of larval and adult preying potentials went upward in parallel, starting from Po generation to F1 to increase slightly from F1 up to F2 which confirmed the depth of positive correlation between them. The fertility rate took a special trend by reversing its decline in F1 than in Po, while increased at the end of F2 (which was the 1st cross mating generation), around the high proportion of high parameter of predation in spite of a weak negative correlation between them, which confirms the fact that (a genetic link between two negative parameters do not prevent their increase or improvement with the preservation of their negative correlation indicating that there was no change in thestructure of the genetic type).

As shown in fig. (4), the relations prevailing among the four positive linkages correlated attributes were weakly positive in natural strains between fertility and the length of the body on one hand and both fertility and productivity, adaptability and longevity on the other. The productivity and sustainability of life and relatively length of the body rose apparently from Po to F1 and continued to rise less frequently starting from F1 to F2 in parallel through the three generations. Also, the rest of the increased parameters of Po to F1, emphasized the balance and stability of these indicators in relation to connectivity on one hand and visibility within the mechanism of genetic structure on the other.

The cross mating technique (allogamy) used in the present research had proved effective in accordance with the results of those applied for predatory rearing in specialized centers (De Bach, 1972 and Ferragut *et al.,* 1987). This method, which was not different from the theories of genetics and genetic improvement used in animal breeding capacity, like cows, sheep, rabbits and poultry in order to avoid the negative effects of continuous rearing of the same strain that result after several generations and led to 50 % ruins in the major biological parameters; fertility, preying potential, developmental period, reproduction and survival (Ayal. *et al.,* 1987). As well, the research proved that the local strains of the studied predatory species *C. septempunctata* had their owned genetic bases valid for conducting of genetic improvement proposed by De Bach, 1972, Dospekhov, 1985 and Ferragut *et al.,* 1987.

Comparing the numerical values obtained in the course of this research with similar research results, a decline in fertility rate, preying potential and survival rate were found, while equivelant values of the developmental period and the length of female were recorded due to the low efficiency of the local strain (Ferragut, *et al.,* 1987, Evans, 2000 and Gaspar., *et al.,* 2000).

In conclusion, the obtained results could be summarized as follows:

1- The local species, *C. septempunctata* is considered of the large predator which is very effective and efficiently preying on aphids and reduce their population densities in the fields and protected crops.

2- Reproduction of *C. septempunctata* was stable, balanced and without any fundamental changes in the values of the most important biological parameters, particularly when the rearing process was carried out by specialists and the random allogamy by mating among all males and females in different groups.

3- The local predatory species, *C. septempunctata* was characterized by a stable and balanced genetic structure, therefore the allogamy technique used in its rearing led to maintain the predator numerical values of the most important multi-gene biological characteristics. As well, the predator had a genetic base for special operations and artificial selection for genetic improvement.

4- A study of prevailing linkages showed a strong negative correlation between fertility and preying potential of the predatory 4th larval instar, medium negative correlation with preying potential of the adults. As well as a strong positive correlation between fertility and the length of the body, its effect included equally all parameters.

5- The presence of a strong positive correlation between the female body length and fertility allowed artificial morphological selection alternating with artificial selection to increase the fertility rate due to the ease of the first selection and speed of the selection of large parents and excluding the small ones at the conduct of rearing and improvement of the predatory species *C. septempunctata*

6- The best patterns of genetic improvement and selection to be applied on the predatory species was based on the value of the selective Guide (I) which takes into account the improvement and raising of the values of all parameters measured by using the following equation:

$$I = AX1 + BX2 + ... ZXn$$

Where: I value the selective Guide
A, B ..., Z = numerical constants.
X1, X2 ...., Xn mean values of the most important biological parameters and morphological multi-gene involved in the pattern of the past genetic improvement.

So the existence of genetic linkages between some negatively correlated important parameters to be improved and increasing its numerical value in order to raise efficiency and obtaining effective predator vitality.

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