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Testing Genetic Build Response of Egyptian Laboratory Strain of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) at Random Allogamy and Inbreeding Mating Techniques

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ABSTRACT

Effect of two mating techniques (random allogamy and inbreeding (brother-sister) on some effective morphobiological parameters of the Egyptian laboratory strain of the predator, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), when reared on the citrus mealy bug, *Planococcus citri* (Risso) at the Lattakia Center for Rearing Natural Enemies, was studied. The predator's Egyptian strain was obtained from the Biological Control Mass-rearing Laboratory, Faculty of Agriculture, Cairo University, Giza, Egypt through personal communication. The study was carried out at 25±2 °C, 60±10 % RH and 16:8 h (L: D) for six generations. Morphobiological parameters of different generations and for both techniques were compared with the parent generation (PG). Obtained results showed that there were insignificant differences in all morphobiological parameters between PG and F6, using random allogamy mating. Morphobiological indicators of females were more affected by the mating technique and recorded significant decrease using inbreeding mating in F3. Female length response for inbreeding mating in F3 and F6 was -4.91 and -9.41%, respectively, while it increased to -3.28 and 1.23%, using random allogamy. Fecundity, reproduction, female longevity and preying potential of female showed negative depression in F3. The fecundity and reproduction parameters response for inbreeding mating in F3 and F6 were -52.36, -51.3% and -53.57 and -54.0%, respectively. Respective response to random allogamy mating technique was more effective than the inbreeding but it was not enough to improve these parameters. Results refer to the unstable genetic structure of the Egyptian laboratory strain of the predator which was inbred on it repeatedly for generations of in closed cages.

Key words: *Cryptolaemus montrouzieri*, Biological parameters, Inbreeding (Brother-sister) mating, Random Allogamy, Genetic response, Egyptian strain.

INTRODUCTION

Mealy bugs are sap-sucking insects that cause severe economic damage to a wide range of crops (Nagrare *et al*., 2009). Biological control plays a significant role in the management of mealy bug species. *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), known as "Mealy bug destroyer." is a general predator of mealy bugs and scale insects. This predator is adapted to the temperatures existing under tropical conditions (Luck and Forster, 2003). *C. montrouzieri* is a native to Australia. It was introduced into California, USA in 1892 for control of the citrus mealy bug (Calusen, 1915). Following the success in the control of the mealy bugs in California, this predatory species was introduced into more than 40 countries and it has been used in different biological applications: (classical and augmentative) to control Pseudococcidae (Cock *et al.*, 2010).

It was introduced to Turkey from USA in 1965 to control *Planococcus citri* on citrus trees (Oncuer and Bayhan, 1982). Also, it was introduced to Syria from Turkey in 1995 and has been reared at Lattakia Center for Rearing Natural Enemies at Tartous Research Center (Al-Khateeb and Raie, 2002, and Al-Khateeb and Asslan, 2007). *C. montrouzieri* was introduced into Egypt from France in 1923, for the control of *Pseudococcus filamentosus*, *P. sacchari* and *Phenacoccus hirsutus* and it was reared and released in many orchards of Egypt but it failed to adapt, probably due to poor overwintering and to the attack of ants. (Tawfik, 1997). In 2005, the predator was introduced again into Egypt from Syria (personal communications) and it has been reared at the Biological Control Mass-rearing laboratory, Faculty of Agriculture, Cairo University, Giza, Egypt. Rearing program of *C. monrouzieri* in both Syria and Egypt aimed to mass rear the predator and to release it periodically during the season.

Because of lack of references on the genetic structure of the predatory strains and its relationship to the most important morphobiological parameters, this study was one of a series conducted on both the Syrian and Egyptian strains of *C. montrouzieri* in order to improve its morphobiological parameters by using random allogamy technique (Al-Khateeb *et al.* 2012).

The present study was carried out to:

1. Determine the most important morphological and biological parameters of the predator, affected with inbreeding and random allogamy mating techniques.
2. Estimate the effect of the allogamy mating technique on the genetic structure of the Egyptian

strain and to evaluate its response for artificial genetic improvement process.
3. Find the best technique to maintain the bio-efficiency indicators of the predator and improve them by using random allogamy technique.

MATERIALS AND METHODS

The study was carried out for the first time in Syria, Lattakia Centre for Rearing Natural Enemies and Biological Control Studies and Research Centre, Faculty of Agriculture, Damascus University, during 2011-2012 on the Egyptian laboratory strain of *C. montrouzieri*. 50 adults of the predator were obtained from the Biological Control Mass-rearing laboratory, Faculty of Agriculture, Cairo University, Giza, Egypt in order to carry out this study.

Rearing and Propagation of the host insect, *Planococcus citri* (Risso)

Potato was used as a host for *P. citri*. Successful rearing required using suitable variety of potato; Sponta has proved to be one of the best varieties for rearing *P. citri*, due to its ability to withstand the conditions of storage, speed to break dormancy stage at a temperature of 4±1 °C and its speed growth giving branches in a short period.

1. **Production of host potatoes:** was carried out under a dark condition, at a temperature of 10-12 °C and 60 ±10% RH inside plastic containers until branches reach 10-15 cm.
2. **Production of *P. citri*:** preferable species because of its short life-cycle and its fecundity under the conditions of 25±2 °C; 60±10% RH and 16:8 (L: D) Light: Dark, using the same plastic containers which had been used in the first stage. Potato stems (10-15 cm long) were prepared to be infested with *P. citri*, using slices of smooth clean papers. The leaves were transferred to darkness to obtain new growths. Repetition of this process was undertaken twice a day to get a pure infestation. It needed 30-45 days to reach the complete infestation and obtaining different instars of *P. citri*

Rearing and Propagation of *Cryptolaemus montrouzieri* Mulsant

The techniques of Smith and Armitage (1931) and Witcomb and Bell (1964) were used for rearing *C. montrouzieri*. Adults of the predator were released on the branches of infested potatoes with *P. citri* (in the second nymphal instar), under the mentioned conditions.

Rearing and Formation of Parental Pairs

Rearing and formation of parental pairs was conducted on the Egyptian laboratory strain of *C. montrouzieri* using two techniques:

1. Formation of generations using inbreeding mating, and
2. Formation of crossing generations using random allogamy mating.

The experiments were carried out using a glass cage, covered with a soft cloth. 10 replicates were tested; each had 2 potatos sprouts infested with *P. citri* and one pair (1♂+1♀) of *C. montrouzieri*/ cage. Females and males were isolated from the stock culture of the laboratory to establish the first emerged parent generation (PG) which represented the laboratory strain. After the new adults of *C. montrouzieri* emerged in the containers, they were distributed into new containers and divided into two statistical groups, every group consisted of 10 replicates. The first represented the first generation of brother-sister mating (F1) and the second represented the first crossing generation/ random allogamy mating (F1). These processes were repeated for six generations. 10 newly hatched larvae were isolated from each replicate and from every generation and then transferred to Petri dishes with branches of potatoes infested with *P. citri* to develop until emergence of *C. montrouzieri* adults in order to study the effect of both techniques on some of the important morphological and biological parameters of the predator.

Replicates of random allogamy technique were carried out as follows:

- 1st cage: ♂ from 1st cage × ♀ from 2nd cage.
- 2nd cage: ♂ from 2nd cage × ♀ from 1st cage.
- 3rd cage: ♂ from 3rd cage × ♀from 4th cage.
- 4th cage: ♂ from 4th cage × ♀ from 3rd cage.
- 5th cage: ♂from 5th cage × ♀ from 6th cage
- 6th cage: ♂from 6th cage × ♀ from 5th cage
- 7th cage: ♂from 7th cage × ♀ from 8th cage
- 8th cage: ♂from 8th cage × ♀ from 7th cage
- 9th cage: ♂from 9th cage × ♀ from 10th cage
- 10th cage: ♂from 10th cage × ♀ from 9th cage

Replicates of inbreeding mating were undertaken as follows:

- 1st cage: ♂ from 1st cage × ♀ from 1st cage.
- 2nd cage: ♂ from 2nd cage × ♀ from 2nd cage.
- 3rd cage: ♂ from 3rd cage × ♀from 3rd cage.
- 4th cage: ♂ from 4th cage × ♀ from 4th cage.
- 5th cage: ♂from 5th cage × ♀ from 5th cage
- 6th cage: ♂from 6th cage × ♀ from 6th cage
- 7th cage: ♂from 7th cage × ♀ from 7th cage
- 8th cage: ♂from 8th cage × ♀ from 8th cage
- 9th cage: ♂from 9th cage × ♀ from 9th cage
- 10th cage: ♂from 10th cage × ♀ from 10th cage

Biological and morphological parameters of *C. montrouzieri* of both techniques, at parent

generation (PG) and crossing generations (F1), (F3), and (F6) were estimated. Studied morphological parameters were; length of females, length and width of 3rd instar of larvae, using millimeter lens and females' weight, using digital balance. Studied biological parameters included larval developmental period of the predator's and generation time, longevity, fecundity, reproduction and preying potential of the 3rd larval instar and adult. Preying potential for each of male and female was estimated by offering 100 nymphs/3rd nymphal instar of *P. citri* in Petri dishes (9 mm diameter), covered with pieces of fine net (2-mm mesh) to provide air ventilation. Ten replicates of males and females from each generation and for each mating technique were used. The Petri dishes were checked daily and the number of nymphs consumed by *C. montrouzieri* was recorded for three days and then the average daily predation rate was calculated. Parameter response for each mating technique at the end of the 3rd and 6th generation was calculated as described by Asslan, 1990.

Experimental design and Statistical analysis

Experiments were carried out using (Randomized Complete Block Design). Obtained data were subjected to ANOVA test using the computer software package SPSS V.18 to determine Duncan's multiple range tests and the (Least Significant Differences) LSD at 5% probability level.

RESULTS AND DISCUSSION

1. **Morphological parameters of the Egyptian laboratory strain of *C. montrouzieri*, using inbreeding mating technique**

Numerical data of the studied morphological parameters of *C. montrouzieri*, the Egyptian laboratory strain, using brother-sister mating technique for six generations under laboratory conditions were summarized in table (1).

Results in table (1) show that there were non-significant differences in all the morphological parameters between PG (parent generation) and F1 using both mating techniques. Averages of female length recorded significant decrease among the three generations (PG, F1 and F3), using the two mating techniques. Female weight recorded significant decrease among (PG, F1 and F3) using brother sister technique and insignificant decrease when allogamy technique was used. Larval length and width recorded non-significant differences among all generations using the two mating techniques. Comparing the values of the morphological parameters between F3 and F6 and between F1 and F6 showed the effect of allogamy technique on maintaining the stability and balance of the numerical values of some morphological indicators as there were insignificant differences in all morphological parameters between PG and F6 using allogamy technique but recorded significant decrease of female parameters using brother sister technique. Female length response for brother sister mating in F3 and F6 were -4.91 and -9.41%, respectively, while it raised to -3.28 and 1.23% using random allogamy technique. As well, female weight response for brother sister mating was -26.19% in F6 but it was positive (+4.13%) using random allogamy. Therefore, the female morphological indicators were highly affected by the mating technique. These findings agree with what reported by Asslan 1990 and Asslan *et al.*, 2008.

Dynamic Changes of Biological Parameters of the Egyptian Laboratory Strain of *C. montrouzieri* and in Relation to Mating Technique

Results in table (2) showed that there were non-significant differences in all biological parameters between PG and F1 when using both mating techniques. Also, there were non-significant differences in some biological parameters (preying potential and longevity of male and preying potential of 3th larval instar) among the three generations (PG, F1, and F3). The most important values of biological parameters (fecundity, reproduction, female longevity, preying potential of female and larval developmental period) decreased, significantly in F3 by using brother sister mating and then dropped sharply. Fecundity decreased from 158.9±49.57 and 126.3±58.87 eggs/ female in PG and F1, respectively to 75.7±22.47 in F3. As well was the reproduction from 132.9±43.24, 109.8±56.00 in PG and F1, respectively to 61.7±21.80 eggs/ female in F3. The results also showed that there were non-significant differences in the values of all biological indicators between F3 and F6. These were expressed by high negative values of parameters response using inbreeding at the end of the 3rd generation and were much depressed in F3 than in F6, such as fecundity and reproduction rates. The fecundity and reproduction parameters response for brother sister mating in F3 and F6 were -52.36%, -51.3% and -53.57% and -54.0%, respectively (Table 2 and figs. 1 & 2). As well, longevity of females decreased significantly in F3 when compared with PG and in F3 and F6 as it was -42.94 and -54.17%, respectively (Table 2, figs. 3 & 4).

Preying potential of *C. montrouzieri* females (no. of consumed *P. citri* nymphs/ female/ day) decreased significantly in F3 when compared with PG and showed non-significant difference between F3 and F6, as recorded -21.40 and -23.7% in F3 and F6, respectively. Larval developmental period at brother-sister mating was shortened by -13.74%

Table (1): Numerical values (mean ±SE) of morphological parameters of the Egyptian laboratory strain of *C. montrouzieri* using brother-sister and allogamy mating techniques for six successive generations under laboratory conditions

Parameter	Mating technique	PG	F1	F3	Parameter response for mating technique in F3 %	F6	Parameter response for mating technique in F6 %
Female length (mm)	Brother-sister mating	A 4.89±0.11a	A 4.8±0.23ab	A 4.65±0.15b	-4.91	B 4.43±0.17c	-9.41
	Random Allogamy	A 4.88±0.12a	A 4.81±0.19ab	A 4.72±0.20b	-3.28	A 4.94±0.10a	1.23
Female Weight (mg)	Brother-sister mating	A 12.6±1.3a	A 12.4±2.00a	A 10.7±1b	-15.08	B 9.3±1c	-26.19
	Random Allogamy	A 12.1±1.4ab	B 11.00±1.1b	A 11.4±1.7ab	-5.79	A 12.6±1.9a	4.13
Larval length (mm)	Brother-sister mating	A 8.27±0.68a	A 8.23±0.72a	A 7.98±0.71a	-3.51	A 8.01±0.38a	-3.14
	Random Allogamy	A 8.48±0.89a	A 8.41±0.92a	A 8.41±0.60a	-0.83	A 8.42±0.95a	-0.71
Larval width (mm)	Brother-sister mating	A 3.83±0.55a	A 3.61±0.41a	A 3.33±0.62a	-13.05	A 3.36±0.74a	-12.27
	Random Allogamy	A 3.79±0.46a	A 3.72±0.46a	A 3.68±0.68a	-2.90	A 3.88±0.52a	2.37

Table (2): Numerical values (mean ±SE) of biological parameters of the Egyptian laboratory strain of *C. montrouzieri* and their responses for two mating techniques

Parameter	Mating technique	PG	F1	F3	Parameter response for mating technique in F3 %	F6	Parameter response for mating technique in F6 %
Fecundity	Brother-sister	A 158.9±49.57 a	A 126.3±58.87 a	B 75.7±22.47 b	-52.36	B 77.4±29.69 b	-51.3
	Random Allogamy	A 149.6±57.09 a	A 143.2±40.55ab	A 104.9±44.41 b	-29.88	A 143.4±33.14 ab	-4.1
Reproduction	Brother-sister	A 132.9±43.24 a	A 109.8±56.00 a	B 61.7±21..80 b	-53.57	B 61.2±27.49 b	-54.0
	Random Allogamy	A 131.40±55.38 a	A 122.6±35.19 ab	A 92.8±42.13 b	-29.38	A 125.60±26.74 ab	-4.4
Male Longevity (days)	Brother-sister	A 129.3±53.73 a	A 93.3±35.14 ab	B 94.3±44.21 ab	-27.07	A 68.2±33.11 b	-47.25
	Random Allogamy	A 118.5±52.64 a	A 113.6±45.96 a	A 97.9±51.60 a	-17.38	A 76.5±36.39 a	-35.44
Female Longevity (days)	Brother-sister	A 117.6±48.14 a	A 87.8±40.08 ab	A 67.1±58.91 b	-42.94	B 53.9±33.99 b	-54.17
	Random Allogamy	A 112.2±35.97 a	A 97.3±45.20 a	A 84.7±50.70 a	-24.51	A 104.5±34.64 a	-6.86
Preying potential of 3rd larval instar (*P. citri* nymphs/ day)	Brother-sister	A 25.17±4.48 a	A 24.43±5.03 a	A 21.67±3.13 ab	-13.91	A 19.73±1.96 b	-21.6
	Random Allogamy	A 23.87±4.22 a	A 25.67±3.30 a	A 23.53±3.68 a	-1.42	A 22.33±3.93 a	-6.5
Preying potential of female (*P. citri* nymphs/ day)	Brother-sister	A 36.73±3.41 a	A 35.17±6.33 a	B 28.87±4.77 b	-21.40	A 28.03±5.37 b	-23.7
	Random Allogamy	A 36.07±6.73 a	A 36.17±4.36 a	A 34.87±6.50 a	-3.33	A 32.13±8.15 a	-10.9
Preying potential of male (*P. citri* nymphs/ day)	Brother-sister	A 35.83±3.99 a	A 34.20±7.08 a	A 29.03±8.56 ab	-18.98	B 25.73±8.07 b	-28.2
	Random Allogamy	A 36.93±4.45 a	A 35.83±6.63 a	A 34.23±7.43 a	-7.31	A 32.97±6.46 a	-10.7
Total Larval developmental period	Brother-sister	A 13.1±1.29 a	A 12.1±1.20 ab	B 11.30±1.49 b	-13.74	A 11.70±1.77 b	-10.7
	Random Allogamy	A 13.4±1.65 a	A 13.1±0.99 a	A 13.5±1.84 a	0.75	A 12.7±1.77 a	-5.2
Generation time /days (Adult-Adult)	Brother-sister	A 31.7±1.83 a	A 31.9±2.33 a	A 32.4±1.90 a	2.21	A 31.4±1.84 a	-0.9
	Random Allogamy	A 31.4±2.76 a	A 31.8±1.69 a	A 30.9±2.13 a	-1.59	A 31.3±2.11 a	-0.3

N=10

Means followed by the same small letter in a row are not significantly different at 5% level of probability N=10

Means (for each parameter) followed by the same capital letter in a column are not significantly different at 5% level of probability.

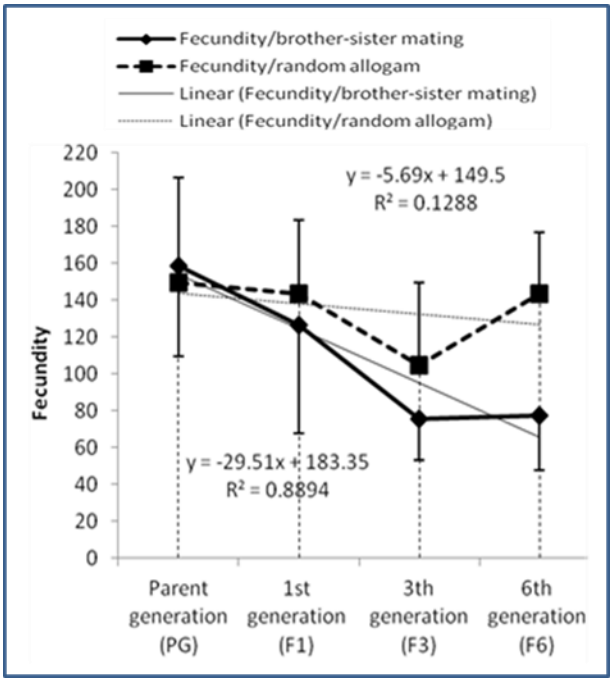

Fig. (1): Total deposited eggs of the Egyptian Laboratory Strain of *C. montrouzieri* female in brother sister and random allogamy mating techniques (regression line for each technique).

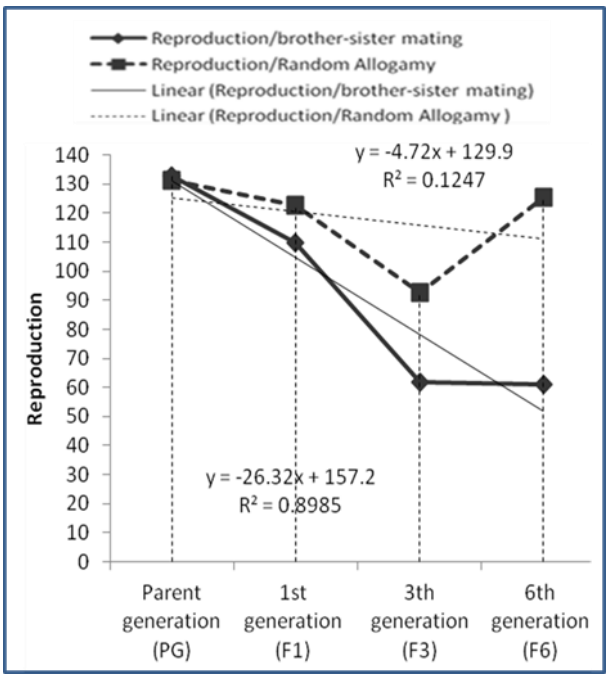

Fig. (2): Total hatched eggs (reproduction) of the Egyptian Laboratory Strain of *C. montrouzieri* female in brother sister and random allogamy mating techniques.

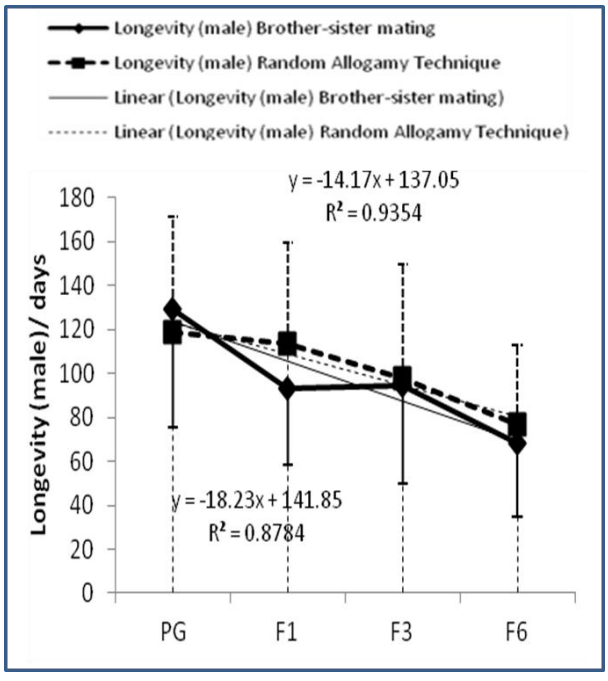

Fig (3): Male longevity of the Egyptian Laboratory Strain of *C. montrouzieri* in brother sister and random allogamy mating techniques (regression line for each technique).

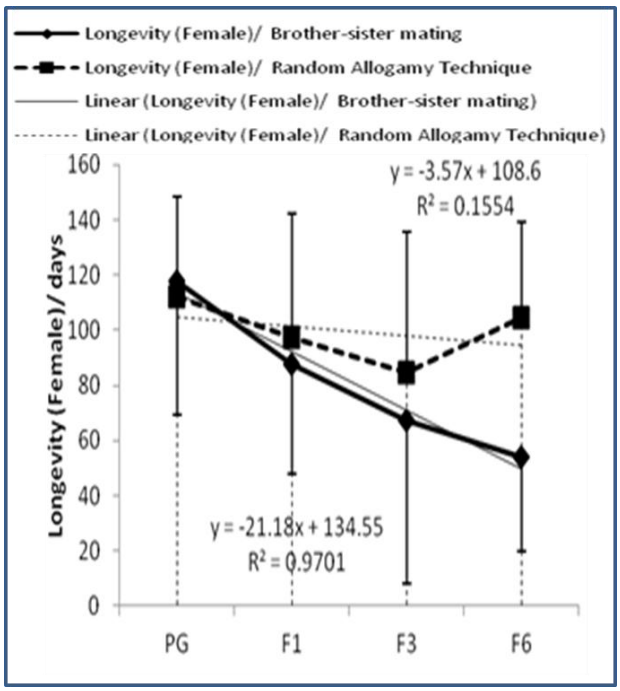

Fig (4): Female longevity of the Egyptian Laboratory Strain of *C. montrouzieri* in brother sister and random allogamy mating techniques (regression line for each technique).

and -10.7% in F3 and F6, respectively than the PG.

Because there was insignificant difference between F3 and F6, and the depression of the most parameters was strong and effective in F3, this refers to the collapse and the deterioration of the major biological parameters at the end of F3 using brother-sister mating and confirms the tendency to avoid brother-sister mating from mass-rearing of *C. montrouzieri* in the Department of Biological Control in Giza.

Statistical analyses showed that there were insignificant differences in all biological parameters among all the studied generations (PG, F1, F3 and F6) using random allogamy, except fecundity and reproduction rates, as they recorded significant decreases in F3 when compared with PG and F1. Fecundity and reproduction rates were -29.88% and -53.57 in F3, but this decrease did not continue until the F6 when insignificant differences occurred in F6, when compared with PG and F1 as the values raised to -4.1% and -4.4% in F6 (Figs. 3 & 4).

Despite the insignificant decrease in most of the morphobiological parameters in F6, compared with parent generation (PG), the random allogamy technique was able to achieve stability and balance in F6. This is probably due to the technique of rearing *C. montrouzieri*, practiced in Egypt, using limited space cages and does not allow the random mating. This was evident at the end of the 3rd generation, as sharp drops occurred in most of the major morphbiological parameters values. This also refers to the unstable genetic structure of Egyptian laboratory strain of the predator which was maintained for several generations based on brother-sister mating inside closed cages.

Obtained results agree with the findings of George and Craig (1964) who stated that to get rid of the disadvantages of inbreeding (brother-sister mating), crossing mating technique could be used to improve the important indicators of Mosquito. Watanabe and Anderson (1972) reported significant differences in some parameters' values for six generations on *Drosophila.* Ayal *et al.* (1987) stated that mass rearing requires necessarily use of random allogamy mating technique/ cross mating 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 chromosome or more and Asslan *et al.* 2008), who reported that after three crossing generations of *Coccinella septempunctata* (L.), insignificant differences were found in all morphobiological parameters. Thornhill (1993), and Keller and Waller (2002) stated that inbreeding can negatively affect a number of fitness components in a variety of organisms because it can lead to, for example, reduced viability, fecundity, growth rate or physiological efficiency. Inbreeding depression was demonstrated in laboratory reared bumble-bees for brood viability, colony size and egg-laying in queens (Plowright and Pallett 1979, and Beekman, *et. al.* 1999).

In conculsion and to improve general situation of the Egyptian laboratory strain, it is recommended to start mass-rearing of *C. montrouzieri* inside isolated and equipped rooms for not less than ten generations. Also, it is advisable to carry out a random allogamy technique with another strain to become ready to start genetic improvement operations.

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