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# MAINTAINING AND DEVELOPING VARROA-TOLERANT HONEY BEE SURVIVORS AS INDICATED BY SELECTIVE BREEDING PARAMETERS

[32]

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## ABSTRACT

The maintaining and developing of varroa-tolerant honey bee survivors were achieved via selective breeding at the apiary attached to the Faculty of Agriculture-Ain Shams University. This was adopted using Artificial Insemination for both selected and unselected (control) parents and completed till the fourth generation. The results showed that the accumulation reduction of infestation percentages by varroa increased from one generation to the second. These infestation percentages were 11.4%, 26.2%, 34.9% and 43.4% for the first, the second, the third and the fourth generations, respectively. When the realized heritability (effectiveness of selection) rates were estimated, it demonstrates a slightly high level for the third generation ($rh^2$= 0.68) than the other three tested generations i.e.: $rh^2$= 0.52 for the first, 0.44 for the second and 0.55, for the fourth generation. In addition, it appears that the third generation harbour the best values of selection differential, intensity of selection and response to selection.

**Key words:** Selection, Realized heritability, Artificial Insemination, Honey bee, Varroa mite

## INTRODUCTION

Since manifestation of *Varroa jacobsoni* and its rapid local dispersion in honey bee colonies. Several related subjects were studied by the following authors to evaluate the relationship between varroa mite and the bee survivors i.e.: the action level of infestation and the corresponding population density of progeny structure of varroa mite (**Kyntschev, 1985; Ritter & Leclereq, 1987; Kulincevic *et al* 1988; Hoffmann, 1992; Medina, 1998 and Gomez & Munoz, 1999**), effects of climatic factors (**Moretto *et al* 1991; Romaniuk *et al* 1993 and Garcia-Fernandez *et al* 1995**) and thermoregulation within honeybee colonies (**Sasak, 1989 and Bienefeld *et al* 1995**).

When the initial Varroa infestation was considered as "heavy invasion rate", the beekeepers were forced to protect their colonies using different insecticidal

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treatments without planning. These procedures were affect the acute appearance of Varroa-tolerance infestation **(Trouiller and Moosbeckhofer, 1997)**.

On the other hand, it appears that the natural resistance to Varroasis due to differentiation among bio and ecotypes or hybridization of honey bee individuals **(Buchler, 1990; Krol, 1990; Elbassiouny, 1998 and Rosenkranz, 1999)**, and the stereotype behavioural pattern of hygienic behaviour among bee colonies play an important role for naturally conspicuous reduction of infestation level by Varroa mite. **(Fuchs and Bienefeld, 1991; Eguaras *et al* 1994; Moretto, 1997; Spivak, 1997 and Szabo, 1998 & 1999)** So the quite safety way must be pass through breeding honey bee procedures for aquired resistant to Varroa mite **(Rinderer *et al* 1997 and Erickson *et al* 1998)**.

The present work aimed to maintain and develope Varroa-tolerant honey bee population under field conditions via selective breeding without using other mite control strategies.

## MATERIAL AND METHODS

Ten breeder colonies of honey bee naturally tolerant to varroasis were chose from different localities (regions) via Kaluobia - Giza - Middle Egypt and Upper Egypt, moreover ten normal colonies (control) were also chose. The twenty colonies were attached to the apiary of Faculty of Agriculture, Ain Shams University at Shoubra El-Kheima.

### Breeding programme

The recommended breeding programme based on artificial insemination (AI) for both tolerant and control groups to prevent over mating was adopted as the following:

- The selected ten colonies (parents), all were used as a sires and dames (closed population) for the first generation, the best five colonies (less infestation level) in the next generation (from ten colonies each) were chosen as a parents for the following generation and the other five colonies were excluded and so on.
- For the unselected group (control), the ten colonies were used for the successive generation.

### Insemination procedure

**Preparing drones:** For both tested groups (selected and unselected (control)) mature drones were caught and replaced together in queenless colony for about seven days in order to be nursed. The pooled collected drones were used for semen collection to inseminate the virgin queens.

**Preparing queens:** Virgin queens were reared randomly from the colonies of each group separately. Virgin queens (20 from each group) 5 – 7 days old were inseminated with about 8 µl of semen obtained from the pooled collecting drones. After AI, queens were left undisturbed for about a week in three frame nucleus colonies until they start egg laying .The best successfully inseminated (10) queens from each group were introduced into permanent colonies (7-8 frames).

Percentage of Varroa infestation on adult bees, for each generation, was estimated after 10 weeks from introducing the inseminated queens in the permanent colonies (during that time the offspring of the introduced queens had replaced most workers of the original queens) and performed on all 20 colonies (10 selected and 10 unselected (control)) at 15 days intervals for two months time period.

The resistance to varroasis in selected colonies was calculated by adopting the following model:

$$Y_{ijk} = \mu + g_i + t_j + (gxt)_{ij} + e_{ijk} \qquad (1)$$

where:

$Y_{ijk}$ = yield for generations(i); inspection periods(j) and replicates(k).
$\mu$ = generation mean
$g_I$ = generation effect
$t_j$ = time effect (inspection periods)
$e_{ijk}$ = error

Furthermore, the regression value for the percentage of infestation by Varroa mite and the corresponding generation was calculated to estimate the differences from one generation to the next. The difference when the regression values were considered for the selected and unselected groups could yield information on the efficacy of selection. Accordingly t-test between the two variables i.e.: the selected and unselected colonies depending on the regression coefficient was adopted as follows:

$$t_b = b_{non\ sel.} - b_{sel.} / \ error(b_{non\ sel.} - b_{sel.}) \qquad (2)$$

where: b = regression coefficient

The selection differential, response to selection, realized heritability and intensity of selection were estimated as follows (**Collins, 1986**):

S = Population mean – mean parental (selected) value (3)
R = Parental population – mean offspring (4)
$h^2r$ = R / S (5)
i = S / $\delta$p (6)

where:

S = selection differential
R = response to selection
$h^2r$ = realized heritability
i = intensity of selection
$\delta$p = phenotypic standard deviation

## RESULTS AND DISCUSSION

The percentages of infestation by Varroa mite individuals in the unselected (control) colonies were fluctuated from generation to generation yielded significant differences between each others. This values were 14.42 ± 0.62%, 16.56 ± 0.84%, 13.24 ± 0.59%, 10.94 ± 0.49% and 12.69 ± 0.42 for parents and the successive generations, respectively (Table, 1 and Fig. 1).

For the selected group (Table, 2 and Fig. 1), the percentages of infestation by Varroa in parents were comparatively significantly lower than parents of unselected (control) group (t =9.475) which averaged 7.11 ± 0.37 %. For the following generations was determinated in the offspring of the next generation as follows:

- For the first generation, the values for the offspring averaged 8.00 ± 0.59% while for the selected parents it averaged 6.30 ± 0.20%.
- In the second generation, the offspring values averaged 7.14 ± 0.67% but the selected parents demonstrate 5.25 ± 0.19%.
- In the third generation, the offspring values averaged 6.62 ± 0.71% and the selected parents values averaged 4.63 ± 0.42%.
- In the fourth generation, the offspring values averaged 5.40 ± 0.47% and the selected parents values averaged 4.02 ± 0.26%.

Table 1. Mean percentages of infestation by varroa mite on adult bees for parents, first, second, third and fourth generations in the unselected (control) group

| Colonies | Parents | Generations | | | |
|---|---|---|---|---|---|
| | | First | Second | Third | Fourth |
| 1 | 13.95±1.03 | 18.35±0.63 | 12.85±1.17 | 12.32±1.23 | 12.47±0.93 |
| 2 | 13.35±1.42 | 16.80±1.10 | 11.60±0.76 | 11.00±1.31 | 13.20±1.04 |
| 3 | 16.85±1.05 | 20.95±2.05 | 15.45±1.40 | 10.10±0.94 | 11.75±1.03 |
| 4 | 11.47±1.08 | 19.30±0.61 | 10.82±1.34 | 14.37±1.20 | 14.82±1.41 |
| 5 | 15.00±1.52 | 15.80±0.59 | 13.90±1.13 | 9.25±1.50 | 10.42±0.82 |
| 6 | 14.40±1.04 | 17.47±1.02 | 13.60±1.22 | 9.17±0.67 | 14.15±0.84 |
| 7 | 10.85±1.08 | 16.00±0.37 | 9.82±1.09 | 9.47±0.74 | 11.47±0.50 |
| 8 | 16.85±1.08 | 14.75±0.40 | 15.60±1.18 | 10.67±0.98 | 13.15±0.80 |
| 9 | 16.10±1.52 | 15.50±0.65 | 15.05±1.61 | 12.20±0.81 | 13.95±1.14 |
| 10 | 15.35±2.65 | 10.72±0.91 | 13.70±0.55 | 10.90±0.70 | 11.55±1.07 |
| General mean (± s.e.) | 14.42±0.62 | 16.56±0.84 | 13.24±0.59 | 10.94±0.49 | 12.69±0.42 |
| F value | | | 10.637** | | |
| L.S.D. | | | 1.834 | | |

t between parents (control and selected) = 9.475**

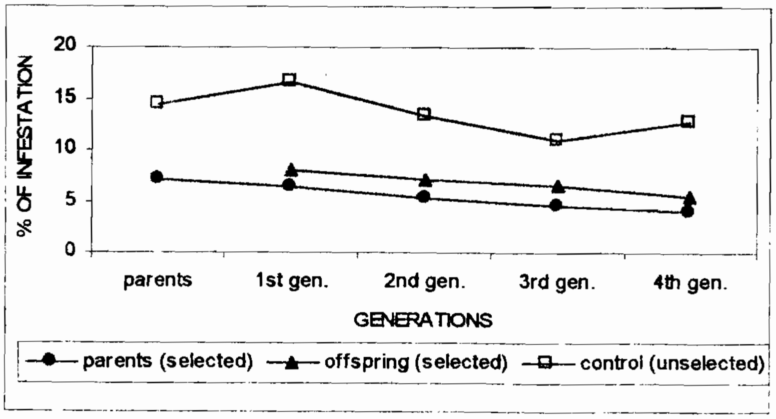


Fig. 1. Percentages of infestation by varroa mite on adult bees of unselected (control) and selected (parents and offspring) groups

Table 2. The fluctuations in the mean percentages of infestation by varroa mite on adult bees for parents, first, second, third and fourth generations in the selected group

| Colonies | Parents | Generations | | | |
|---|---|---|---|---|---|
| | | First | Second | Third | Fourth |
| 1 | 8.40±0.70 | 8.17±0.66 | 7.65±0.70 | 3.95±0.81* | 6.62±1.07 |
| 2 | 7.87±0.94 | 9.55±0.99 | 8.40±1.04 | 9.55±1.09 | 6.44±1.14 |
| 3 | 5.85±0.54 | 7.00±1.13* | 10.04±0.89 | 7.80±1.07 | 4.06±0.43* |
| 4 | 9.10±0.68 | 11.07±0.48 | 5.43±0.50* | 9.90±0.81 | 7.72±1.22 |
| 5 | 6.32±0.73 | 6.50±0.80* | 5.37±0.43* | 5.38±1.04* | 3.60±0.58* |
| 6 | 6.22±0.88 | 6.40±0.77* | 11.02±0.69 | 6.93±1.06 | 6.05±1.23 |
| 7 | 8.00±0.58 | 10.37±0.60 | 4.90±0.80* | 6.05±0.75* | 7.07±1.09 |
| 8 | 5.22±0.76 | 5.90±0.60* | 4.65±0.63* | 8.87±1.54 | 3.32±0.67* |
| 9 | 6.92±0.93 | 5.70±0.58* | 8.02±0.70 | 3.52±0.47* | 4.10±0.71* |
| 10 | 7.20±1.13 | 9.35±0.83 | 5.90±0.62* | 4.25±0.83* | 5.02±0.47* |
| General mean (± s.e.) | 7.11±0.37 | 8.00±0.59 | 7.14±0.67 | 6.62±0.71 | 5.40±0.47 |
| Selected mean (± s.e.) | 7.11±0.37 | 6.30±0.20* | 5.25±0.19* | 4.63±0.42* | 4.02±0.26* |
| Progress of Each generation | | 11.4% | 16.7% | 11.8% | 13.2% |
| Accumulation | | | 26.2% | 34.9% | 43.4% |

* selected parents for next generation

Table 3. Analysis of variance figures for the successive four generations and time of inspection of infestation percentages by varroa mite individuals

| Source of Variance | d.f. | Selected | | | | No selected | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | MSQ | F | Pr > F | H-F | MSQ | F | Pr > F | H-F |
| Generations | 3 | 47.266 | 2.79 | 0.0541 | | 204.167 | 12.65 | 0.0001 | |
| Error G | 36 | 16.913 | | | | 16.144 | | | |
| Time | 3 | 2.330 | 2.90 | 0.0381 | 0.0381 | 5.040 | 3.52 | 0.0176 | 0.0197 |
| G x T | 9 | 3.706 | 4.62 | 0.0001 | 0.0001 | 6.288 | 4.39 | 0.0001 | 0.0001 |
| Error T | 108 | 0.802 | | | | 1.433 | | | |

When the analysis of variance for the percentages of infestation by varroa mite was carried out, it yielded significant differences between the selected and the unselected groups (Table, 4). Differences among generations of the two groups, revealed also a distinct variation among the successive four generations, but more significant for the unselected group. When the time of inspection was considered a considerable influence for both groups was obtained. Moreover, the interaction between generations and time of inspection demonstrates noticeable variation. This means, that the reduction of Varroa mite infestation affected both the generations and the time of inspection.

The variance between the two groups depending on the regression coefficient ($t_b$ = 1.208) however, has no significant difference. On the other hand, the distance between the regression lines of both groups for each generation (Fig. 2) represented the genetic factors (inherited part) that maintained by selection, which were 7.31,8.56,6.10,4.32,7.29 for parents, first, second, third and forth generations, respectively. So, the variance depending on the inherited part (the difference between the general means of the two groups, Table 3) revealed highly significant values for the differences (t =55.678)

$$\{t = (\Sigma\mu_{non\ sel.} - \Sigma\mu_{sel.}) / \sqrt{(MSQ_{non\ sel.}/n + MSQ_{sel.}/n)}$$

where : $\Sigma\mu$ = general mean, MSQ Mean of squares}

**Selection differential (S):** The selection differentials for the successive four generations were 1.70, 1.89, 1.99 and 1.38, respectively (Table, 6). The results showed that the S shaped curve start to increase in the second generation then reaching its maximum during the third generation, then decreased obviously during the last generation.

It is a well known fact that, increasing S indicate that the superiority of tested parents. So the third generation seems to be the most proper generation for selection in this respect.

**Response to selection (R):** As the same manner for **S** and **i**, the response to selection **R** for the third generation seemed to be the best (1.37) with wide variance compared with the other generations: 0.89, 0.84, and 0.77 for the first, the second and the fourth generations, respectively (Table, 6).

**Realized heritability ($h^2r$):** The heritability values were used to predicate the response ability to the selection, where it was the inherited part of the selection differentially. It was noticed again that the $h^2r$ for the third generation ($h^2r$= 0.68) was slightly higher than the other three generations. In spite of the value of $h^2r$ started with 0.52 for the first generation and ended with 0.55 for the forth generation, nearly the same values (Table 6).Generally, the realized heritability for the selected group (which could be estimated as = actual gain (initial parents – last generation) /sum of selection differential) were 0.246.

**Intensity of selection (i):** In spite of selecting 50% of the offspring as a parents for the next generation (starting from the first generation and continuous until the fourth generation). The intensity of selections varied from one generation to the other generation. These values

Table 4. Mean of time of inspections within generations for infestation percentages by varroa mite in the unselected (control) and selected groups

| Generations | Time of inspections | | | | |
|---|---|---|---|---|---|
| | Unselected (control) | | | | |
| | T1 | T2 | T3 | T4 | Mean ±s.e. |
| G1 | 15.90$^{A}$ | 16.74$^{A}$ | 15.74$^{A}$ | 17.08$^{A}$ | 16.56±0.84 |
| G2 | 12.43$^{B}$ | 14.86$^{A}$ | 12.44$^{B}$ | 12.93$^{B}$ | 13.24±0.59 |
| G3 | 10.64$^{B}$ | 10.60$^{B}$ | 10.96$^{B}$ | 11.59$^{B}$ | 10.94±0.49 |
| G4 | 13.50$^{AB}$ | 11.99$^{B}$ | 12.38$^{B}$ | 12.91$^{B}$ | 12.69±0.42 |
| Mean ± s.e. | 13.12±0.95 | 13.55±1.20 | 12.88±0.88 | 13.63±1.03 | 13.36±1.02 |
| MSD | 2.984 | 2.760 | 2.346 | 2.762 | |
| | Selected | | | | |
| G1 | 7.69$^{A}$ | 8.32$^{A}$ | 7.98$^{A}$ | 8.02$^{A}$ | 8.00±0.59 |
| G2 | 6.72$^{A}$ | 7.28$^{A}$ | 6.98$^{AB}$ | 7.57$^{AB}$ | 7.14±0.67 |
| G3 | 7.70$^{AB}$ | 6.38$^{A}$ | 6.13$^{AB}$ | 6.27$^{AB}$ | 6.62±0.71 |
| G4 | 4.59$^{B}$ | 6.44$^{A}$ | 5.09$^{B}$ | 5.49$^{B}$ | 5.40±0.47 |
| Mean ± s.e. | 6.67±0.63 | 7.10±0.39 | 6.54±0.53 | 6.84±0.50 | 6.79±0.47 |
| MSD | 2.891 | 2.85 | 2.474 | 2.329 | |

MSD = Minimum Significant Difference

Table 5. Estimate the regression coefficient for unselected (control) and selected groups

| Polynomial Contrast | | Averaged Variable | |
|---|---|---|---|
| | | Unselected (control) | Selected |
| Contrast Estimate (b) | | - 2.958 | - 1.860 |
| Hypothesized Value | | 0 | 0 |
| Difference (Estimate – Hypothesized) | | - 2.958 | - 1.860 |
| Std. Error | | 0.635 | 0.065 |
| Sig. | | 0.000 | 0.007 |
| 95% Confidence interval | Lower Bound | - 4.246 | - 3.179 |
| for Difference | Upper Bound | - 1.669 | - 0.541 |

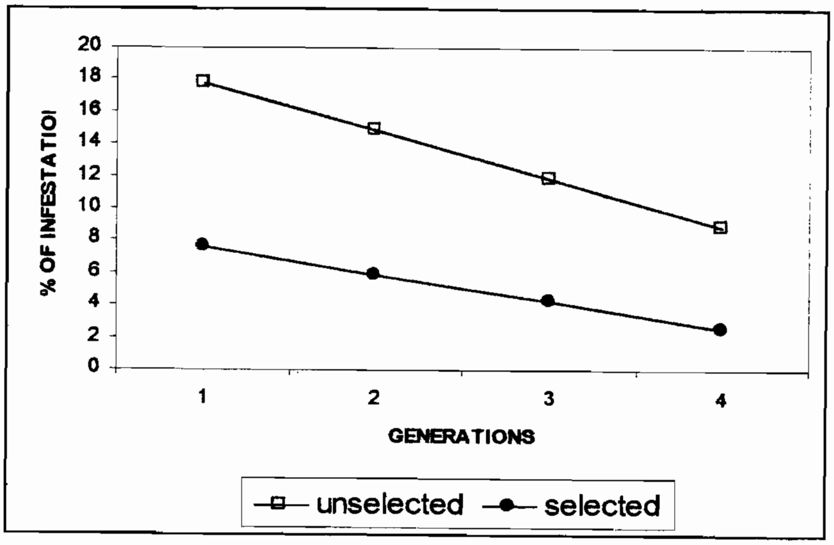


Fig. 2. Regression lines of percentage of infestation by Varroa mite on generations for selected and unselected (control) honey bee colonies

Table 6. Estimates of selection differential, intensity of selection, response to selection and realized heritability for infestation percentages by varroa mite in the first, second, third and fourth generations in the selected group

| Generation | Mean of offspring | Mean of selected parents | Selection differential (S) | Intensity of selection (i) | Response to selection (R) | Realized heritability ($h^2r$) |
|---|---|---|---|---|---|---|
| Parents | | 7.11 | | | | |
| 1st generation | 8.00 | 6.30 | 1.70 | 3.69 | 0.89 | 0.52 |
| 2nd generation | 7.14 | 5.25 | 1.89 | 4.29 | 0.84 | 0.44 |
| 3rd generation | 6.62 | 4.63 | 1.99 | 2.12 | 1.37 | 0.68 |
| 4th neration | 5.40 | 4.02 | 1.38 | 2.37 | 0.77 | 0.55 |

were 3.69 for the first, 4.29 for the second, 2.12 for the third and 2.37 for the fourth (Table, 6).

Increasing intensity of selection, refer to the reliable increasing inbreeding coefficient and relationship between relatives. In general it could be concluded depending on the estimated parameters, that the third generation was the best generation when these values were considered followed by the fourth, the first and the second generations.

**Progress of generations:** When the reduction of infestation percentage by varroa (Table, 2) for each generation was estimated separately it appears that, the second generation had the highest progression value (16.7%). The fourth generation (13.2%). Come next both the first and the third generations harbour almostly the same values, hence they were 11.4% and 11.8%, respectively. According to the findings of the following authors, these variability values may be due to the simultaneous effects of hybridization (**Buchler 1990, Krol 1990 and Elbassiouny, 1998**) or hygienic behaviour (**Fuchs and Bienefeld 1991, Spivak 1997 and Szabo 1998-99**).

On the other hand, the reduction of infestation percentages by varroa increased from generation to generation but to a certain extent. They were 11.4%, 26.2%, 34.9% and 43.4% for the 1st, the 2nd, the 3rd and the 4th generations, respectively.

It was found in the progression values for the third generation that it was no quite high when compared with the second and fourth generations; but dominants the completely tested generations and harbour the selection differential, intensity of selection, heritability and response to selection.

The obtained results showed that it is seems possible to produce and maintain varroa-tolerant strains of honey bee out of domestic stock (**Erickson *et al* 1998**) and sustain the infestation level much lower (**Rinderer *et al* 1997**).

From the economical point of view it could be concluded that, for practical selection, the commercial beekeepers under field conditions can develop this character in their bees without artificial insemination by considering drones from colonies with low infestation levels of varroa and excluding those from colonies with high infestation levels of varroa by simply removing the drone broods and replacing their virgin queens gradually from appropriate centers.

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# الاحتفاظ وتطوير صفة تحمل افراد نحل العسل لطفيل الفاروا عن طريق الانتخاب

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**عادل محمد البسيونى[١]**

**١ – قسم وقاية النبات – كلية الزراعة – جامعة عين شمس – شبرا الخيمة – القاهرة – مصر**

الاحتفاظ بصفة المقاومة فى نحل العسل لطفيل الفاروا وتنميتها تمت عن طريق الانتخاب بمنحل كلية الزراعة–جامعة عين شمس–القاهرة– مصر باستخدام التلقيح الآلى لكلا من مجموعة الآباء المنتخبة (مختارة من مناطق مختلفة فى مصر) و مجموعة المقارنة (عشرة طوائف لكلا منهما)، وتم تتبع هذه الصفة حتى الجيل الرابع.

أظهرت النتائج أن نسبة الانخفاض فى الإصابة بالطفيل علي النحل البالغ كانت تزداد من جيل الى جيل مقارنة بالآباء المختارة, حيث كانت ١١,٤% فى الجيل الأول و٢٦,٢% فى الجيل الثانى و٣٤,٩% فى الجيل الثالث و٤٣,٤% فى الجيل الرابع. أيضا أظهرت النتائج أن تقديرات المكافئ الوراثى المحقق $rh^2$ (افضل الطرق لمقارنة كفاءة الانتخاب) تشير إلى ان قيم الجيل الثالث (٠,٦٨) أعلى من قيم باقى الأجيال حيث كانت (٠,٥٢) للجيل الأول و(٠,٤٤) للجيل الثانى و(٠,٥٥) للجيل الرابع. بالإضافة إلى ذلك فان قيم الفارق الانتخابى و شدة الانتخاب والاستجابة للانتخاب كانت أعلى فى الجيل الثالث عن باقى الأجيال.

**تحكيم:** أ.د. جميل برهان الدين السعدنى أ.د محمد عطية عويس