

HYGIENIC TEST AS A PARAMETER OF HONEY BEE RESISTANCE TO INFECTIVE BROOD DISEASES

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INTRODUCTION

By selecting bees resistant to infective brood diseases different level of cleaning behavior of bees is used. The cleaning behavior of bees is apparent in their ability to remove diseased or dead larvae or pupae from brood combs and this way to prevent formation and cumulating of spores.

First information on a possible behavioral resistance of bees are by Jones and Rothenbuhler (1964). For the purpose of measurement it is possible to imitate the situation by killing brood using cyanogas or freezing (Milne, 1982), or by squeezing a needle in single brood cells (Newton & Ostasiewski, 1986). The quicker bees remove killed brood from cells the better is the precondition of resistance of bees to brood diseases.

We constructed a mathematical formulae for calculating a parameter which would characterise the hygienic behavior of a colony using data on course of cleaning killed pupae. We also analysed the genetic action on the parameter of hygienic test.

METHODS

We used the way of killing pupae by squeezing a needle through cell cups by Newton & Ostasiewski (1986). We killed pupae usually on the area of 10 x 10 cells in the tested bee colonies. In the beginning of the test the number of all cells in the tested area (P) and number of cells free of pupae (N0) we recorded and also the place of tested comb and area in it for simple determination. In one, two and three days after killing pupae the number of cells quite cleaned (N1, N2, N3) we recorded and at the last control also the number of quite sealed cells (NL) in the tested area. Also time at each control (t0, t1, t2, t3) we wrote. We constructed the computational formulae to express the mean number of hours needed for cleaning each killed pupae:

$$\text{HYG} = \frac{(N1-N0) \cdot (T1/2) + (N2-N1) \cdot [(T1+T2)/2] + (N3-N2) \cdot [(T2+T3)/2] + (P-N3-NL) \cdot (T3+12)}{(N1-N0) + (N2-N1) + (N3-N2) + (P-N3-NL)} + \frac{NL}{(N3-N0)/T3}$$

where: T1 is the time interval from t0 to t1, T1 = t1 - t0
T2 = t2 - t0
T3 = t3 - t0 (hours)

after some improvement:

$$\text{HYG} = \frac{(N1-N0).(T1/2)+(N2-N1).[(T1+T2)/2]+(N3-N2).[(T2+T3)/2]+(P-N3-NL).(T3+12)}{P - N0 - NL} + \frac{NL . T3}{N3-N0 \text{ (hours)}}$$

The formulae assumes the cumulative and always between two controls the linear course of the number of cleaned cells in the tested area of a brood comb. For example the part of formulae $(N2-N1).[(T1+T2)/2]$ products the number of quite cleaned cells from time t1 till time t2 by mean time interval from killing pupae till theirs quite cleaning by bees. The part of formulae $(P-N3-NL).(T3+12)$ counts the number of cells that are not quite empty by the last control and at the same time they are already uncapped by bees, and products it with time $T3+12$. This is the estimated mean time in which at the last control found uncapped and non cleaned pupae will be cleaned out. The number 12 (hours) is correct for control intervals about 24 hours. By different intervals it must be changed with half of the length of the interval. The whole greater (left) part of the formulae expresses the average time fallen to every cleaned cell.

The cells stayed quite capped at the last control content either living, by squeezing only injured pupae or dead pupae that bees have not began to remove yet. Ignoring them would cause an error of the resulted value of parameter HYG. For it would not be necessary to control the course of their another cleaning by bees it is assumed that bees will remove these rest capped pupae by the same speed at which they cleaned killed pupae from the beginning of the test till the last control. The speed is mathematically expressed by formulae $NL / [(N3-N0)/T3]$ and is added to calculated speed of removing pupae in the first three time intervals. The graphic expression of the course of cleaning cells is apparent in the fig. 1.

The resulting parameter HYG (hours) expresses the average time that bees need for the quite removing of each killed pupae and is a simple parameter of behavioral resistance of bees to brood diseases. If the first control is made 24 hours after killing pupae and all cells in the testing area are quite cleaned the HYG value is 12 hours.

RESULTS AND DISCUSSION

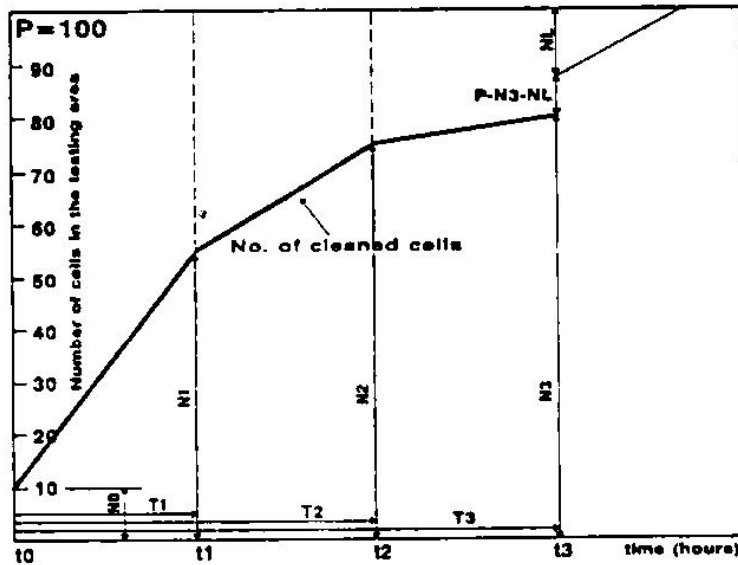
In 1990 to 1994 we measured the hygienic test values of 295 bee colonies in most cases with instrumentally inseminated queens. If a colony was repeatedly tested the average value of repeated measurements was used. The set of measured values had a great bias from normal distribution: mean was 53,8 h and median 30,0 h by standard deviation 80,4 h, see fig. 2. That is why we transformed the original data using natural logarithms ($\ln x$). The mean of transformed data was 3,516, median 3,401 and standard deviation 0,845. Thus the set of transformed values has nearly normal distribution as is apparent in fig. 3. In another analysis we used only transformed data.

Using analysis of variance we determined the genetic basis of the hygienic test (tables 1 and 2). By sorting colonies into groups by mothers and also by fathers there were highly significant genetic differences between these genetic groups in the hygienic test ($P < 0.001$). Thus the selection on the improved cleaning behavior of bees by removing dead brood from a brood combs in the honey bee nest using the described hygienic test and this way also the selection of bee populations for the resistance to infective brood diseases is possible.

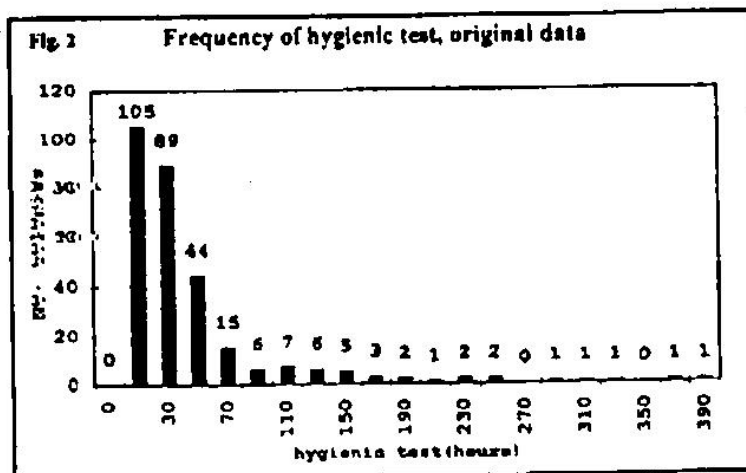
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- MILNE CP (1982): Laboratory measurement of honeybee brood disease resistance. Uncapping and removal of freeze-killed brood by newly emerged workers in laboratory cages. *J. Apic. Res.* 21 (2) 111-114.
- NEWTON DC, OSTASIEWSKI NJ (1986): A simplified bioassay for behavioral resistance to American foulbrood in honey bees (*Apis mellifera* L.). *Amer. Bee Journal* 126 (4) 278-281.

Fig. 1 The course of removing killed pupae during the hygienic test



Explanations are in the text.



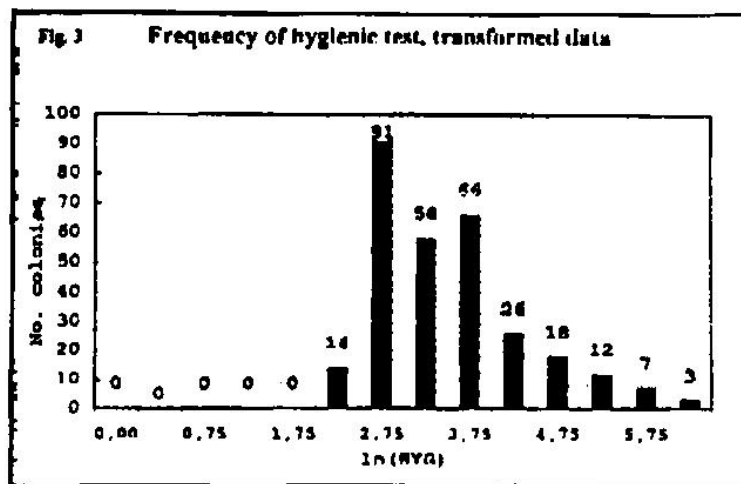


Table 1.

Analysis of variance of the hygienic test; the influence of dam queens (families by mother of queens)

Source of variance	SS	d.f.	MS	F-ratio	Significance
Mother queen	82.593	44	1.877	4.113	0.0000
Error	98.586	216	0.456		
Total	181.179	260			

Table 2.

Analysis of variance of the hygienic test; the influence of sire queens (families by father, i.e. drone queen)

Source of variance	SS	d.f.	MS	F-ratio	Significance
Father queen	42.008	23	1.826	3.025	0.0000
Error	112.892	187	0.604		
Total	154.900	210			