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Original article

Changes in a population of *Apis mellifera* L. selected for hygienic behaviour and its relation to brood disease tolerance

M. Alejandra PALACIO[a]*, Emilio E. FIGINI[b], Sergio R. RUFFINENGO[a], Edgardo M. RODRIGUEZ[b], Marcelo L. DEL HOYO[b], Enrique L. BEDASCARRASBURE[b]

[a] Unidad Integrada INTA, Facultad de Ciencias Agrarias, UNMdP, PROAPI, C.C. 276, 7620 Balcarce, Argentina
[b] Facultad de Ciencias Veterinarias, UNCPBA, Campus Universitario Paraje Arroyo Seco, PROAPI, 7000 Tandil, Argentina

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Abstract – Honeybee colonies were evaluated for hygienic behaviour using a pin-killed brood assay. Presence and absence of visual symptoms of brood diseases were recorded. Colonies that removed more than 80% of dead brood after 24 hours were selected for queen and drone production and new colonies were evaluated for hygienic behaviour. This procedure was repeated yearly from 1992 through 1997. The degree of total hygienic behaviour (brood removed) and partial hygienic behaviour (brood uncapped but not totally removed) were determined. Colonies were classified as hygienic and non-hygienic and these data were related to the incidence of brood diseases. Total hygienic behaviour increased in the population after four years of selection on queens without mating control from 66.25% in 1992 to 84.56% in 1997. Hygienic colonies had a lower frequency of brood diseases when compared to non-hygienic colonies. It is suggested that this trait can be used as a selection criterion in queen breeders' apiaries.

hygienic behaviour / *Apis mellifera* / selection / brood disease / tolerance

1. INTRODUCTION

Most of the features of economic importance in beekeeping are the result of the behaviour of the whole colony. The colony is not an individual or a population in the usual sense. It is a family composed of a mother (queen), one or more fathers (drones), their daughters (workers) and sons (drones). A queen usually copulates with

* Correspondence and reprints
E-mail: palacio@vet.unicen.edu.ar

ten to seventeen drones, so the colony is a super-family with a common queen mother and sub-families that have different drones as fathers [10]. Thus, the study of the social behaviour of the colony is more complex than that of individuals.

One of the behaviours that has been well described in honeybee colonies is hygienic behaviour. This behaviour is the ability of workers to uncap cells and remove dead or disease brood from them. It was first described by Rothenbuhler [8] when he observed that inbred lines were resistant to American Foulbrood because they removed diseased brood. In contrast, dead brood remained in cells of colonies headed by inbred lines which were susceptible. From this contrast in removal ability, it was hypothesized that there were genetic differences between colonies for hygienic behaviour. The behaviour of F1 honeybees from the crossing of the two inbred lines and from the backcross of F1 drones to the hygienic inbred line was analyzed and it was hypothesized that this behaviour was controlled by two pairs of recessive genes, u (uncapping) and r (removing) [9].

Moritz [6] analyzed Rothenbuhler's original data and concluded that uncapping was controlled by one gene with two alleles, but that removal behaviour might be controlled by two genes (r1 and r2). He also suggested that there could be some kind of interaction between them.

Because the bees eliminate the focal infection from the colony, this behaviour has been related to brood disease resistance [3, 8, 10–13].

Brood diseases are one of the most important restrictions to beekeeping development in Argentina. Diseases usually weaken colonies and sometimes kill them. At present, management practices and chemicals are used to control brood diseases. Chemicals are not always effective, and when used in excess or at the wrong time they leave residues in honey.

American foulbrood is the most serious brood disease of honey bees. Though the best methods to control this disease are management practices (shaking honeybees, sterilizing hive materials, etc.) many beekeepers use oxytetracycline for its control [2]. However, Alippi [1] has detected resistance of *Paenibacillus larvae* and *Bacillus alvei* to oxytetracycline. In recent years tolerance to this drug was detected in some areas in the USA [5, 13].

Chalkbrood caused by the fungus *Ascosphaera apis* is also present in Argentina and though affected colonies usually do not die it has had negative effects on the colonies. There is no chemical treatment for this disease.

Because of these problems, the objectives of this work were:

(a) to determine the change in hygienic behaviour in a selected population without mating control, and

(b) to study the relationship between hygienic behaviour and brood diseases.

2. MATERIALS AND METHODS

The work was carried out during five years (1992–1996) in Las Animas Apiary in Tandil (Buenos Aires Province, Argentina, – 37° LS).

2.1. Determination of the change in hygienic behaviour in a selected population without mating control

The experiments were carried out with an initial population of sixty five colonies headed by naturally mated queens. Queens were from different apiaries in Buenos Aires province where commercial beekeeping is concentrated. Although massive queen introductions have been made from different origins, *A. m. ligustica* was the most common subspecies introduced in this area.

The colonies were kept in standard Langstroth hives and evaluated for hygienic

behaviour between October to July each year.

To quantify hygienic behaviour a pin-killed brood assay was used, where all the capped cells present in an area of 10 cm × 5 cm were counted (x). Later, capped brood cells were perforated using a pin to kill the brood.

The treated comb was replaced in the original colony and after 24 hours the number of uncapped cells with dead brood inside (z) and the number of cells that remained capped (y) were recorded.

Using these values, measures of the total hygienic behaviour (THB) and partial hygienic behaviour (PHB) were obtained. THB was the number of cells of dead brood that were removed by the honeybees divided by the total number of cells of brood killed. PHB was the number of cells of dead brood that were detected and uncapped by honeybees but not totally removed in 24 hours divided by the total brood killed.

$$\text{T.H.B} = \frac{x - y - z}{x} \times 100$$

$$\text{P.H.B} = \frac{x - y}{x} \times 100.$$

Colonies were evaluated for hygienic behaviour once a month for at least six months.

The median values for all six hygienic tests were calculated. The colonies that registered THB over 80% in at least three measurements were selected and used as breeder colonies for queen production.

Daughter queens were introduced in four-frame nuclei. They mated naturally and after forty days the colonies were evaluated for hygienic behaviour as described above. Each year new colonies from beekeepers were introduced to the program for evaluation. These colonies were received in October each year and the number varied each year.

The procedure described above was repeated from October to July in 1993–94, 1994–95 and 1995–96. In 1996–97 the daughter colonies and the whole population were evaluated for hygienic behaviour, but no mother colony was selected.

THB and PHB average values were obtained for the whole population (population), for the colonies selected each period (mothers), and for colonies with daughter queens (daughter) each year and a Chi-square test was used to compare initial and final populations.

2.2. Relation between hygienic behaviour and resistance to brood disease

While measures of hygienic behaviour were taken, the general state of the colony and presence or absence of visual symptoms of brood diseases (chalkbrood, American foulbrood and European foulbrood) were recorded. All colonies in the first year, and a sample size between 50 and 60 colonies the following years, were used to record brood diseases symptoms. Colonies were classified as hygienic (THB > 80%) or non-hygienic (THB < 80%) following previous work [4] and these data were related to the presence of brood diseases using a Chi-square test.

3. RESULTS

3.1. Determination of change in hygienic behaviour in a selected population without controlled mating

Table I gives the median values of THB and PHB for the whole population (mother colonies + daughter colonies + new colonies from beekeepers), for selected mothers and daughter colonies in each year. As these variables were not normally distributed, the median was used as the best average estimator (value).

THB in the initial population was 66%. After four years of selection without mating control and with the introduction of new

Table I. Total Hygienic Behaviour (THB) and Partial Hygienic Behaviour (PHB) median values (in percentages) for total population (mother, daughters and other colonies from beekeepers), for selected colonies (mothers) and daughter colonies (daughters) for each period.

	Population			Mother			Daughter		
Period	N	THB	PHB	N	THB	PHB	N	THB	PHB
92–93	65	66	92	6	93	98	–	–	–
93–94	113	63	88	12	74	95	26	72	88
94–95	151	82	96	13	93	98	67	82	94
95–96	167	84	94	8	88	96	109	93	94
96–97	105	85	93	–	–	–	48	86	91

N = number of colonies.

colonies each year, THB increased to 84% in the population indicating an improvement of 18%. THB was higher each year for the population except in 1993–94 when the median THB was 63%.

PHB includes colonies that detected and uncapped dead brood but only removed brood partially or not at all. PHB values were high in the initial population (1992–93 = 96%). Selection was made for THB and the colonies selected (mothers) always had PHB values above the population median. However, PHB was not improved during the following years.

In Figure 1, the distribution of the variable THB in the initial population (1992–93) and final population (1996–97) is presented. Both populations initially had an asymmetrical distribution and after selection this asymmetry was more pronounced. In the initial population, 28 of 65 colonies (43%) had THB values over 80% and in the final population, 77 of 104 honeybee colonies (74%) had values over 80%. These differences were statistically significant ($p < 0.001$).

In Figure 2, the distribution of the variable PHB in the initial population (1992–93) and final population (1996–97) is presented. The PHB frequency distribution was asymmetrical, but a weak difference was detected between initial and final populations. In the initial population, 59 of 65 honeybee colonies (91%) had PHB values over 0.8 and in the final population 98 of 104 honeybee colonies (96%) were over this PHB value. These differences were not statistically significant ($p = 0.1474$).

3.2. Relation between hygienic behaviour and brood disease resistance

In Table II, colonies evaluated in all years were classified as hygienic (THB > 80%) or non-hygienic (THB < 80%), and by the presence or absence of brood diseases or American foulbrood within the two groups. All years were considered together.

Hygienic colonies had a lower frequency of brood diseases (n = 287, $p < 0.001$). Only 9 of 109 hygienic colonies had visual symptoms of brood disease (8.00%). Brood diseases were detected in 44 of 178 non-hygienic colonies (24.70%).

Hygienic colonies had a lower incidence of American foulbrood. (n = 287, $p < 0.001$). The percentage of hygienic colonies affected by this disease was 1.8% whereas in non-hygienic colonies it was 10.1%.

4. DISCUSSION

Message and Gonçalves [4] work with Africanized honeybees detected a variability

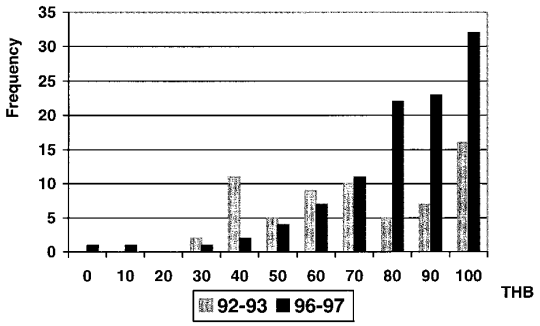

Figure 1. THB in the initial (92–93) and final population (96–97).

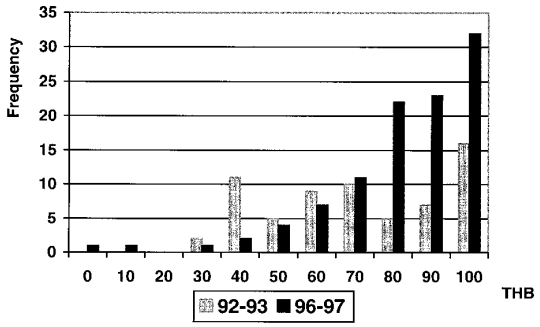

Figure 2. PHB in the initial (92–93) and final population (96–97).

Table II. Number and percentage of hygienic and non-hygienic colonies with and without brood disease and American foulbrood symptoms.

	Brood disease		American foulbrood		
Group	Presence	Absence	Presence	Absence	Total
Hygienic	9 (8.26%)	100 (91.74%)	2 (1.83%)	107 (91.74%)	109
Non-hygienic	44 (24.72%)	134 (75.28%)	18 (10.11%)	160 (89.89%)	178
Total	53	234	53	234	287

for hygienic behaviour and suggested that selection programs for brood disease resistance would be feasible. Oldroyd [7] suggested that the hygienic behavioural morph existed in Australia's commercial bee strains and that selective breeding should be able to produce suitable genotypes.

The colonies used in the present experiment had been selected by beekeepers from their own apiaries on the basis of 'good performance' (brood area, no disease presence, gentleness). Despite the previous selection for good performance, variability for hygienic behaviour was detected in the initial population.

A clear increase in hygienic behaviour was obtained after four years of selection for this trait. The increase was possible even

though new colonies were incorporated to the program, and all queens mated freely (without instrumental insemination).

Median THB values for the whole population in the second year 1993–94 (64%) were lower than those of the initial population (66%) perhaps because many new colonies were introduced for evaluation and many of them had low initial THB values. These values were so low that the median value for selected mothers that year was less than 80%, (74%). In that year, colonies with at least two (and not three) THB values over 80% were selected. However, all six evaluations were considered to calculate the median value. In following years, THB was improved in the population reaching a median value of 84% in the period 1996–97.

Rothenbuhler [9] stated that both hygienic genes (uncapping and removing) were recessive. In this work, hygienic genes were present not only in selected queens but in drones that were produced from those colonies too. Though queens did not mate in an isolated area, the frequency of hygienic genes increased in the apiary by increasing the number of hygienic colonies each year.

Trump, Thompson and Rothenbuhler [14] observed that some colonies that had hygienic and non-hygienic honeybees removed dead brood as quickly as colonies with hygienic honeybees exclusively. They said that for colony level expression of hygienic behaviour, 13–50% of the bees in the colony must carry the genes for the behaviour which may explain the improvement of this behaviour in our population. In this way, it was possible to obtain at least some hygienic honeybees in the colonies and so the colonies behaved as hygienic.

The improvement obtained in the population was less each year. Perhaps it is not possible to improve THB over 80% with naturally mated queens in this mating place.

When PHB was considered, the median values were higher than THB in all the populations. A high PHB value indicates that dead brood was detected and uncapped in twenty-four hours but was not removed completely. Selection was made for THB, and though both variables are related, no improvement for PHB was obtained. PHB values were high in the initial population and most colonies were able to detect and uncap dead brood. We suggest that selection in our population, had an effect on genes related to dead brood removal but not to uncapping. We also have to consider that the pin-killed brood assay used in this experiment makes a hole in the wax capping, which stimulates more rapid uncapping.

When THB values were related to the presence of brood disease, we can conclude that hygienic behaviour is related to tolerance to brood disease. Hygienic colonies had less disease. This result agrees with Spivak and Gilliam [12] working with *Ascosphaera apis* and with Spivak [11] who reported that hygienic colonies had significantly lower levels of chalkbrood disease and no American foulbrood disease when compared to 46 commercial colonies.

The lower frequency of colonies affected with brood diseases and especially with American foulbrood is very important because this demonstrates that it would be possible to manage honey bees without chemical controls, which are not always effective and do not act on the bacterial spores.

This research demonstrates that it is possible to increase the frequency of the hygienic trait in populations with naturally-mated queens (with no use of instrumental insemination) and it should be considered in selection programs in queen breeders' apiaries. As both genes (uncapping and removing) are recessive some hygienic colonies must be used as breeder colonies for queen production and other colonies selected by this trait must be used for drone production.

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Résumé – Changements dans une population d'*Apis mellifera* L. sélectionnées pour le comportement hygiénique et relation avec la tolérance aux maladies du couvain. Cette étude a été menée pour déterminer le changement survenu dans le comportement hygiénique d'une population d'abeilles domestiques sélectionnées sans contrôle de l'accouplement et pour étudier la relation entre comportement hygiénique et maladies du couvain. Chaque mois on a évalué le comportement hygiénique de colonies d'abeilles de différentes origines en tuant le couvain avec une épingle. On a déterminé (a) le comportement hygiénique total (CHT) : couvain entièrement éliminé par les abeilles/couvain total tué, (b) le comportement hygiénique partiel (CHP) : couvain désoperculé mais pas totalement éliminé/couvain total tué. Les colonies qui avaient un CHT > 80 % lors de trois mesures au moins ont été sélectionnées et utilisées pour la production de reines. Un mois après l'accouplement naturel des reines, on a évalué le comportement hygiénique des colonies. Ces procédures ont été appliquées d'octobre à juillet de 1992 à 1997. Le CHT et le CHP ont été calculés pour la population de départ, pour les colonies sélectionnées à chaque période et pour les colonies avec des reines sœurs. Les colonies évaluées ont été classées en hygiéniques et non hygiéniques et ces données ont été corrélées avec la présence de maladies du couvain. Le CHT de la population initiale était de 66 %. Après quatre années de sélection sans contrôle de l'accouplement et après avoir introduit de nouvelles colonies, le CHT était de 85 % (Tab. I). Une augmentation de 19 % de ce caractère a donc été obtenu. La répartition des variables CHT et CHP dans la population initiale et la population finale était asymétrique, mais après la sélection cette différence était encore plus marquée (Figs. 1 et 2). En corrélant les caractères hygiénique et non hygiénique avec la présence de maladies du couvain et avec la loque américaine, on trouve que ces maladies sont moins fréquentes chez les colonies hygiéniques (Tab. II). Nos résultats montrent que le comportement hygiénique est lié à la tolérance aux maladies et qu'il est possible d'augmenter la fréquence de ce caractère dans les populations en effectuant une sélection sur les reines fécondées naturellement.

***Apis mellifera* / comportement hygiénique / sélection / maladies du couvain / tolérance**

Zusammenfassung – Durch Selektion auf hygienisches Verhalten in einer Population von *A. mellifera* bewirkte Änderungen und ihre Beziehung zur Krankheitstoleranz. In dieser Untersuchung sollte in einer Population ohne Paarungskontrolle die durch Selektion bewirkte Änderung des hygienischen Verhaltens und die Beziehung zwischen hygienischem Verhalten und Brutkrankheiten bestimmt werden. Honigbienenvölker verschiedener Herkünfte wurden jeden Monat mit einem Nadeltest zur Abtötung der Brut auf ihr hygienisches Verhalten untersucht. Es wurde das vollständige hygienische Verhalten (THB, der Anteil vollständig entfernter an der abgetöteten Brut) und das teilweise hygienische Verhalten (PHB, der Anteil entdeckelter, aber nicht vollständig entfernter Brut an der abgetöteten Brut) registriert. Völker mit mehr als 80 % THB wurden für die Königinnenproduktion ausgewählt. Einen Monat nach der natürlichen Begattung der Königinnen wurden das hygienisches Verhalten der Volker untersucht. Dieses Verfahren wurde von 1992 bis 1997 von Oktober bis Juli durchgeführt. THB und PHB wurden für die Ausgangspopulation, für die ausgewählten Völker und für die Völker der Tochterköniginnen ermittelt. Über diesen Zeitraum untersuchte Völker wurden als hygienisch oder nicht-hygienisch eingestuft

und auf Brutkrankheiten untersucht. THB in der Ausgangspopulation betrug 66 %. Nach vierjähriger Selektion ohne Paarungskontrolle oder Einbeziehung neuer Völker war THB auf 85 % angestiegen, dies bedeutet einen Anstieg dieser Eigenschaft in der Population von 19 % (Tab. I). Die Verteilung der Eigenschaften THB und PHB war in der Ausgangspopulation sowie in der Endpopulation unsymmetrisch, nach der Selektion war dies allerdings ausgeprägter (Abb. 1 und 2). Wenn hygienische und nicht-hygienische Völker zu dem Vorkommen von Brutkrankheiten in Beziehung gesetzt wurde, zeigte sich dass diese Krankheiten bei den hygienischen weniger häufig waren (Tab. II). Unsere Ergebnisse zeigen, dass das hygienische Verhalten eine Beziehung zur Toleranz gegenüber Brutkrankheiten aufweist und dass eine Steigerung dieser Eigenschaft in der Population durch Selektion von Koniginnen bei natürlicher Paarung erreicht werden kann.

Hygienisches Verhalten / *Apis mellifera* / Selektion / Brutkrankheiten / Krankheitstoleranz

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