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# QUEEN INTRODUCTION INTO THE QUEENRIGHT HONEY BEE COLONY

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## Abstract

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One of the actual elementary biologic principles of the introduction of queen is that the recipient colony has to be queenless. We accidentally found that a queen can be accepted also in queenright colony with using of the queen excluder. Therefore, we conducted two experiments with the introduction of queen in queenright colony.
Under defined conditions of the experiment and with application of the queen excluder as a separator of queens we successfully introduced queen in the queenright colony. This result is discussed in relation to the general principle that a queen should be introduced only in a queenless colony. It is possible that there are some exceptions advert to the existence of some unknown biologic patterns in the honey bee biology and pheromones.

*Apis mellifera*, honey bee, ethology, beekeeping, introduction of queen, polygyny

The introduction of a queen in the honey bee colony is one of the elementary zootechnical operations within a season. The old queens and/or queens with bad breeding values must be replaced by the young ones. There are several biologic principles that have to be abided (Ambrose, 1992). One of the principle is that the colony with introduced queen or replaced queen (requeened colony) must to be queenless (Laidlaw, 1992).

There are several natural factors influencing result of this process (Tarpy *et al.*, 2000). An individual virgin queen's fighting success may depend on a number of attributes, including her size and weight, pheromonal signals, and use of piping signals (characteristic sounds made by a queen that could act as an advertisement of fighting ability and suppress the emergence of rivals). Virgin queens are also at an advantage if they emerge earlier, since they have the opportunity to eliminate younger rivals that are still developing (Schneider *et al.*, 2001).

The workers of the colony may also influence the outcome of the contest, and will be under selection pressure to do so in ways that maximise the workers' inclusive fitness – the extent to which their genes are passed to the next generation (Schneider *et al.* 2001). Though the workers appear to show "little, if any, nepotism" (Tarpy *et al.*, 2000) during the rearing of larval queen cells, studies have shown that a queen's relatedness to the workers does affect the outcome of the polygyny reduction stage of the queen replacement process.

The mechanism by which workers might influence the outcome of contests between emerged virgin queens is the vibration signal, in which a worker grasps a virgin queen and rapidly vibrates her body for a second or two. Schneider *et al.* (2001) found a relationship between the rate at which emerged virgin queens were vibrated and their chance of ultimate success over their rivals. This raises the possibility that the vibration signal could act as a means through which workers influence the result of contests between virgin queens.

In order to decrease the fighting ability of queens, two thirds of one of their mandibles was cut off with scissors (Dietemann *et al.*, 2008). Such operated queens did not contest with each other (even 3 queens). They refrained from engaging in lethal contests that typically characterize their reproductive dominance behaviour and coexisted peacefully within a colony.

The introducing of queens is a simple method but with uncertain result in spite of the abidance

of the biologic principles (McCutcheon, 2001). We found (Přidal, 1999, so far unpublished observation) that there were several exceptions to these principles and a queen could be accepted also in the queenright colony. The aim of this paper is to describe conditions and results of two experiments with the introduction of a queen in the queenright colony as a qualitative proof.

## MATERIAL AND METHODS

The two experiments with the queen introduction in the queenright honey bee colony (*Apis mellifera* L.) were conducted.

### The first experiment

Location of the apiary: the Czech Republic, reg. Bohemian-Moravian Highlands, cadastral area Markvartice u Třebíče, 550 m above the see level. The honey bee colony (strain Vigor) was kept in Langstroth hive (frame height 185 mm) with five magazines.

The colony No. S15 was freshly after swarming on 23rd May 2009. The eggs of the old swarmed out queen were still present and there was only one virgin queen. This virgin queen was marked with paint and number 5 and isolated under the queen excluder in four magazines on the same day (23rd May 2009). The queen No. 5 did not oviposit even on 5th June 2009 because of inappropriate cold and rainy weather with very weak honey flow. On this day, a new queen No. 30 was introduced in queen cage with candy and the cage was placed in centre of the 5th magazine with brood above the queen excluder. The control of the introduction was realized on the 20th June 2009.

### The second experiment

Location of apiary: the Czech Republic, reg. Bohemian-Moravian Highlands, cadastral area Příbram na Moravě, 432 m above the see level. The honey bee colony (strain Vigor) was kept in Třeboňský hive (two types of frames – 390 × 275 mm – high magazines and 390 × 170 mm low magazines) with two deep magazines as a brood chamber and three low magazines as a honey chamber. The course of weather was cold and rainy and with very weak honey flow.

The first introduction of a queen was realized in the queenright colony No. P19 on 7th June 2009. The old queen was taken away from the colony and the new young queen No. 18 was put in a brood chamber in the queen cage with candy under the queen excluder in the lower of the high magazines. On the 13th June 2009 the introduction of the queen No. 18 was controlled with positive result, however, the queen still did not lay eggs and no comb was built on the queen cage. During checking of the queen introduction, the five emergency queen cells were found and immediately destroyed. Consequently, the lower of the high magazines was separated from the upper one by the queen excluder. A new young queen No. 42 was put in the queen cage with candy into the upper magazines with capped brood. The control of the introduction was realized on 18th June 2009.

Both introduced queens were with intact mandibles to eliminate their inability to potential fights contrary to Dietemann *et al.* (2008).

## RESULTS

### The first experiment

The queen No. 30 introduced into the colony No. S15 was accepted and oviposited a lot of eggs (brood nest on the six frames). The drone comb was built around the queen cage. The queen No. 5 oviposited too. The colony S15 had two ovipositing queens separated by the queen excluder and none of them was injured or otherwise damaged due to possible fight between queens through the excluder. The queen No. 5 was removed from the colony. The queen No. 30 was still present in the colony and oviposited on 5th July 2009.

### The second experiment

The queen No. 42 introduced in the colony No. P19 was accepted and oviposited (about 3 $dm^2$ of eggs). The colony was fully quiet. The drone and worker small combs were built around the queen cage. The queen No. 18 in lower of the high magazines oviposited well, thus, there were two together ovipositing unrelated queens of the strain Vigor separated by the queen excluder in the colony No. P19. On the same day, the queen No. 42 and the queen excluder were removed from the colony. On the 27th June the queen No. 18 was still present and oviposited in the four high combs of its brood.

## DISCUSSION

The results of both experiments show that an unrelated queen can be successfully accepted if the young unrelated mated still non-ovipositing queen or the related virgin queen (sister) is present in the colony. It is not consistent with results of Mangum (1997) and McCutcheon (2001) showing that no virgin or mated queen (always sister of workers from supersedure) can be present in the requeened colony. The biologic rule, e.g. by Laidlaw (1992) that the presence of the queen as a mother of workers is not allowed in requeened colony, is not touched with our results.

The results of this study are rather in relation to methods of the two-queen management system when two queens are present and separated by the queen excluder in the same colony. However, in this management a new queen is usually introduced into the queenright colony with her workers (daughters)/colony (Ambrose, 1992a).

It is possible that the results of our experiments were influenced by low or otherwise changed pheromonal activity of the queen in the colony. The co-

lonies in this study had freshly accepted young unlaying or laying and related or unrelated queen and its pheromonal activity could be changed.

Apšegaité & Skirkevičius (1999, 2000) confirm that amount of (E)-9-oxo-2-decenoic acid (9-ODA), as a queen pheromone component, is low in the virgin queen, higher in freshly mated queen and the highest in older and well ovipositing queens. Apšegaité & Skirkevičius (2003) found that queens with the higher amounts of volatile components than amount of 9-ODA (typical of the virgin queens and inductive of an aggressive behaviour of workers to queens) were rather rejected in recipient colony than queens with very low content of the volatile components. It is possible that queens in this experiment were acceptable for workers in all cases because of the similar condition/physiology status of the introduced queens. Apšegaité & Skirkevičius (1995) showed that qualitative differences in composition of queen pheromone extracts are more important than only quantitative ones. Similar tendencies in this principle were showed by Winston *et al.* (1998) that synthetic queen and worker *Apis mellifera* pheromones did not improve the rate of successful requeening.

Skirkevičius & Skirkevičienë (1999) found that sensitivity of the worker's pheromonal receptors is variable within a year. The greatest sensitivity of worker bee chemoreceptors is also in May to July. Therefore, it can be excluded that the introduced queens were ignored in June, namely in colony No. P19. However, it cannot be precluded that a colony in the after-swarming condition is more tolerant to the presence of more queens while there is no laying queen. Moreover, there is an effect of the queen excluder preventing direct fight between queens. This experiment resulted in acceptation of queens without any their damage in spite of the fact that the queens are able to fight through the queen excluder with result of damage of their wings or legs.

We observed accidentally the acceptation of the laying queen in the queenright colony in 1999 (Přidal, 1999 – so far unpublished observation). The displaced queens (old and otherwise inconvenient queens) were temporarily placed in the cage during requeening as a reserve for case that a queen in her colony would not be accepted. The old queen can be returned securely in her colony any time later. The next introduction of the queen can be repeated for a week later. The cages with displaced queens were placed in the honey chamber above the queen excluder in colonies where no queen was changed in the actual year (stowage colony). The workers of the stowage colony take care of these queens until a new queen is accepted in requeened colony. This is time for elimination of old queen from the stowage colony. During this elimination the stowage queen escaped into the honey chamber by mistake. We did not search this queen with the opinion that the workers of the stowage colony will kill this queen when their queen-mother has laid under the queen excluder. Three weeks later we found this queen. She was laying (5 frames with her brood) and without somatic marks of fight with queen under the queen excluder which was also in good condition. This introduction of queen "with live ammunition" is absolutely unusual. Low pheromonal activity queen or low sensitivity of pheromonal receptors of workers in this case cannot be expected with respect to the results of Apšegaité & Skirkevicius (2000).

Within introduction of queens is eliminated the natural replacement of queens as it occurs in case of swarming, afterswarming or supersedure. In these processes, virgin queens are vibrated by workers and they are piping (Schneider et DeGrandi-Hoffman, 2008). This phenomenon is important for the polygyny reduction and selection of the queen. It is possible that this fact is an advantage within the artificial introduction of the queen. No queen is preferred through the vibrating signals by workers. This pressumable advantage can be expected namely in the case of introduction of two or more queens.

The results of Dietemann *et al.* (2008) suggest that weak queens exploit an alternative reproductive strategy and provide an explanation for rare occurrences of queen cohabitation in nature. However it is not the only one case of the queens' coexistence. The two queens can even overwinter in the same colony during supersedure that sometimes continues from summer as late as spring. The observations of this study were carried out on unrelated queens, therefore, nepotism, supposed during supersedure, was eliminated. Results of this observation indicate that there are still further factors influencing the possibility of the coexistence of queens in the same colony.

In spite of the fact that the introduction of a queen is a nonrandom process (Tarpy *et al.*, 2000) it is still problematic zootechnical operation under multifactorial impact. It is advisable to look deeper into this matter and to bring more light into this problem. It is possible that there are some chances of improvement for this operation or for the two-queen management system. The results of the above described investigation are the qualitative proof indicating new ways. The next basic research of honey bee biology, pheromones and ethology in requeened colony is needed.

## SUMMARY

The introduction of a queen or the requeening in the honey bee colony is one of the elementary zootechnical operations within a season. There are several natural factors influencing result of this process. The introducing of queens is a simple operation but with uncertain result in spite of the abidance of the biologic principles.

The aim of this communication is to describe conditions and results of two experiments with the introduction of a queen into the queenright colony as a qualitative proof for unknown biologic principle and ethology of the honey bee.
The queen introduction was carried out in two the queenright honey bee colony (*Apis mellifera* L.). In the both colonies a newly accepted queen was present under the queen excluder, however, so far not laying. The introduced laying queen was put in queen cage with candy in the magazine above the queen excluder.
The both queens above the queen excluder were accepted and laying within one week after introduction in spite that colonies were queenright. The queens under the queen excluder laid too.
The result shows that an unrelated queen can be successfully accepted by colony if the young unrelated mated still non-ovipositing queen or the related virgin queen (sister) is present in the colony. It is not consistent with the contemporary knowledges. The method of this experiment could be useful for construction of next experiments and improvement of the requeening methods. In spite of the fact that introduction of queen is a nonrandom process it is still problematic zootechnical operation under the multifactorial impact. Result is discussed in relation to the biology and the biotechnology of the honey bee.

## SOUHRN

Úspěšné přijetí matky ve včelstvu s matkou

Přidání nebo výměna matky je jeden ze základních zootechnických postupů v chovu včely medonosné během sezony. Výsledek tohoto postupu je ovlivněn několika faktory, a proto je jeho výsledek vždy nejistý i při dodržení všeobecných zootechnických zásad.
Cílem tohoto sledování je popsat podmínky a výsledek dvou experimentů s přidáním matky do včelstev s matkou jako kvalitativní důkaz neznámého biologického principu a etologie včel.
Přidání matky bylo provedeno ve dvou včelstvech s matkou včely medonosné (*Apis mellifera* L.). V obou včelstvech byly přítomny pod mateří mřížkou nedávno přijaté dosud nekladoucí matky. Přidávané kladoucí matky byly vloženy ve vyjídací klícce s medocukrovým těstem do středu nástavku na mateří mřížku.
Obě matky byly přijaty a během jednoho týdne po přidání začaly klást navzdory tomu, že pod mateří mřížkou byly již přítomné kladoucí matky.
Výsledek těchto pokusů dokazuje, že včelstvo může přijmout nepříbuznou kladoucí matku, i když ve včelstvu je přítomná mladá nepříbuzná dosud nekladoucí matka nebo příbuzná panuška (sestra). To je v rozporu s dosavadními zootechnickými zásadami přidávání a výměny matek. Tento výsledek je podkladem pro případné další experimenty a zlepšení postupů při výměnách matek. I když přidání matky je nenáhodný proces, jde stále o postup s nejistým výsledkem s ohledem na multifaktoriální vlivy, které na něj působí. Výsledek je diskutován ve vztahu k biologii a chovu včely medonosné.

*Apis mellifera*, včela medonosná, etologie, včelaření, přidání matky, polygynie

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