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ORIGINAL RESEARCH ARTICLE

Sustainable multiple queen colonies of honey bees, *Apis mellifera ligustica*

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Summary

Honey bee multiple queen colonies composed of several mated queens able to move around freely were produced by modulating biological factors that evoke fighting and queen elimination within the colony, mainly by ablating mandibles of queens to avoid inter-queen rivalry. Following this method, 128 colonies in eighteen apiaries were set up with multiple queens, all of which were mated and 6-12 months old. One hundred of the colonies (78.1%) retained all introduced queens. In total, 658 out of 733 queens (89.8%) were accepted after their introduction. The majority of these colonies experienced no queen loss for two months and most were still stable after six months. Of 80 colonies, 55 (68.8%) experienced no queen loss over the winter. These results show that our method is viable to produce sustainable multiple queen honey bee colonies for commercial use. In addition this technique will help to increase our understanding of basic questions of the evolution of sociality, such as division of reproduction and the evolution of polygyny.

Colonias sostenibles de abejas *Apis mellifera ligustica* con múltiples reinas

Resumen

Colonias de abejas con reinas múltiples compuestas por varias reinas fecundadas capaces de moverse libremente fueron producidas modulando factores biológicos que provocan la lucha y la eliminación de la reina dentro de la colonia, principalmente quitando las mandíbulas por ablación de reinas para eludir rivalidad de la reina-reina. Siguiendo este método se establecieron 128 colonias con múltiples reinas en dieciocho colmenares, que fueron apareadas con 6-12 meses de edad. Cien de ellas (78,1%) conservaron a todas las reinas introducidas. En total, 658 de las 733 reinas (89,8%) fueron aceptadas después de su introducción. La mayoría de estas colonias no experimentó ninguna pérdida de reinas durante dos meses y la mayoría seguía siendo estable después de seis meses. De 80 colonias, 55 (68,8%) no experimentaron ninguna pérdida de la reina durante el invierno. Estos resultados muestran que nuestro método es viable para producir colonias de abejas sostenibles con múltiples reinas para uso comercial. Además esta técnica ayudará a aumentar nuestra comprensión de las cuestiones básicas de la evolución de la sociabilidad, como la división de reproducción y la evolución de poliginia.

Keywords: Multiple queen colonies, monogyny, polygyny, mandible, young worker

Introduction

The western (*Apis mellifera*) and the eastern (*Apis cerana*) honey bee are rare cases of insects that have been successfully domesticated. Honey bees play an important economic role as a producer of honey, royal jelly, beeswax and propolis, which have been proven to have a variety of biological activities (Krell, 1996) and have an indispensable role in pollination for agricultural and natural eco-systems (Morse and Calderone, 2000).

A large colony population is necessary for a high yield of hive products (Chen, 2001). Because of this, for many decades attempts in many countries have been conducted in developing methods that increase brood rearing beyond the natural capacity of a normal single queen colony (Kovtun, 1949, 1950; Melnik, 1951; Spoja, 1953; Wallrebenstein, 1958; Haydak and Dietz, 1967). One possibility to achieve this is to create a colony where more than one queen reproduces. Honey bee societies are, however, normally monogynous (Ribbands, 1953). If several queens meet, which may happen because virgin queens emerge simultaneously, or alien queens enter the colony by colony merger (Neumann *et al.*, 2001), they typically fight for reproductive supremacy until only one survives and monopolizes reproduction in the colony (Winston, 1987). During the fights, queens grapple together, using their legs and mandibles to position themselves to sting each other. Stinging is the usual cause of death, but is only successful when queens have a good purchase with their mandibles on their opponents and can position themselves suitably (Butz and Dietz, 1994; Gilley, 2001; Dietemann *et al.*, 2008). Worker honey bees may also play a role during the elimination of supernumerary queens by destroying queen cells (Tarpy and Fletcher, 1998; Hatch *et al.,* 1999), by affecting queen behaviour through immobilization of queens (Gilley, 2001), by attacking queens with balling behaviour (Robinson, 1984) and by interfering with the outcome of inter queen competition through the "vibration signal" (Allen, 1959; Painter-Kurt and Schneider, 1998) or withholding trophallaxis (Tarpy and Fletcher, 1998).

Polygyny may occur naturally during supersedure and swarming, but is only temporary, with monogyny eventually being reestablished by a variety of means (Gilley and Tarpy, 2005) in a period varying from hours to months (Hepburn and Radloff, 1998). The attempts to artificially produce more productive multiple queen colonies therefore required physical separation of the queens to prevent them fighting (Wallrebenstein, 1958; Haydak and Dietz, 1967). Attempts also have been made to obtain free running multiple queen colonies, but their success was variable and none of the methods was widely accepted. For example, Kovtun (1949, 1950) introduced several queens up to 1.5 years old, with wings clipped, into a hive consisting of combs of emerging brood and honey with the empty cells filled with warm water. It was claimed that there must be no workers in the hive otherwise all the queens will be killed (Kovtun, 1949, 1950). This method is not, however, practical since the comb is easily destroyed by warm water due to the thermoplastic properties of wax (Pirk *et al.*, 2004). Secondly, the queens are prone to die due to the lack of care from workers, even if they refrain or survive the fighting. Melnik (1951) introduced three queens sequentially, one queen every two days, into a queenright colony without any treatment. This colony performed less well than single queen colonies for honey production and all the queens were found dead outside the hive when a young queen emerged (Melnik, 1951). Spoja (1953) successfully introduced queens of varying ages with wings clipped by introducing them among worker bees without using cages (Spoja, 1953), but this method did not produce stable multiple queen colonies as many of them did not survive the over wintering period (Spoja, 1953).

Here, we report an efficient method of obtaining stable multiple queen colonies composed of several egg laying and freely moving honey bee queens. Our multiple queen colonies were produced by simultaneously modulating biological factors that normally prevent polygyny in honey bees and providing a suitable social context within the hive. This involved the reduction of the possible sources that evoke fighting and queen elimination within the colony, i.e. removing older intolerant workers (Robinson, 1984) and decreasing the great fighting ability of queens (Dietemann *et al.*, 2008).

Materials and methods

The honey bee colonies used were of the Pinghu strain (from Pinghu County, Zhejiang Province, China) derived from the Italian honey bee (*Apis mellifera ligustica*). This strain has been selected by local beekeepers in recent decades for high royal jelly production. Queens were reared from grafted larvae, were allowed to mate freely and to return to their individual colonies until their egg laying ability was established at 6-12 months of age.

The colonies destined to host the multiple queens were prepared as follows: combs of emerging brood were selected and shaken, which triggers flight in the older bees, while young bees tend to remain on the comb (Sigg *et al.*, 1997). The combs were then placed in the hive box with the young bees still clinging to them. Young bees were used to avoid workers balling and killing the queens, a behaviour typical for older workers (Robinson, 1984). The host hives were placed at a distance (5-10 m) from their original location to ensure that all remaining foragers (older bees) did not re enter them. One to three day old workers were however preferred to freshly hatched individuals which may not be able to care for the queens efficiently (Lindauer, 1953). The amount of combs and bees to be used in the multiple queen colony depended on the number of queens to be introduced. Four to six combs were used for three to six queen colonies. Additional combs of honey and pollen were added beside the brood combs to provide enough food because the colony was deprived of foragers at the beginning.

Two days after the colonies were prepared, queens were taken out of their original colonies and introduced to different locations in the host hives after a third to half of both mandibles were removed with micro-scissors. The ablation of mandibles reduced their propensity to fight and kill each other (Dietemann et al., 2008). In addition, the large abdomens of the egg laying queens might further reduce their ability to fight (Spiewok, 2006).

In order to test the viability of our method, this protocol was implemented in the seventeen household apiaries in Pinghu, Zhejiang and in our experimental apiary in Hangzhou between 2005 and 2008. In each of these apiaries, one to three multiple queen colonies, each

Table 1.

Results

Discussion

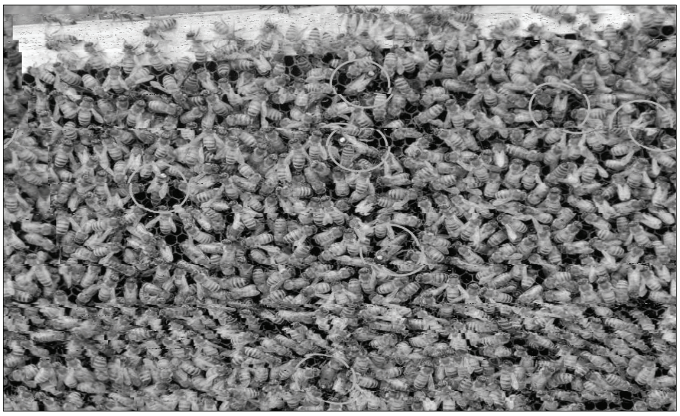

Fig. 1.