Thelytoky in a strain of U.S. honey bees (*Apis mellifera* L.)

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Introduction

Thelytoky is a type of parthenogenetic reproduction where unfertilized eggs develop into females (Suomalainen 1950). Thelytoky is common in the Cape honey bee (*Apis mellifera capensis* Escholtz), but it occurs with considerably lower frequency in European honey bees (*Apis mellifera* L.) (Onions 1912; Jack 1917; Anderson 1963; Ruttner 1976). In colonies with queens most worker ovaries are suppressed by the pheromone 9-oxo-decenoic acid and other substances produced by the queen (Butler and Fairey 1963), or by the presence of unsealed brood (Kropacova and Haslbachava 1971). However, ovaries can develop and workers can lay eggs after the queen and brood are gone (Perepelova 1929; DeGroot and Voogd 1954; Butler 1957; Butler and Fairey 1963; Jay 1970; Kropacova and Haslbachova 1970, 1971). European workers generally lay unfertilized haploid eggs that develop into males (drones). In rare instances, virgin queens and laying workers produce diploid eggs that develop into females (Mackensen 1943).

Given the high frequency of thelytoky in Cape bees, the relatively rare occurrence in domestic stocks of European bees is unexpected, since populations capable of thelytoky have an advantage over those in which laying worker eggs develop exclusively into drones (Ruttner 1977). Without thelytoky, the survival of a colony rests completely on the successful mating of a single queen which must leave the hive to mate. If this queen does not encounter drones or does not return to the hive, a replacement cannot be produced because female larvae of a suitable age for queen rearing no longer exist, and because the first queen to emerge usually destroys the other queen cells in the colony. However, if brood from laying workers could be raised into queens the colony would have a facultative survival mechanism in case the virgin queen is lost. Thelytoky should occur with greater frequency in populations exposed to conditions that reduce the chances of a queen either taking or returning from a mating flight (Moritz 1984).

A strain of honey bees (hereafter referred to as LUS) has been established from a breeding program in which virgin queens were introduced into broodless colonies (i.e., eggs and larvae did not exist in the colony) from November to March in southern Arizona. The purpose of the breeding program was to select for bees that would rear queens and drones at that time of year. Inclement weather and limited numbers of drones can occur during Arizona winters and prevent queens from successfully mating. Thus, introducing virgin queens at this time of year exerts pressure that could cause the frequency of thelytoky in the population to increase. The purpose of this study was to test for the existence of thelytoky in LUS and determine the frequency of this trait. In addition, observations of worker bees in queenless LUS colonies were made to compare their behavior with that reported to occur in Cape bees.

Methods and Materials

Eighteen queenless four or five frame nucleus colonies of LUS were established using two frames of brood (ranging in age from eggs to pupae) from queenright LUS colonies and two to three frames of honey and pollen. The adult bees covering these frames were included. Different LUS colonies were used to establish each nucleus colony. As controls,

Abstract

A strain of U.S. domestic honey bees (*Apis mellifera* L.) with the ability to rear workers and queens using the eggs of laying workers has been isolated. Previously, thelytoky was assumed to occur rarely in honey bees with the exception of the South African Cape bee (*A. mellifera capensis*). Our thelytokous line, hereafter referred to as LUS, was developed from commercial stocks of European honey bees. Comparisons of worker behavior and ovarian development were made among queenless colonies of LUS and two arrhenotokous lines hereafter referred to as CP and cd. LUS had a significantly lower percentage of workers with developed ovaries at the time when eggs from laying workers first appeared in cells than either CP or cd. All three lines constructed queen cells and deposited laying worker eggs in them, but viable queens emerged only from LUS. The CP line did not rear larvae in the queen cells but in some instances the cd line did. However, the cd bees destroyed the queen cells either prior to or soon after capping them. Comparisons between behaviors of queenless LUS colonies and those reported to occur in queenless Cape bee colonies also are discussed.

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three queenless nucleus colonies of a panmictic array of commercial bee lines maintained as a closed population (CP) (Page and Laidlaw 1982; Severson et al. 1986) and six colonies of honey bees carrying the Cordovan (cd) mutant color marker (Laidlaw and Page 1984) were established using the procedure described above. Entrances of the queenless colonies were covered with screen mesh for 24-48 hours after being established to prevent bees from drifting back to their parent colonies. All queenless colonies were examined three to four times weekly while brood from the previous queen was present so that queen cells from the brood could be destroyed. After all the previous queens' brood had emerged, the colonies were examined twice weekly to determine when workers began laying eggs. When eggs from laying workers first appeared in the colonies (i.e., when one or more eggs were seen), 10-20 workers were sampled and dissected to determine the percentage with developed ovaries. The first appearance of eggs was chosen as a means to standardize the time when workers would be sampled, since the percentage of workers with developed ovaries can change over time in queenless colonies (Anderson 1963). Ovaries were considered to be developed if developing eggs were visible in the ovarioles. The brood from laying workers present in either worker or drone cells was sexed while in the pupal stage by removing the cell's cap, and determining gender by the morphology of the head capsule. The presence of queen cells with larvae being actively tended by workers was noted along with whether the cells had a queen emerge or were destroyed by the workers.

Observations of bees on the frames were made during colony inspections. We avoided the use of smoke during these inspections whenever possible to minimize disruption to the workers on the frames. Sometimes during an inspection bees were seen biting each other, or with their abdomens in the cell assuming an egg laying position. We sampled LUS bees being bitten and dissected them to determine if they had ovary development. Whether workers assuming the egg laying position always deposited an egg in the cell also was determined. To conduct more detailed observations of queenless LUS colonies, two frame observation hives were established using one frame of brood and another of pollen and honey along with the adult bees on the frames. The activity of bees on the frames was observed twice daily once in the morning and afternoon, for 30-60 min. intervals. Observations were begun when all the brood from the previous queen had emerged. The observation hives were not included among the colonies used to test for thelytoky.

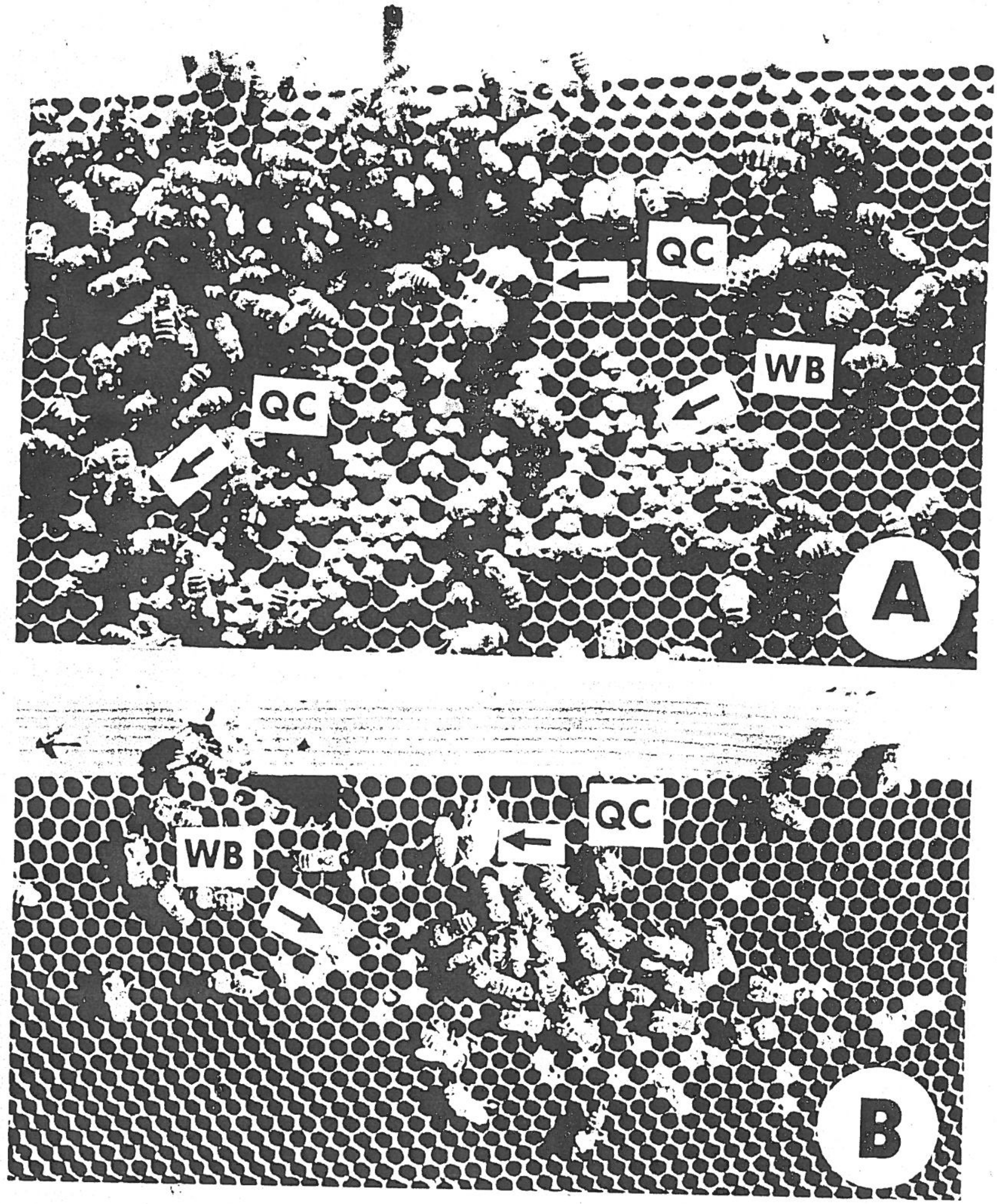

Figure 1. Queen cells (QC) and worker brood (WB) from a queenless colony of non-thelytokous European bees (A) and thelytokous LUS (B). The worker brood and capped queen cell in B were produced from the eggs of laying workers, while in A they are from the brood of the queen that had been removed.

Results

Once all the brood emerged in queenless LUS, CP, or cd colonies, worker bees were scattered over the frames giving the colony the distinctive appearance associated with the queenless state. Upon closer examination of bees from the 4-5 frame nucleus colonies and in the observation hives sometimes workers were seen grasping each other with their mandibles. In a LUS observation colony, workers were seen pulling nestmates out of the cells in which they had inserted their abdomens. On other occasions, in the observation hives we saw eggs being eaten by nestmates immediately after the laying worker removed her abdomen from the cell. In the observation hives and the nucleus colonies some bees assumed an egg laying position in a cell, but did not lay an egg. In nucleus and observation colonies we observed bees remaining stationary with their wings spread while nestmates bit them on the dorsal surface of the abdomen and the thoracic area (particularly at the points where the wings articulate). This behavior occurred in LUS, CP, and cd colonies and has been previously described in queenless colonies by Velthuis (1970). LUS from nucleus colonies that were being bitten by other workers were examined for ovary development; 26.7% of these bees had developed ovaries (colonies sampled = 5, total bees examined = 15, SD = 11.4%). We attempted to sample bees being bitten in CP and cd colonies and examine them for ovary development, but sample sizes were too small to obtain meaningful results. Dead bees on the bottom boards of seven LUS colonies were examined and an average of 1.5% of the dead bees per colony had developed ovaries (bees examined = 65, SD = 1.5%). Examination of workers selected at random from the queenless test colonies indicated that an average of 27.1% of the LUS workers had developed ovaries when eggs first appeared in the colony (Table 1). This was a significantly lower percentage than either CP or cd (60.0% and 44.0% respectively).

Of the 18 colonies of LUS tested for thelytoky, 55.6% reared worker brood from the eggs of laying workers, and 50% reared queens. Queens from the brood of laying workers emerged only in the 4-5 frame nucleus colonies, and never in the observation hives. In the nucleus colonies, sometimes a patch of worker brood was produced and the queen cell was constructed within that patch (Fig 1.). A queen cell positioned among worker brood is commonly seen in a colony that is requeening itself in the conventional manner using brood from the previous queen. However, some queen cells from thelytokous LUS colonies were located at the very top of the frame. Neither CP or cd constructed queen cups in this region. A queen produced from laying worker eggs successfully mated and produced worker and drone brood. However, eight of the nine queens produced from workers' brood either did not return to the hive after a mating flight, or were critically injured during artificial insemination.

Queenless CP colonies reared only drones, although queen cells were constructed and eggs from laying workers were placed inside them. The eggs did not hatch, and often were gone the next day. Similarly, cd colonies produced only drones from laying worker eggs, although some colonies reared larvae in queen cells. These queen cells were larger and longer than those produced by LUS or commonly seen in colonies rearing queens from a mated queen's brood. During colony inspections the cd workers were observed crawling over the capped queen cells just as the LUS bees did in their colonies. However, within 3-5 days in the cd colonies the queen cells were torn down by the workers.

Discussion

LUS were selected from commercial European honey bee stock, indicating that thelytoky may exist as part of the overall *Apis mellifera* gene pool. However, reports indicate that in managed colonies thelytoky is expressed at a very low frequency (Mackensen 1943). This may be because beekeeping practices inadvertently select against thelytoky. For example, swarming and supercedure can be minimized through various management techniques, and thus the possibility of a colony becoming queenless due to the loss of a virgin queen can be reduced. If colonies lose their queens and do not have brood to produce replacements, the queens often are replaced with new ones by beekeepers. Hence, there is no selective pressure for thelytoky in colonies managed in this manner. Conversely, the conditions under which the LUS strain was derived may have inadvertently selected for thelytoky. Virgin queens introduced into broodless colonies during the winter may not have been accepted by the workers in some cases, while in others the queens may not have mated or were lost on mating flights. Some of the colonies that survived may have done so because they requeened themselves with brood from laying workers. The winter requeening procedure was repeated annually using queens produced from brood of colonies that survived the previous year's winter requeening. If thelytoky was at a low frequency in the LUS strain at the beginning of the breeding program, the fre-

Table 1

Types of progeny reared from the eggs of laying workers in queenless colonies of U.S. honey bees. Tucson, Arizona.

Colony type	No. of colonies observed	% workers with developed ovaries ± sd	% colonies rearing drones	% colonies rearing workers	% colonies rearing queens	No. of queen emerged
CP	3	60.0 ± 24.5 a	100.0	00.0	00.0	0
cd	6	44.0 + 3.3 a	100.0	00.0	20.0	0
LUS	18	27.1 + 15.0 b	100.0	55.6	50.0	9

Means followed by the same letter are not significantly different at the 0.05 level as determined by Duncan's [1951] multiple range test.

quency possibly was increased because of continued selection followed by the production of new queens from brood of the survivors.

Unfortunately, all but one of the queens produced from laying worker brood were lost before they could begin egg laying. Still, queens reared from the brood of LUS laying workers apparently have the potential to mate and produce worker and drone brood. We stopped finding eggs in the colonies once the queen emerged and was present in the hive. The colony whose queen successfully mated, behaved like any other colony with a new queen. After mating the queen began laying worker brood which was cared for by the adult workers in the colony. The colonies that reared queens but lost them did not rear others. The colonies subsequently dwindled and died or were robbed by workers from other colonies thus causing the LUS workers to abandon the hive. Colonies composed of 4-5 frames of workers and brood apparently have only one chance at rearing a queen from laying worker brood. If the queen is lost, the workers will not produce another perhaps because the workers are too old, the colony is too weak, or some combination of both. Whether a colony that had a larger population at the time of queen removal would have enough bees of the appropriate age to rear another queen from laying worker brood if they lost the first one needs to be tested.

When queenless nucleus colonies were inspected, the use of smoke was minimized to limit the disruption of the bees. Still, opening a colony is disruptive because it changes colony temperature and perhaps odor and pheromone levels within the hive's environment. We cannot be sure of the repercussions of opening colonies on the workers' behaviors we observed on the frames. Observation hives enabled us to make more detailed behavioral observations of LUS workers in queenless colonies without having to open the colony. However, how well the results from the observation hives mirror the behaviors of bees in the nucleus colonies is not known. Workers in observation hives reared fewer larvae into adults compared to the nucleus colonies, and never reared queens. Perhaps the populations were too small or temperatures could not be properly maintained in the observation hives for brood rearing to approach the levels seen in the nucleus colonies.

There are both similarities and differences between laying workers of Cape bees and LUS. Cape bees can have workers with developed ovaries while brood is present (Anderson 1963). We have not found this to occur in LUS (DeGrandi-Hoffman unpubl. data). Internal fighting among nestmates following the removal of a queen and a subsequent increase in the number of dead bees on the bottom board occurs in Cape, LUS, CP, and cd bees. As in Cape bees, most of the dead LUS bees did not have ovary development. In Cape bees an average of 28% of the workers have developed ovaries 13 days after queen removal, and in LUS the average is 27% when eggs from laying workers are first seen (Anderson

1963). Significantly fewer workers in queenless LUS colonies have developed ovaries compared to CP or cd, suggesting that worker ovaries might be more effectively suppressed by the presence of laying workers in LUS (Velthuis 1970). Cape bee workers lay unfertilized diploid eggs because during anaphase II the egg pronucleus and the central descendent of the first polar body fuse to form a diploid zygote nucleus (Verma and Ruttner 1983). Whether a similar cytological mechanism exists in LUS is yet to be determined.

A honey bee colony's ability to requeen itself with the eggs of laying workers requires not only that some workers can lay diploid eggs, but that the workers can foster the cooperation from nestmates needed to construct a queen cell and rear the egg into a queen. When laying workers developed in CP or cd colonies, often queen cells were constructed and sometimes eggs were deposited inside them. However, the eggs were either cannibalized by other workers or left unattended and did not hatch. Other than in LUS, the greatest cooperation among individuals to rear a queen from laying worker eggs was in cd bees where workers actively cared for the larvae in the cells. Queen cells were capped in some instances, but were destroyed soon afterwards. Our study indicates that attempts at requeening occur in non-thelytokous lines of honey bees, but apparently these bees lack some of the physiological and behavioral attributes needed to rear a viable queen.

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