chamber (25 °C and 95% RH) for 48 h to induce fungal growth. After the incubation period, fungal growth on media and from larvae incubated in the moist chamber were examined under light microscope using mounts stained with Cotton-Blue-Lacto-Phenol. Fungus identification was done using the corresponding keys for Deuteromycota (Barnett & Hunter, 1998) and Ascomycota (Hanlin, 1998). The fungal growth was isolated and regrown on fresh media using culture method 1. Sizes of 50 randomly chosen sexual structures of *A. apis* were measured.

The most common fungi isolated by the first method were *A. apis*, followed by *Penicillium* sp. (table 1). The fungi *Beauveria* sp. and *Cladosporium* sp. were less common. In the second method, most of the colonies were isolated, and the fungi genera were almost the same as in the first method (table 1). The fungi isolated in this study included both saprophytes (*Penicillium* sp. and *Cladosporium* sp.) and bee pathogens (*A. apis* and *Beauveria* sp.) (Barnett & Hunter, 1998; Hanlin, 1998; Medina & Mejia, 1999).

A. apis was observed growing on most of the media, but on PDA, PDA+P, and V_8 the production of spore cysts and spore balls was scarce. To induce production of those structures, it was necessary to transfer cultures to the medium W-A. In all media, the colonies were initially white and cottony, then white to silver fruiting bodies grew under the aerial mycelium. When the fungus was cultured on W-A the mycelium it showed poor growth, but in about 5–8 days produced abundant spore cysts.

A. apis cultures on W-A produced spore cysts containing numerous spore balls full of one cell hyaline, ellipsoid and smooth ascospores in both mature fungus cultures and moist chamber incubated larvae. There was variability among isolates in spore cyst diameter (58 ± 15 µm), spore ball diameter (13 ± 3 µm) and ascospore length (3 ± 0.4 µm). According to Hanlin (1998), the morphological characteristics confirm the presence of *A. apis* in the analysed brood samples.

Beauveria sp. was only isolated using the smashed larvae method. The *Beauveria* sp. colonies were white, with a fluffy to powdery appearance and conidiophores grouped in clusters. The observed conidia were one cell, hyaline, rounded to ovoid, and produced on conidiophores with zigzag ends, as described by Barnett & Hunter (1998).

In conclusion, *A. apis* the causal agent of chalkbrood, as well as some other saprophytic and potential pathogenic fungi, were found associated with AHB in Costa Rica. Despite our findings, however, *A. apis* remains uncommon and its impact on apiculture in Costa Rica appears minimal. We anticipate its occurrence may increase in situations where *V. destructor* is not adequately managed, and feel that future studies into the interaction between *A. apis* and *V. destructor*, particularly under tropical conditions, are needed.

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Unusual queen cell construction and destruction in *Apis mellifera* from far-eastern Russia

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Honey bee (*Apis mellifera*) colonies produce queen cells during three fundamental reproductive processes. (1) Queen cells (swarm cells) are produced by colonies prior to colony reproduction by swarming. (2) Queen cells (supersedure cells) are also produced by colonies that are replacing an existing queen. (3) After a queen is lost, workers construct emergency queen cells. In both the swarming and the emergency queen replacement processes, developing queens generally mature to emergence or until they are detected by a virgin queen that emerged earlier (Winston, 1987). However, the fate of queen cells during an apparent supersedure process is more varied. Allen (1965) observed that some colonies occasionally destroyed unsealed queen cells and more rarely destroyed sealed queen cells. Subsequently, some of these colonies built new queen cells and then successfully superseded. Also, several colonies destroyed some but not all of their sealed queen cells prior to supersedure (Allen, 1965).

Members of our research group both in the USA and in Russia (Kuznetsov, personal communication) made observations suggesting that some colonies of honey bees we have studied in far-eastern Russia, including some imported to the USA (Rinderer *et al.*, 2001), chronically constructed queen cells then destroyed the cells before virgins emerge without superseding the queen. Otherwise, these colonies appeared to be 'normal'. That is, they were similar in size (worker bees and brood nest) and brood

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TABLE 1. Numbers of queen cells destroyed on various days of development for five colonies of Russian honey bees

Developmental day	Colony number					Number destroyed	Percentage destroyed
	1	2	3	4	5		
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	2	1	0	0	0	3	4.9
5	6	1	2	0	1`	10	16.3
6	1	10	1	1	0	13	21.3
7	2	4	0	1	0	7	11.4
8	0	1	0	0	0	1	1.6
9	1	3	2	3	0	9	14.7
10	1	3	0	2	0	6	9.8
11	1	0	0	1	0	2	3.2
12	2	0	0	0	0	2	3.2
13	0	0	0	0	0	0	0
14	6	0	1	0	0	7	11.4
15	0	0	0	0	0	0	0
16	0	0	0	1	0	1	1.6
Total	22	23	6	9	1	61	100

quality (numbers of cells in sealed brood areas not containing brood) to colonies in the same apiaries which did not show this behaviour. Although observations of specific colonies were made weeks apart, some colonies always seemed to be constructing some queen cells and simultaneously destroying others. This suggested that the behaviour was chronic and hence unusual. This observational study was conducted to determine the validity of these initial observations and to determine the age of the occupants of the queen cells when they were destroyed.

Initially, *c.* 100 Russian honey bee colonies were evaluated for unusual chronic production and thereafter premature destruction of queen cells. Five colonies were found between 20 June and 15 July 2002 that had apparently normal queens (no noticeable morphological or behavioural defects and laying normal brood patterns) in normal colonies as previously described that also had queen cells in various stages of construction and destruction. In each colony the queen was confined to a brood chamber consisting of two 10-frame hive bodies (16.19 x 45.40 cm) separated from one or two honey storage chambers by a queen excluder. Frames were numbered to facilitate obtaining records concerning the exact position of queen cells. The colonies were observed in Baton Rouge, Louisiana, USA, every other day from 15 July 2002 to 30 July 2002 during a weak nectar and pollen flow. Frames were examined for the presence of queen cells and new queen cells in the form of a cell cup containing an egg. When queen cells were found, notes were made regarding the position of the cell cups on the frame. In addition, paint marks were placed on wooden frames near the cells to facilitate re-examination on subsequent days.

The age of the queen cell occupants was determined by comparing the first day when a cell cup with an egg was observed with the first day that the cell cup contained a larva. Cells that contained an egg on the first and third day and a young larva on the fifth day had occupants with ages known to the day. Cells that contained an egg on the first day and a larva on the third day at the most were one day older than estimated by the size of the larva on the third day. The ages of occupants of queen cells that were larvae when the study began were estimated by comparing their size to the size of queen larvae of a known age according to Winston (1987). The age of the 12 queen cells that were already sealed when the colonies were first examined could not be determined, so we destroyed them on the second day of observation. There were two other sealed cells destroyed by the colony by the second day of observation. While egg removal from cell cups was observed, it was not recorded as cell destruction.

Four of the five colonies continued to construct and destroy queen cells during the observation period (22, 23, 6 and 9 cells per colony). One colony made and destroyed one cell then made one other cell and used it for supersedure after the queen gradually stopped laying eggs. A total of 61 queen cells were observed that contained larvae or pupae with known or estimated ages (table 1). All of these cells were destroyed prior to expected emergence, and no virgin queens were found in the four colonies that did not supersede. Although the colonies produced and destroyed different numbers of cells, the destruction of cells followed a similar pattern. Colonies started new queen cells within two days after having destroyed one or more cells and often had younger cells at the time that older cells were being destroyed. The colony which superseded after their queen stopped laying eggs destroyed one cell and then built another which emerged. The other four colonies continued the process of producing and destroying queen cells in the presence of the original queen which continued to produce eggs.

The destruction of queen cells usually began by the cell being chewed from the side, even with unsealed cells. The larva or pupa in the cell was then removed through a side or the bottom hole in the cell. The cell was further destroyed and reduced to either a queen cup or nothing. We did not record the exact process of the destruction of all the queen cells. However, two cells were observed and recorded as being chewed from the end of the cell where queens emerge. One of these cells developed from a cell cup which was observed to have an egg on the first and third day, allowing the age of the developing queen to be known to the day. On the 14th day of development the cell was found with an opening in the bottom having a chewed edge and no pupal remains. No virgin was found in the colony. The initial destruction of this cell must have begun on the 13th day or at the latest early on the 14th day. While it might have been possible that a virgin emerged two to three days early from this cell, it is more likely that the cell was destroyed before a virgin emerged. The other cell was observed to be chewed from both the end and the side on day 16. No virgin was found in the colony. Both colonies continued with their original queen.

This construction and destruction of queen cells is unusual in three respects. It is chronic, cell destruction often occurs with sealed cells, and some destroyed sealed cells have the appearance of a cell which has emerged a day or more earlier. This contrasts with the observations of Allen (1965) who saw the occasional destruction of cells which were usually not sealed. However, we agree with the general conclusion of Allen (1965)

that the presence of queen cells in a colony does not necessarily indicate a queen change will occur.

The presence of queen cells and cells which appear to have emerged are used in beekeeping to signal a need for actions to prevent swarming or to replace a failing queen with a queen of a desired stock rather than allow the colony to supersede. The unusual queen cells we observed throughout the brood nest are likely to be interpreted as supersedure cells rather than swarm cells. Beekeepers may unnecessarily re-queen some Russian colonies if they attempt to prevent supersedure by requeening. Supersedure is a frequent occurrence in honey bee colonies (Butler, 1957), usually has a favourable outcome for the colony, and might be profitably ignored by beekeepers. Supersedure can often occur without beekeepers noticing the event. General requeening of all colonies on a yearly or every other year schedule may be a better strategy to assure that colonies have queens of a desired stock.

The adaptive value of this unusual queen rearing behaviour is not clear. The seasonality of far- eastern Russia provides a short growing season with a limited opportunity for honey production followed by a winter dearth that may last up to six months (Crane, 1990). Perhaps these queen cells, in various stages of development, provide an opportunity for colonies to more quickly replace a queen during the short active season should she fail.

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A survey of *Varroa destructor* strains on *Apis mellifera* in Turkey

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This report extends the work of Çakmak *et al.* (2003), who surveyed the occurrence of varroa and tracheal mites in the Republic of Turkey. Their study revealed that tracheal mites occur only rarely in Turkey, whereas varroa mites are abundant. However, the initial report did not include information about the strains (haplotypes or mitochondrial genotypes) of *V. destructor* found in Turkey. Two major strains of *V. destructor*, one native to Korean and one native to Japanese *A. cerana*, have colonized *A. mellifera*. The Japan strain, which has been found on *A. mellifera* in Japan and South America (Anderson & Trueman, 2000) is less virulent and causes less damage to the colony than the Korea strain which has been identified in North America, Europe and Asia (Anderson & Trueman, 2000). Here we report the strains of *V. destructor* found in Turkey as background information for beekeepers in this area.

Restriction fragment length polymorphism (RFLP) analyses were performed on 50 mite samples collected from 10 colonies from eight localities in Turkey (fig. 1, right). DNA extraction was performed on each mite according to Qiagen's DNEasy protocol for animal tissue utilizing DNA binding columns (Qiagen, Valencia, CA, USA). A portion of the cytochrome oxidase I (COI) gene was amplified by PCR using primers V51: 5′-GTAATTTG-TATACAAAGAGGG-3′ and V1400: 5′-CAATATCAATAGAA-GAATTAGC-3′. Each 50 µl PCR reaction contained 10 µl DNA, 0.5 µl 10 mM stock solution of each primer, 5 µl 10X PCR reaction buffer (Promega, Madison, WI, USA), 8 µl 25 mM $MgCl_2$ (4 mM final concentration), 0.5 µl dNTP stock (10 mM each dATP, dCTP, dGTP, dTTP; Pharmacia polymerization mix), 0.25 µl (approximately 1 unit) *Taq* polymerase (Promega, Madison, WI, USA). Thirty-five cycles were run using the profile: 94°, 20 seconds, 40°, 1 minute 30 seconds, 2 minute ramp to 72°, 72°, 1 minute. PCR products were digested with restriction enzymes *Xho*I and *Sac*I. Ten µl of amplified COI gene were diluted with 5µl of H_2O and digested with 0.5 µl of either *Xho*I or *Sac*I. The digestion products were separated by electrophoresis through ethidium bromide-stained 2% agarose gels and visualized on a UV transilluminator. These two restriction enzymes can distinguish Korea and Japan strains of *V. destructor*; both possess an *Xho*I restriction site in the amplified fragment of COI but only the Japan strain possesses the *Sac*I site.

Eighteen samples were used for DNA sequencing. PCR products were gel purified using Qiagen's QIAquick Spin (Qiagen,

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