

Original article

**Occurrence and distribution of *Ascosphaera apis*  
in North America: chalkbrood in feral honey bee  
colonies that had been in isolation  
on Santa Cruz Island, California for over 110 years<sup>1</sup>**

M Gilliam<sup>1\*</sup>, BJ Lorenz<sup>1</sup>, AM Wenner<sup>2</sup>, RW Thorp<sup>3</sup>

<sup>1</sup> US Department of Agriculture, Agricultural Research Service, Carl Hayden Bee Research Center, 2000 East Allen Road, Tucson, AZ 85719;

<sup>2</sup> Department of Biological Sciences, University of California, Santa Barbara, CA 93106;

<sup>3</sup> Department of Entomology, University of California, Davis, CA 95616, USA

(Received 23 March 1997; accepted 30 July 1997)

**Summary** — Twenty strains of *Ascosphaera apis* were cultured from larval mummies and discolored larvae from feral honey bee colonies that had been in isolation on Santa Cruz Island, California for over 110 years, long before chalkbrood was first reported from the USA in 1968. Disease symptoms and enzyme patterns of *A. apis* differed from those found on the mainland. Island strains showed little variability. These results and the history of the bees suggest that the pathogen accompanied the bees to the island and that the bees survived chalkbrood by a combination of hygienic behavior and microorganisms that inhibit *A. apis* which were isolated from larvae and stored pollen.

***Ascosphaera* / chalkbrood / honey bee / *Penicillia***

## INTRODUCTION

Santa Cruz Island, California, USA, located 37 km from the mainland off the coast of Santa Barbara, is mountainous and has a Mediterranean climate. In 1987, a program was initiated to remove all colonies of feral

European honey bees, *Apis mellifera* L, from the essentially uninhabited island in an effort to restore native bee populations and pollination systems in the Channel Islands National Park (Wenner et al, 1995). Santa Cruz Island is the largest (25 000 hectares) of the five Northern Channel Islands and the

\* Correspondence and reprints  
Fax: (1) 520 670 6493

<sup>1</sup> Mention of a proprietary product or company name does not constitute an endorsement by the US Department of Agriculture.

only one that has ever had honey bees (for more than 120 years in 1995) with no apparent interchange of bees between the island and mainland since the original introduction (Wenner et al, 1995).

Wenner and Thorp (1993) reviewed both the history of honey bees on the island and recent research. An unknown beekeeper brought honey bees to Santa Cruz Island around 1873 but abandoned them well before 1880. Subsequently, the offspring of the original introduction became feral and spread over the entire island. The honey bees are remarkably uniform over the island, and allozyme testing suggested that there was a small founder population (RE Page, Jr, pers comm). By 1995, a total of 280 colonies in original cavities and from swarm traps had been located (Wenner et al, 1995). Most colonies were located in cliff faces (Wenner and Thorp, 1993), and those that were situated where observations could be made had excellent brood patterns and appeared healthy. Unfortunately, access to these feral colonies was generally limited by their location in rock cavities, and one could not see beyond the entrance. Therefore, it was impossible to conduct complete surveys for bee diseases. For these same reasons, no attempts were made to survey feral colonies from the mainland coastal areas.

In 1990, fungal growth on comb cells and on larvae and mummies typical of chalkbrood infection was observed in a few feral colonies on Santa Cruz Island. At this time, the objective of the larger study on the island was first to locate and then to destroy honey bee colonies and not to collect them. Thus, no colonies had been manipulated, no swarming had occurred for several years because of a drought, and no combs or empty bee hives had been moved to the island. Bees were never introduced from the mainland during the course of the study. Since chalkbrood, a fungal disease of honey bees, caused by *Ascosphaera apis* (Maassen ex Claussen) Olive and Spiltoir, was not

reported from the USA until 1968 [for a review see Gilliam and Vandenberg (1997)], we wished to determine whether *A apis* was responsible for any of the observed fungal growth and whether chalkbrood was present in this honey bee population that had been in isolation for over a century. A positive result would indicate that chalkbrood may have been present in North America in an island ecosystem, long before it was first reported from the mainland. To achieve this goal, we aimed to examine abnormal larvae and larval mummies for pathogens and to isolate and identify microorganisms from these samples.

## MATERIAL AND METHODS

Representative combs were removed in July 1990 from honey bee colonies in rock cavities on the island, sealed in separate double plastic bags, refrigerated and hand-carried to Tucson. All subsequent studies including observations, uncapping and removal of cadavers from cells, isolations of microorganisms and taxonomic determinations of microbial isolates were performed under sterile conditions in a laminar flow hood at the Carl Hayden Bee Research Center in Tucson. This virtually eliminated any possibility of contamination of island material with mainland fungi or other microorganisms.

The cells and their contents were examined. In addition to fungal growth in comb cells and on larvae, larval mummies were noted. These were white, black, green or brown in color. The presence of sunken cappings, scattered brood patterns, brown discolored larvae and collapsed brown larvae suggested that bacterial diseases might also be present. Moreover, there was evidence of hygienic behavior (uncapping and removal of diseased and dead brood) by adult worker bees, a genetically determined trait that is the primary mechanism of resistance to chalkbrood (Gilliam et al, 1988). The scattered brood patterns were most probably due to removal of abnormal brood since small holes in cappings (the first step in hygienic behavior) and partially removed cappings were seen on cells containing dead brood.

The contents of 28 selected cells (14 capped and 14 not capped) were subjected to detailed

microbiological analyses for diagnoses of bacterial and fungal diseases and to isolate microorganisms. Thus, 11 larval mummies of various colors, 15 discolored tan to dark brown larvae (nine intact, five collapsed and one hard), and two bee bread (stored pollen) samples were collected and processed using sterile techniques throughout. Each was first transferred to a separate sterile vial. Wet mounts and Gram stains of each sample were prepared and examined microscopically, and fungal elements were noted and measured. The remainder of each sample was homogenized in 0.5 mL of sterile distilled water in a glass tissue grinder. Each homogenate was then streaked onto one plate of each of the following Difco® media: Sabouraud dextrose agar with 0.2% yeast extract (SDA-YE), Czapek solution agar (CZ) nutrient agar (NA), and brain-heart infusion agar with 0.01% thiamine hydrochloride (BHITHC). Plates of SDA-YE were incubated at 35–37 °C under 10% carbon dioxide for isolation of *A apis* and other fungi. Plates of CZ were incubated aerobically at 25 °C for isolation of molds. NA and BHITHC were incubated aerobically at 37 °C to isolate bacteria; BHITHC was included to test for *Bacillus larvae*, the causative agent of American foulbrood disease, and for other bacteria having fastidious growth requirements. All plates were examined for the 2-week incubation period. As colonies appeared, they were examined microscopically in wet mounts and Gram stains and transferred to fresh plates of the same medium on which they were isolated, except that molds other than *A apis* were transferred to CZ. Pure cultures were confirmed microscopically and maintained on slants of the above media under the same incubation conditions used for initial isolations.

Measurements of morphological structures, separation of mating types and mating tests of *Ascosphaera* isolates and reference strains were conducted according to Christensen and Gilliam (1983). Spore cyst data were analyzed using SAS-ANOVA and student's *t*-test (SAS Institute Inc, 1985). Positive identification of *A apis* and assignment of mating types were based on compatibility in mating tests with five reference strains (Gilliam and Lorenz, 1993; Gilliam et al, 1994). Enzymes of selected isolates and reference strains were analyzed with the API ZYM system (Analytab Products, Plainview, NY) using the methods of Gilliam and Lorenz (1993). Other microbial isolates were characterized by macroscopic and microscopic morphology, and Peni-

cillia were tested and identified according to Raper and Thom (1968).

## RESULTS

No bacterial diseases or their pathogens were found. However, frass and two cocoons of the greater wax moth, *Galleria mellonella*, were observed on a single small comb section containing two black chalkbrood mummies.

Twenty strains of *A apis* were isolated from eight samples (two black mummies in uncapped cells, two collapsed discolored larvae in capped cells, and two discolored larvae in uncapped cells and two in capped cells). Seven of these were mated strains, five were plus strains, and eight were minus strains. Five samples contained all three types, two had a mated and a minus strain, and one contained only a minus strain. Spore cysts of *A apis* were observed in three additional samples (a green mummy and two black mummies in uncapped cells), and possible *A apis* spores were seen in another green mummy from a capped cell with no growth of *A apis* on culture. Thus, at least 11 of 28 samples were positive for *A apis*.

Two white mummies (one from an uncapped and one from a capped cell) yielded no microbial growth, although microscopic examination revealed fungal mycelia in both. Seven mummies (four from uncapped cells and three from capped cells) from which *A apis* was not cultured contained other fungi, primarily *Penicillia*. These mummies were white (*Penicillium brevi-compactum*), green (*P cyclopium* var *echinulatum* with or without *P brevi-compactum*), dark brown (an unidentified brown fungus, *Penicillium cyclopium* var *echinulatum*, *P brevi-compactum* and *Penicillium* sp), or black (Mucorales). Thus, they were the color of the predominant fungus that was present. *Ascosphaera apis* was the only microorganism present in two black mummies and one discolored larva. Other sam-

ples positive for *A apis* yielded one to three additional microorganisms, primarily *P brevi-compactum* and *P cyclopium* var *echinulatum*.

Of the 28 total samples, *P brevi-compactum* was isolated from 15, *P cyclopium* var *echinulatum* from 13, yeasts from seven, unidentified *Penicillium* spp from six, *Bacillus* spp from five, *P corylophilum* from five, an unidentified brown fungus from two, Mucorales from two, *Streptomyces* sp from one, and an unidentified bacterium from one. Both bee bread samples contained *P cyclopium* var *echinulatum*, and one yielded *P brevi-compactum*, *P corylophilum*, an unidentified brown fungus, a yeast, and *Bacillus* sp in addition.

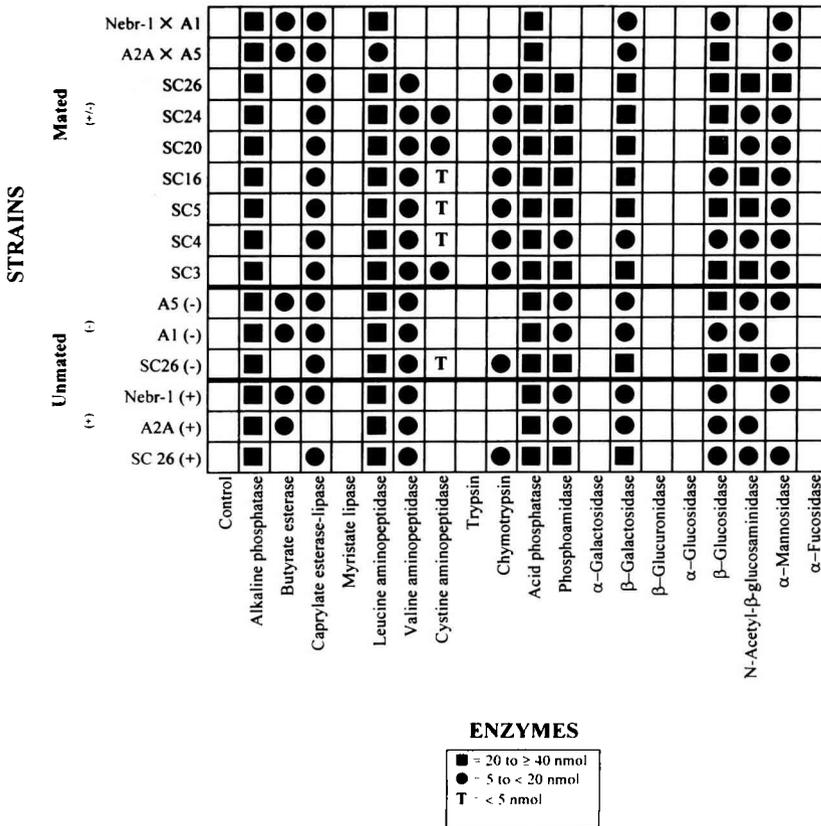
Preliminary measurements of morphological elements of *A apis* were conducted on the wet mounts prepared from both the original samples and 8-day cultures of *A apis* on the isolation media. Spore cyst sizes ranged from 45–120  $\mu$  in the original samples and 25–130  $\mu$  in the cultures. Spores were 2.5–4.0  $\times$  1.0–2.5  $\mu$ , and spore balls were 10–20  $\mu$ .

In a more detailed analysis, a total of 490 spore cysts were measured from five of the seven sporulated strains isolated from the Santa Cruz Island bees and from two fresh isolates from managed honey bee colonies in Tucson (table I). All spore cysts were from cultures of the various strains that were grown, maintained, and measured under exactly the same conditions on plates of

**Table I.** Spore cyst diameters of *Ascospaera apis* strains from managed honey bee colonies in Tucson and feral honey bee colonies on Santa Cruz Island.

Strain	Source <sup>a</sup>	Spore cyst size ( $\mu$ m) $\pm$ SD (n = 35)	Significance of SCI strains compared to	
			HBC4	A2A X A2
<i>4 days of incubation</i> <sup>b</sup>				
HBC4	T	50.9 $\pm$ 14.3		
A2A X A2	T	55.1 $\pm$ 12.3		
SC5	SCI	59.6 $\pm$ 13.0	0.05	NS
SC16	SCI	55.3 $\pm$ 11.1	NS <sup>c</sup>	NS
SC20	SCI	62.6 $\pm$ 15.7	0.05	NS
SC24	SCI	66.2 $\pm$ 14.1	0.05	0.05
SC26	SCI	59.4 $\pm$ 17.9	0.05	NS
<i>14 days of incubation</i>				
HBC4	T	61.1 $\pm$ 21.3		
A2A X A2	T	57.4 $\pm$ 18.3		
SC5	SCI	58.7 $\pm$ 21.8	NS	NS
SC16	SCI	62.8 $\pm$ 19.8	NS	NS
SC20	SCI	53.1 $\pm$ 19.8	NS	NS
SC24	SCI	56.8 $\pm$ 14.9	NS	NS
SC26	SCI	69.6 $\pm$ 19.5	NS	0.05

<sup>a</sup> T, fresh *A apis* isolates from managed honey bee colonies in Tucson; SCI, *A apis* strains from feral honey bee colonies on Santa Cruz Island. <sup>b</sup> Aerobic conditions at 25 °C on Sabouraud dextrose agar with 0.2% yeast extract. <sup>c</sup> NS, not significant ( $P = 0.05$ ).



**Fig 1.** Results of the API ZYM enzyme test system with nine selected island (SC) strains and six reference strains.

SDA-YE incubated aerobically at 25 °C for 4 and for 14 days. Results revealed significant differences in the sizes of spore cysts from four of the five *A apis* strains in younger cultures from island bees compared to a strain (HBC4) from managed honey bee colonies. In 14-day-old cultures, these differences vanished. In comparison to another Tucson strain (A2A X A2), only one island strain differed at 4 days and another at 14 days. In each of these cases, island strains of *A apis* were significantly larger than mainland strains. Differences in spore cysts sizes at 4 and 14 days were sig-

nificant ( $P = 0.05$ ) for some strains (SC20, SC24, SC26 and HBC4) but not others (SC5, SC16 and A2A X A2). Spore cysts of strains SC20 and SC24 were significantly smaller at 14 days compared to 4 days. In SC26 and HBC4, they were significantly larger at 14 days. These differences between strains may reflect varying maturation times for cysts of different strains.

Results of enzyme analyses of nine selected island strains and six reference strains are shown in figure 1. All island and reference strains produced alkaline phosphatase, leucine aminopeptidase, acid phos-

phatase,  $\beta$ -galactosidase and  $\beta$ -glucosidase. None produced myristate lipase, trypsin,  $\alpha$ -galactosidase,  $\beta$ -glucuronidase,  $\alpha$ -glucosidase, or  $\alpha$ -fucosidase. Some differences between island and reference strains occurred with the other enzymes. Most notable were the production of chymotrypsin by all island strains but no reference strains and the production of butyrate esterase by all reference strains but no island strains.

## DISCUSSION

Microscopic and microbiological analyses revealed chalkbrood but no bacterial disease. *Ascosphaera apis* was isolated and identified from larval mummies and from both intact and collapsed discolored larvae. Thus, the symptoms of chalkbrood in the island bees differed from those in mainland bee colonies since brown discoloration and collapse of larvae are not generally recognized signs of the disease. The strains of *A apis* from island bees mated with mainland strains, confirming the identity of *A apis*.

Sizes of spores and spore balls of the *A apis* strains isolated were in general agreement with previously reported measurements (Rose et al, 1984; Bissett, 1988; Alonso Rodriguez et al, 1993). Mean diameters of spore cysts of some strains of *A apis* from island bees were significantly larger than those in the reference strains in younger cultures but not in 14-day cultures with one exception. All but two of the mean diameters of the spore cysts were below previously reported means of 65.8–82, but this was also the case with strains recently isolated from managed colonies of honey bees in Tucson where the mean diameters were  $47.4 \pm 15.0$  to  $63.6 \pm 19.7 \mu$ ;  $n = 270$  (Gilliam, 1995). Thus, more variability appears to exist in the mean diameters of spore cysts of *A apis* than has been generally thought. Previous measurements of 2800 spore cysts from 23 strains of *A apis* from feral and managed

colonies of honey bees and from a carpenter bee demonstrated that spore cyst size of some strains but not others differed somewhat depending on media, culture age, incubation temperature and the addition of  $\text{CO}_2$  (Gilliam, 1995). However, the trends of these differences varied among the strains. Thus, it was concluded that strain differences seemed to be the most important factor influencing the size of spore cysts. The present results appear to confirm this conclusion.

Enzyme analyses from both mated and unmated island strains revealed some differences from the reference strains and from our previous results (Gilliam and Lorenz, 1993; Gilliam et al, 1993; Gilliam et al, 1994). All island strains were negative for butyrate esterase, most were positive for cystine aminopeptidase, and all produced chymotrypsin. We previously found only one *A apis* strain that did not produce butyrate esterase or that produced cystine aminopeptidase. Only a few previously encountered strains produced chymotrypsin. Also, all island strains produced valine aminopeptidase, phosphoamidase, N-acetyl- $\beta$ -glucosaminidase and  $\alpha$ -mannosidase while production of these enzymes is variable in the reference strains and in our previously tested strains. All island stains were remarkably similar in their enzymology in both the enzymes produced and their levels. This indicates little variability in the pathogen in contrast to strains from honey bees and carpenter bees from the North American mainland but might be related to the number of strains analyzed.

The present results along with the history of the honey bees on Santa Cruz Island indicate that the pathogen probably accompanied the bees to the island. This view is supported by the uniformity of the island bees, the lack of interchange of bees between the island and the mainland after the original introduction of honey bees around 1873, the differences in symptoms of chalkbrood

in island and mainland bees, and the differences in enzymology of island and mainland strains of *A apis*. We were extremely careful not to introduce *A apis* to the island nor to contaminate the material collected from the island. Thus, bees or bee equipment were not introduced to the island, island colonies were not manipulated, and all laboratory observations and studies were conducted under sterile conditions. Therefore, it appears that chalkbrood may have been present in North America in a population of honey bees isolated on Santa Cruz Island long before the disease was reported from the mainland. Chalkbrood apparently was not a major problem for the island bees since they had survived for over 110 years at the time of the present study in 1990, and most colonies were strong and healthy. This is probably due to the hygienic behavior of the bees and also possibly to the presence of microorganisms that are antagonistic to the chalkbrood pathogen. These mechanisms of tolerance or resistance to the pathogen were evidenced by holes in cappings and partially removed cappings on cells containing dead brood and by the isolation of taxa of microorganisms (Penicillia, Mucorales, *Bacillus* spp) that were previously shown to be antagonistic to *A apis* (Gilliam et al, 1988; Gilliam, 1990).

Our results indicate possible antagonism by the non-*A apis* microorganisms. For example, of the 17 samples with no *A apis*, other microorganisms, primarily fungi, were isolated from 15, and two contained only non-viable fungal mycelia. Yet these larvae had symptoms identical to those of larvae from which *A apis* was isolated. Also, of the three mummies with spore cysts of *A apis* that were not viable, one yielded *P breviscompactum*, *P cyclopium* var *echinulatum*, and *Streptomyces* sp; *Bacillus* spp and Mucorales were both isolated from the other two. Thus, the pathogen may have been inhibited or killed by these organisms. This would explain why no *A apis* was isolated even when spore cysts were observed.

Although we cannot rule out the possibilities that these microorganisms may be saprophytes living on larvae dead from other causes or that *A apis* was overgrown by them, it is unlikely that they are pathogens since they are common associates of honey bee colonies (Gilliam et al, 1988; Gilliam et al, 1989). Future research will attempt to answer these questions by determining the potential of these isolates for chalkbrood control.

The bees of Santa Cruz Island may be a source of stock that has tolerance to chalkbrood. Unfortunately, since the goal of the project on the island was to eliminate and not to transfer bees to the mainland, much of the population was destroyed before the present work was completed. However, efforts continue to secure queens from the island for breeding and subsequent evaluation. We are also attempting to obtain additional samples of chalkbrood and *A apis* from island colonies for analyses.

## ACKNOWLEDGMENTS

We thank M Spivak and WT Wilson for reviewing the manuscript.

**Résumé — Présence et répartition d'*Ascospaera apis* en Amérique du Nord : du couvain plâtré dans des colonies sauvages d'abeilles mellifères restées isolées sur l'île de Santa Cruz, Californie, pendant plus de 110 ans.** Un apiculteur introduisit des colonies d'abeilles mellifères, *Apis mellifera* L, au centre de l'île de Santa Cruz aux environs de 1873, mais les abandonna avant 1880. La descendance des colonies originelles devint sauvage et se répandit sur la majeure partie des 25 000 ha de l'île au cours des 110 années qui suivirent, sans qu'il n'y ait apparemment de nouvelle introduction d'abeilles. Une analyse allozymique des abeilles sauvages a suggéré que la population fondatrice était petite.

Le couvain plâtré, mycose des abeilles mellifères causée par *Ascosphaera apis*, a été signalé pour la première fois aux États Unis en 1968. En 12 ans il s'est étendu à toute l'Amérique du Nord.

En 1990, au cours d'opérations destinées à éliminer toutes les abeilles de l'île de Santa Cruz, on a remarqué le développement de champignons sur des cellules des rayons et sur des larves provenant de colonies sauvages. La présence d'opercules affaissés, de couvain disséminé et de larves brunes déformées suggérait en outre l'existence de maladies bactériennes. Des rayons représentatifs ont été prélevés, des cellules et leur contenu examinés et le contenu de 28 cellules sélectionnées (14 operculées et 14 non operculées) a fait l'objet d'analyses microbiologiques détaillées afin de diagnostiquer les maladies bactériennes et fongiques et d'isoler les organismes responsables. L'examen de 11 larves momifiées, de 15 larves décolorées et de deux cellules de pollen n'a pas révélé de maladies bactériennes ni d'agents pathogènes. Néanmoins *A apis* était présent dans onze des échantillons (cinq momies, deux larves décolorées et déformées et quatre larves décolorées intactes) à la fois dans des cellules operculées et des cellules non operculées. *Penicillium brevicompactum* et *P cyclopium* var *echinulatum* sont les organismes les plus fréquemment isolés des momies qui ne renfermaient pas *A apis* ou des momies qui renfermaient du mycelium d'*A apis* non viable.

Vingt souches d'*A apis* ont été isolées. Elles ont été identifiées par la mesure des structures morphologiques et par des tests de fécondation avec des souches référencées. Les diamètres des sporocystes des souches de l'île correspondaient en général à ceux des souches du continent, à quelques exceptions près pour lesquelles les sporocystes des souches insulaires étaient significativement plus grands (tableau I).

L'analyse de 19 enzymes de souches insulaires fécondées et non fécondées a

montré des différences par rapport aux souches continentales mais une remarquable similitude parmi les souches insulaires, à la fois dans la qualité et la quantité des enzymes produits (fig 1). Ceci est l'indication d'une faible variabilité de l'agent pathogène dans l'île, contrairement à ce qui se passe pour les souches du continent nord-américain. Les différences les plus notables entre les souches insulaires et continentales portaient sur l'absence de butyrate estérase et sur la présence de cystine aminopeptidase et de chymotrypsine chez les souches de l'île de Santa Cruz.

Ces résultats, ainsi que l'histoire des abeilles sur l'île, montrent que l'agent pathogène a probablement accompagné les abeilles sur l'île et que celles-ci ont survécu au couvain plâtré par un comportement hygiénique, qui se manifeste par des petits trous dans les opercules, par l'élimination partielle des opercules des cellules renfermant du couvain mort et par des microorganismes antagonistes d'*A apis* qui ont été isolés dans les échantillons. Il se peut donc que le couvain plâtré ait été présent sur l'île de Santa Cruz longtemps avant que la maladie ne soit signalée sur le continent.

#### *Ascosphaera apis* / *Penicillia* / États Unis / répartition géographique / historique

**Zusammenfassung — Vorkommen und Verbreitung von *Ascosphaera apis* in Nordamerika: Kalkbrut in wildlebenden Honigbienenvölkern, die seit mehr als 110 Jahren auf der Insel Santa Cruz, Californien isoliert waren.** Ein Imker brachte 1873 Honigbienen (*Apis mellifera*) in das Zentrum der Insel Santa Cruz, aber kümmerte sich schon vor 1880 nicht mehr um sie. Die Nachkommen der ein oder zwei Ursprungsvölker verwilderten und besiedelten die 25 000 Hektar der Insel fast vollständig. Allozymteste zeigten, daß es sich um eine kleine Gründungspopulation gehandelt hat.

Kalkbrut, eine Pilzkrankheit der Honigbienen, die durch *Ascospaera apis* hervorgerufen wird, wurde 1968 zum ersten mal in den Vereinigten Staaten nachgewiesen. Innerhalb der nächsten 12 Jahre wurde sie in ganz Nord Amerika gefunden.

Bei den Bemühungen, 1990 alle Honigbienen von der Insel Santa Cruz zu entfernen, wurde Pilzwachstum in Wabenzellen und auf Larven der wilden Völker bemerkt. Eingesunkene Zelldeckel, unregelmäßige Brutflächen und eingefallene braune Larven ließen vermuten, daß es auch bakterielle Krankheiten gab. Von repräsentativen Waben wurden Zellen samt Inhalt untersucht und der Inhalt von 28 ausgesuchten Zellen (14 verdeckelte und 14 unverdeckelte) einer genauen mikrobiologischen Analysen zur Diagnose von Bakterien- und Pilzinfektionen unterzogen und die Mikroorganismen isoliert.

Die Ergebnisse von 11 larvalen Mumien, 15 verfärbten Larven und 2 Pollenzellen zeigten weder eine bakterielle Erkrankung noch Erreger. Allerdings wurde in 11 der Proben *A apis* sowohl in verdeckelten wie unverdeckelten Zellen nachgewiesen (fünf Mumien, zwei eingefallene verfärbte Larven, und vier intakte verfärbte Larven). *Penicillium brevi-compactum* und *P cyclopium var echinulatum* waren die häufigsten Isolate von Mumien, die entweder kein *A apis* oder nicht lebensfähiges *A apis* enthielten.

Zwanzig Stämme von *A apis* wurden isoliert. Sie wurden durch Messung der morphologischen Struktur und durch Kreuzungstest mit Referenzstämmen identifiziert. Die Durchmesser der Sporencysten der Inselstämmen stimmten bis auf einige Ausnahmen, bei denen die Sporencysten signifikant größer waren (Tabelle I), mit den Festlandsstämmen überein.

Analysen von 19 Enzymen von gepaarten und ungepaarten Inselstämmen zeigten Unterschiede von den Festlandsstämmen sowohl in der Art der Enzyme als auch in

ihrem Niveau (Abb 1), aber es gab auffällige Ähnlichkeiten innerhalb der Inselstämmen. Damit zeigte sich auf der Insel eine im Gegensatz zu den Stämmen vom nordamerikanischen Festland geringe Variabilität der Pathogene. Die deutlichsten Unterschiede zwischen Insel- und Festlandsstämmen entstanden durch die Abwesenheit von Butyratesterase und durch das Vorkommen von Cystin-Aminopeptidase und Chymotrypsin in den Stämmen von Santa Cruz.

Diese Ergebnisse zeigen zusammen mit der Geschichte der Honigbienen dieser Insel, daß die Krankheitserreger wahrscheinlich zusammen mit den Bienen auf die Insel kamen, und daß die Bienen Kalkbrut durch eine Kombination von zwei Faktoren überlebt haben: durch Hygieneverhalten, das durch die kleinen Löcher in den Zelldeckeln und durch teilweise Entfernung von Zelldeckeln von Zellen mit toter Brut zu erkennen ist, und durch antagonistische Mikroorganismen wie *Penicillium* spp, *Mucorales* und *Bacillus* spp, die aus den Proben isoliert werden konnten. Demnach scheint die Kalkbrut in Nordamerika in der isolierten Population auf der Insel Santa Cruz bereits lange vor der Entdeckung dieser Krankheit auf dem Festland aufgetreten zu sein.

***Ascospaera apis* / Kalkbrut / Honigbienen / Penicillia**

**REFERENCES**

Alonso Rodríguez JM, Puerta Puerta F, Hermoso de Mendoza Salcedo J, Rey Pérez J, Gil Anaya MC, Hermoso de Mendoza Salcedo M (1993) La ascoseferosis de la abeja melífera en España. Estudio micológico de 47 brotes de la enfermedad. *Rev Iberoam Micol* 10, 39-46

Bissett J (1988) Contribution toward a monograph of the genus *Ascospaera*. *Can J Bot* 66, 2541-2560

Christensen M, Gilliam M (1983) Notes on the *Ascospaera* species inciting chalkbrood in honey bees. *Apidologie* 14, 291-297

Gilliam M (1990) Chalkbrood disease of honey bees, *Apis mellifera*, caused by the fungus, *Ascospaera*

- apis*: a review of past and current research. *Proceedings and Abstracts of the Fifth International Colloquium on Invertebrate Pathology and Microbial Control*, Adelaide, Australia, 398-402
- Gilliam M (1995) Problems in the identification of *Ascosphaera apis*. *XXXIV International Congress of Apiculture, Lausanne, Switzerland*. Apimondia Publishing House, Bucharest, Romania (in press)
- Gilliam M, Lorenz BJ (1993) Enzymatic activity of strains of *Ascosphaera apis*, an entomopathogenic fungus of the honey bee, *Apis mellifera*. *Apidologie* 24, 19-23
- Gilliam M, Vandenberg JD (1997) Fungi. In: *Honey Bee Pests, Predators, and Diseases, Third Edition* (RA Morse, K Flottum, eds), AI Root, Medina, Ohio (in press)
- Gilliam M, Lorenz BJ, Buchmann SL (1994) *Ascosphaera apis*, the chalkbrood pathogen of the honey bee, *Apis mellifera*, from larvae of a carpenter bee, *Xylocopa californica arizonensis*. *J Invertebr Pathol* 63, 307-309
- Gilliam M, Prest DB, Lorenz BJ (1989) Microbiology of pollen and bee bread: taxonomy and enzymology of molds. *Apidologie* 20, 53-68
- Gilliam M, Lorenz BJ, Prest DB, Camazine S (1993) *Ascosphaera apis* from *Apis cerana* from South Korea. *J Invertebr Pathol* 61, 111-112
- Gilliam M, Taber S III, Lorenz BJ, Prest DB (1988) Factors affecting development of chalkbrood disease in colonies of honey bees, *Apis mellifera*, fed pollen contaminated with *Ascosphaera apis*. *J Invertebr Pathol* 52, 314-325
- Raper KB, Thom C (1968) *A Manual of the Penicillia*. Hafner, New York
- Rose JB, Christensen M, Wilson WT (1984) *Ascosphaera* species inciting chalkbrood in North America and a taxonomic key. *Mycotaxon* 19, 41-55
- SAS Institute, Inc (1985) *SAS Users Guide: Statistics, Version 5 Edition*. Cary, North Carolina
- Wenner AM, Thorp RW (1993) The honey bees of Santa Cruz. *Bee Cult* 121, 272-275
- Wenner AM, Thorp RW, Barthell JF (1995) Varroa mites in an island ecosystem. *Am Bee J* 135, 831-832