Proceedings of the American Bee Research Conference

The American Association of Professional Apiculturists (AAPA) sponsored two research conferences in 2001. The first met in San Diego, California on January 12-13 in conjunction with the annual meeting of the American Beekeeping Federation. The second met at the Donaldson Brown Conference Center in Port Deposit, Maryland on September 8-9. The 2002 research conference will be held in conjunction with the annual meetings of the Canadian Honey Council (CHC) and the Canadian Association of Professional Apiculturists (CAPA) in Niagra Falls, Ontario on December 5-6, 2002. The following are abstracts from the 2001 meetings.

1. Adams, L. R.,[a] E. G. Rajotte,[a] & N. Ostiguy[a] - THE PROPER TIME TO PLACE *OSMIA CORNIFRONS* IN AN ORCHARD: A DEGREE-DAY MODEL APPROACH - *Osmia cornifrons* (Megachilidae), also known as the Japanese hornfaced bee, is a solitary bee commonly used for pollination in commercial Japanese apple orchards. *O. cornifrons* was successfully introduced into the Eastern U.S. in the 1970s, but has not been adopted by U.S. apple growers despite estimates that it is 80 times more effective at pollinating apple blossoms than are honey bees. This study seeks to facilitate the commercial adoption of *O. cornifrons* as an apple pollinator by establishing the degree-day relationship for its development and then use that information combined with predictive weather maps to let growers know when to introduce diapausing *O. cornifrons* into the orchard.

The degree-day concept is widely used in the field of pest insect management. This concept states that insect development is facilitated by the accumulation of time at a temperature at or above the temperature conducive for development. We can use this idea to determine how long it takes the diapausing adult *O. cornifrons* to emerge, given a set of temperature conditions.

One hundred and fifty cardboard tubes filled with diapausing adult *O. cornifrons* were obtained from a common over-wintering site. After removal from the tubes, bees were sorted into individual plastic cups and placed in one of five growth chambers set at constant temperatures representing the range of field ambient temperatures experienced by bees during mid- to late spring. Bees were observed thrice daily for emergence. Once a bee emerged, its sex, weight, days to emergence, tube number, and position within the tube were recorded. Using the days to emergence we established an emergence line. The developmental rate can be expressed as the following line: 1/days= -0.0332 + 0.055*temperature, with $R^2 = 0.99$. The developmental threshold was extrapolated from this line and found to be 6.04 degrees Celsius.

The degree-day relationship established in the lab was then validated in the field. In late spring 2000, three groups of bees in shelters were placed in three locations in the State College area. Each shelter contained 70+ bees and two data loggers to record hourly temperatures. Bees were checked daily and the sex, weight, days to emergence, tube number, and position within the tube were recorded. A standard meteorological site for measuring the maximum and minimum daily temperatures was at each location. The data collected at these stations was used to correlate temperatures inside the bee shelter with the ambient temperatures predicted by ZedX, Inc. (Bellefonte, PA), a company that specializes in agricultural weather predictions and modeling.

Using ZedX, Inc. weather predictions and climatological data collected for the past 30 years, we can use our degree-day model to determine when adult *O. cornifrons* will emerge from diapause in any square kilometer of Pennsylvania. The temperature inside the bee shelter is linked to the standard meteorological site, which is then related to recorded climate data. We are able to generate color maps showing when the bees will emerge. In the future we hope to model apple cultivar bloom using predictive models, then overlay the *O. cornifrons* emergence data which will allow us to tell growers when to place their bees in the orchard to achieve maximum pollination.

We thank the Pennsylvania Department of Agriculture for funding this research.

2. Arechavaleta-Velasco, M. E.[b], G. J. Hunt [b], T. Glenn [c] and M. Spivak [d] -GENETIC ANALYSIS OF THE HYGIENIC BEHAVIOR OF BACKCROSS HONEY BEE COLONIES - Honey bee hygienic behavior is a rare example of a behavioral mechanism of disease resistance. Hygienic behavior is a highly desirable economic trait that could reduce the negative effects of diseases and parasites and could diminish the amount of chemical products that are used for their control.

This study was conducted to analyze the hygienic behavior of colonies composed of backcross workers. Two lines of honey bees were selected for either high of low hygienic behavior. One hygienic colony and one non-hygienic were selected. A queen was reared from the hygienic colony, and this queen was artificially inseminated with the semen of one drone reared from the non-hygienic colony to produce a colony with hybrid workers. From this colony 23 virgin hybrid queens were produced. Twelve of these queens were each artificially inseminated with the semen of a single drone that was reared from the original hygienic colony. The remaining eleven queens were similarly inseminated with semen of drones from the original non-hygienic colony. This produced colonies composed of backcross workers. Each queen was introduced into a small colony consisting of two frames of brood, two frames of honey and pollen, and approximately 1 kg of bees. The colonies were kept in single, deep hives in the same apiary. Forty-five days after the queens were introduced, the hygienic behavior of the colonies was tested with the brood freeze-killed method using liquid nitrogen on three different occasions.

Significant differences were found between the two types of backcross and between the colonies. An analysis of the variance components indicate that the main source of variation between the colonies was due to the effect of the type of backcross, suggesting that the variation measured among the colonies was partially genetic in origin.

3. Arechavaleta-Velasco M. E.[b] & G. J. Hunt[b] - GENETIC ANALYSIS OF GUARDING BEHAVIOR OF EUROPEAN HONEY BEES - Honey bee colony defense consists of primarily two distinct behaviors, guarding and stinging. A guard is a bee that patrols the entrance of the hive and inspects bees or moving objects that come close to the hive. Five quantitative trait loci (QTLs) that affect honeybee defensive behavior were mapped as a colony trait in a population derived from Africanized and European bees.

The objective of this study was to analyze the expression of guarding behavior in colonies composed by backcrossed workers derived from defensive and gentle European honey bee colonies and to test for the effect of three QTLs (*sting-1, sting-2* and *sting-3*) on the expression of guarding behavior of individual European honeybees. A queen was reared from a defensive colony and was artificially inseminated with the semen of three drones from the same colony. From this queen a second queen was reared and inseminated with the semen of a drone from a gentle colony. From this queen nine hybrid queens were reared and divided in two groups. Six queens were single-drone artificially inseminated with drones from the defensive colony and three queens were single-drone artificially inseminated with drones of the gentle colony in order to produce two types of colonies composed of backcross workers. Sixty days after the queens were introduced to colonies, guards observed at the entrance of each hive were counted and marked with enamel paint. Twenty-four hours later marked bees that continue guarding were counted and collected for DNA analysis. This procedure was repeated in five different occasions. Samples of nurse bees and foragers were collected from each colony as controls. Two colonies were selected, one from each type of backcross, and the DNA of the sampled bees were screened using molecular markers linked to the defensive behavior QTLs. A t-test to compare the mean of two populations was used to look for differences between the two types of backcross for the number of guards marked and the number of guards collected. A Xi^2 goodness of fit test was used to look for deviations from the expected 1:1 segregation for a colony composed of backcross workers in the genotypes of the collected bees that would indicate an effect of the linked QTL on the behavior.

Significantly more guards were marked and collected from the defensive backcross colonies than from the gentle backcross colonies. Significant deviations from the expected 1:1 segregation were found in the genotypic frequencies of guards of the defensive and gentle backcrosses for the molecular marker linked to *sting-2*. A significant deviation from the expected segregation pattern was observed in the genotypes of guards of the defensive backcross colony for the molecular marker linked to *sting-1*, and in the genotypes of guards of the gentle backcross colony for the molecular marker linked to *sting-3*. No significant deviations from the expected 1:1 segregation were found in control bees (nurse and foragers) for any of the molecular markers tested.

The results showed that the genetic composition of the colonies influence the number of guards in a colony. These results also suggest that these three QTLs affect the expression of guarding behavior in European honey bees. The genetic molecular analysis results showed that *sting-2* has an important effect on the expression of guarding behavior in this population of honey bees. This is the first confirmation of *sting-2* in an independent population and demonstrates that these QTLs can influence guarding behavior in honey bees in the U.S.

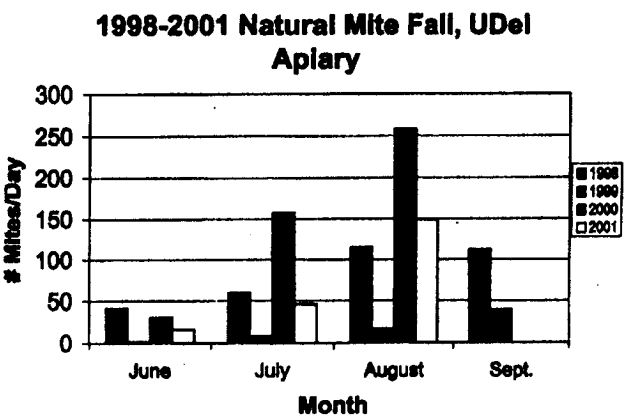
4. Caron, D. M.[d], C. Ziegler[d] & J. Hubner[d] - Determining Treatment Threshold for Varroa Mites - Control of the parasitic Varroa mite is a serious challenge to successful keeping of honey bee colonies for US and world beekeepers. Most beekeepers in the US have or currently are using the pyrethroid fluvalinate (Apistan®) for Varroa mite control (Caron, 1999, *Am. Bee J.* 139:631), but with mite resistance to fluvalinate increasing, the sole approved alternative miticide is coumaphos (Checkmite+ ®), an organophosphate. Some beekeepers are seriously exploring IPM alternatives for Varroa mite suppression (Calderone, 1999, *Bee Culture* 127(4): 27; Caron, 1999, cited above).

Delaplane and Hood (1999 *Apidologie* 30:383) established a natural fall threshold of 59-187 mites overnight onto sticky boards for the Southeastern USA based on tolerable mite levels of 3172-4268 total mites for colonies in the mid 20,000 population range. Our study has focused on defining a treatment threshold based on fall (August – September) mite population assessment while mite levels are still tolerable, but prior to total colony collapse using sticky boards as a monitoring tool.

In 1998, total mite fall (as determined by fluvalinate treatment) in 9 colonies exceeded a conservative 3000 total mite/colony level selected as a working threshold while in 5 colonies (33%), the 3000 total mite population level was not exceeded. The highest natural mite fall in September monitoring of the 5 colonies below 3000 total mites was 43 mites/day. In 1999, mite populations approximated or exceeded 3000 mites in 15 colonies, but were lower in 11 (42%) colonies. If a treatment threshold of 60 mites/colony/day had been utilized, 8 (31%) of the colonies would have been considered not needing fall chemical miticide control treatment. Use of a slightly less conservative 4000 total mite level, would have moved the threshold figure close to 100 mites/day and resulted in 16 of 26 (62%) colonies as not in need of fall miticide treatment.

In 2000 and 2001, mite levels increased very substantially between July and August sampling periods so that use of Apistan to determine total mite population was started one month earlier than in the previous two years. In 2000, all but one colony (of 19 total in study) exceeded the conservative fall target figure of less than 3000 total mites and only one other was below 4000 mites. Natural mite fall monitoring levels were <57 mites/day for these two colonies. Preliminary analysis of 2001 data analysis indicates 4 colonies (18%) as below the conservative 3-4000 total mites with a natural mite fall in mid-August of 53 or fewer mites/day.

We conclude that a mite threshold of 43 - 60 mites/day (natural mite fall monitored for a 3-day interval using sticky boards) would have resulted in a decision in which approximately one-third of the colonies would not be treated in 1998 & 1999 and approximately 20% in 2000 and 2001. Studies are continuing to determine what level of total mite population in colonies might be tolerable in the Mid-Atlantic region and to further challenge potential threshold estimates of <60 mites natural mite fall/day indicates colonies do not require fall miticide treatment.



5. Caron, D. M.[d], M. Frazier[a],& M. Embrey[e] - REGIONAL RESEARCH/ EXTENSION PROGRAM IN APICULTURE - THE MAAREC APPROACH - Extension and Research bee specialists of the land grant universities of New Jersey, Maryland, Delaware, Pennsylvania and West Virginia, form one-third of a MAAREC (Mid-Atlantic Apiculture Research and Extension Consortium) Task Force developed to address the pest management crisis facing the beekeeping industry in the Mid-Atlantic Region. The 16-member Task Force also includes representation from the state beekeeping associations of each of the five states plus the State Department of Agriculture Apiary inspection personnel. The USDA/ARS (Beltsville Bee Lab) completes the Task Force.

The MAAREC Task Force meets twice annually to identify research and extension priorities for apiculture in the Mid-Atlantic Region, review and monitor progress of bee research/extension projects and assist specialists in obtaining funding for a consolidated apiculture. Apiary inspection and beekeeper input through MAAREC and the use of tools such as beekeeper surveys are being used to identify the most effective ways to direct beekeepers in sound management decisions for mite and disease control.

A recent MAAREC survey of over 750 beekeepers in the Mid-Atlantic region showed strong support for research on Integrated Pest management (IPM) and/or non-chemical approaches to honey bee mite and disease control. While chemical treatments are seen as a necessary short-term control tactic, beekeepers are concerned about the rapid development of resistance to these materials, chemical contamination of the hive, and health risks associated with use of control chemicals. The regional project seeks to identify alternatives to chemical controls and promote less reliance on chemical pesticides for mite control. Included is demonstration research of integrated approaches to reduce mite pressure, development of an economic injury level (threshold) for fall mite control decision within an IPM framework and sampling method efficacy for measuring mite numbers in bee colonies.

An integral part of this regional effort is the timely delivery of IPM-based management techniques. Traditional information delivery approaches include 32 MAAREC extension publications, a field guide to ID major pests/diseases, an annual IPM short course, for-sale publications, and a Penn State Beekeeping correspondence course. Interactive information transfer techniques developed by extension specialists of the region include a CD-Rom BeeAware program for diagnosing and trouble-shooting bee problems, a MAAREC website <http://maarec.cas.psu.edu>, and visual teaching aids of slide sets and video. There is also a regional beekeeping newsletter BEEAWARE.

New publications and/or decision support tools specific to the adoption and implementation of IPM for honey bee mites and disease are anticipated. A new "train-the-trainers" beekeeping training manual is available in both paper copy version and as an outline resource to assist beekeeping short course instructors/presenters with outlines of information appropriate in honey bee management instruction (DMCaron, 2001 Beekeeping Resource Manual, MAAREC Misc Publ 2, 57 pp). All new and updated materials generated are made available free of charge to participating MAAREC state beekeeping associations, county cooperative extension offices and apiary inspection services and available to others through posting to the website and through for-sale publications from Penn State University Cooperative Extension.

Follow-up survey tools will be developed to assess IPM adoption by beekeepers and the support of the coordinated educational program in the MAAREC region. Participating extension personnel, in collaboration with researchers, will report the research and extension (IPM adoption) results of this cooperative project in articles in local, regional and nationally-distributed beekeeping journals and at local, state, regional and national beekeeping/grower meetings.

6. Dedej, S.[g] & K. S. Delaplane [g] - INTERACTIONS AND POLLINATING EFFICACIES OF HONEY BEES AND NECTAR-THIEVING CARPENTER BEES - Failure to produce good crops in blueberry is frequently the result of poor pollination. Based on single-bee flower visits, southeastern blueberry bees (*Habropoda laboriosa*) and bumble bees (*Bombus spp.*) are considered the most efficient pollinators of rabbiteye blueberry (Cane and Payne 1990, *Proc. Alabama Agr. Exp. Sta.* 37: 4); however their unpredictability limits their commercial pollination value.

Honey bees are the most numerous visitors in blooming rabbiteye blueberry in south Georgia, followed in descending order by bumble bee queens, bumble bee workers, carpenter bees (*Xylocopa spp.*), and southeastern blueberry bees (Delaplane, 1995, *American Bee Journal* 135, 825-826). However it was thought that honey bees were marginally useful as pollinators (Cane & Pane 1990). This conclusion was later reversed by Sampson & Cane (2000, *J. Econ. Entomol. 93: 1726-1731*) and by our work. In 2000, we demonstrated fruit set up to 80 % in plots tented with honey bees compared with 39.1 % fruit set in open plot.

Nectar thieving by carpenter bees may contribute to pollination problems in rabbiteye blueberry. In order to obtain nectar, carpenter bees rob flowers of rabbiteye blueberry by cutting slits in the sides. Cane and Pane (*1991 Proc. Southeast Blueberry Conference, Tifton, GA*) observed that honey bees learn to use these slits and become secondary nectar robbers. As few as one carpenter bee per 25 bushes or 4 % incidence of punctured corollas can shift 80-90 % of honey bees to robbing. Delaplane (1995) showed that the percentage of robbers was highest in *Xylocopa* species (100 %) followed by *Apis mellifera* (92.3%) in rabbiteye blueberry.

In the present study during the spring and summer 2001, rabbiteye blueberry plants were tented with known numbers of honey bees (0 or 3200), carpenter bees (0, 1 or 2), or both honey bees and carpenter bees following a switchback design in an attempt to determine the effects of the bees, singly or interacting, on flower visiting behavior, fruit-set and fruit quality (Table).

The experiment confirms the practical efficacy of honey bee as pollinators of rabbiteye blueberry with fruit set reaching 75.6 % (A-A-A) compared with only 21.7 % in the open plot. The addition of *Xylocopa* tended to decrease fruit-set, but significantly so only for A-AX-A (1 *Xylocopa*) . Moreover fruit-set was significantly reduced in the tent with *Xylocopa* only (X-X-X). Thus, honey bees appear capable of mitigating negative effects on fruit-set of *Xylocopa* nectar thievery. It remains unclear whether Xylocopa is a net contributor or limiter on fruit quality in rabbiteye blueberry. On one hand, fruit set in X-X-X was numerically (but not significantly) higher than either the open plot or tent without bees. However X-X-X was significantly lower than open plot for speed of ripening, berry weight, and seeds per berry. Sucrose content of juice did not differ among treatments.

NA	Open plot	21.7 ± 3.6 (44) b	80.7 ± 5.3 (25) a	1.37 ± 0.1 (25) a	14.6 ± 1.4 (25) bc	13.0 ± 0.4 (25) a
NA	No bees	17.6 ± 3.9 (34) b	51.8 ± 10.4 (17) abc	0.84± 0.1 (17) c	0.5 ± 0.2 (17) f	15.4 ± 0.5 (17) a
NA	A-A-A	75.6 ± 3.9 (40) a	31.3 ± 5.1 (19) cd	0.84 ± 0.1 (38) c	18.6 ± 1.3 (38) ab	11.3 ± 0.2 (38) a
2	X-X-X	31.7 ± 4.1 (34) b	39.3 ± 8.4 (26) cd	0.95 ± 0.1 (28) c	3.1 ± 0.6 (26) ef	13.8 ± 0.3 (28) a
1	A-AX-A	37.2 ± 5.2 (38) b	69.7 ± 8.6 (30) ab	1.35 ± 0.1 (32) a	23.0 ± 1.8 (32) a	10.7 ± 0.3 (32) a
1	AX-A-AX	57.1 ±5.2 (28) a	39.8 ±14.5 (28) bcd	1.25 ± 0.1 (28) ab	7.2± 1.0 (28) de	15.9 ±4.1 (28) a
2	A-AX-A	70.1 ± 4.7 (36) a	14.2 ± 3.4 (35) d	0.99 ± 0.1 (35) bc	12.2 ± 1.3 (35) cd	12.3 ± 0.3 (35) a
2	AX-A-AX	72.4 ± 4.4 (40) a	16.6 ± 4.0 (40) d	1.06 ± 0.1 (40) abc	11.8 ± 1.0 (40) cd	10.8 ±0.2 (40) a
2	AX-AX-AX	69.4 ± 4.1 (37) a	23.9 ±5.1 (38) cd	1.07 ± 0.1 (37) abc	10.3± 0.7 (37) cd	12.0 ±0.3 (36) a

Table. Mean value of characters measured ± standard errors, parentheses = n. Column means with the same letter are not different at the α=0.05 level. During three consecutive weeks of flowering, *Apis* (A), *Xylocopa* (X), or both (AX) were present in tents. The table indicates the bees present and their sequence. Fruit characters were measured at harvest.

7. DeGrandi-Hoffman, G [h]. - UPDATE ON AFRICANIZED HONEY BEE RESEARCH - Africanized honey bees (AHB) have displaced resident European honey bee (EHB) populations in many areas where they have immigrated. In southern Arizona, where this research was conducted, African matrilines and patrilines compose the vast majority of the feral honey bee population. The goal of our research program is to identify factors in the biology of AHB that have enabled them to replace EHB populations.

We divided our research into two major studies. The first study is directed at identifying factors could contribute to the loss of European patrilines. We designed studies to determine whether African patriline queens have selective advantages over Europeans that, during times of queen replacement, increase their chances of becoming the new queen. We compared European and African queen development time, worker behaviors toward developing and emerged queens of both patrilines, and sperm utilization by queens mated to both African and European drones. We found that African patriline queens have shorter development times and a greater probability of emerging before European patriline queens. African patriline workers vibrate queen cells and emerged queens and feed developing queens more often than European patriline workers. Cells with African patriline queens are vibrated more often by workers and emerged first in more than 90% of our observations. Finally, both African and European matriline queens inseminated with sperm from equal numbers of European and African drones produced predominately African patriline workers. In summary, all the factors we examined that could influence the patriline of a new queen showed a clear advantage for queens with African patrilines.

The second area of research was directed at determining how European matrilines are replaced by African matrilines. We found that some African matriline colonies contain workers that lay diploid eggs. This trait is called thelytoky. When the colony becomes queenless, it can requeen itself with brood from laying workers. Other traits we have observed in thelytokous African bees are: (1) some workers have mature ovaries in queenright colonies; (2) supersedure queen cells and queen cups with eggs in them are frequently seen even when a laying queen is present; (3) when a colony requeens itself with the offspring of a laying worker, workers continue to lay eggs and rear queens for several weeks after the queen emerges. We also suspect that colonies with thelytokous workers produce a form of worker called "intercastes". Intercastes resemble small virgin queens. The attributes of intercastes we have uncovered thus far are that they: (1) emerge from worker cells, (2) do not care for brood, (3) are not queen attendants, (4) forage but are not scouts, (5) are frequently seen in colonies that are being robbed, (6) can invade colonies either individually or in groups, and (7) do not necessarily have developed ovaries. Currently, we are looking at how intercastes invade colonies. The information we obtain from our studies will provide a foundation for designing management strategies to maintain EHB colonies in AHB areas and to more successfully introduce EHB queens in colonies that have become Africanized.

8. Delaplane, K. S.[g] - PROGRESS REPORT: INTEGRATING MITE MITIGATION MEASURES WITH PUBLISHED ECONOMIC THRESHOLDS FOR *VARROA DESTRUCTOR* - One of the explicit goals of researchers working with integrated pest management of *Varroa destructor* is to reduce or ultimately eliminate the beekeeping industry's reliance on synthetic acaricides. If beekeepers can chronologically delay chemical treatments as long as possible this not only reduces overall chemical use, but enables mites through genetic recombination and reproduction over time to conserve their chemical susceptible genes (see Metcalf, 1982. *In* Introduction to insect pest management, 2d. ed, John Wiley). Numerous IPM practices against varroa have been developed, and when coupled with a research-derived economic threshold, hold promise as the key to delaying chemical treatments. A collaborative project between the Univ. Georgia, Univ. Tennessee, and Clemson Univ. is examining the efficacy of apiary isolation (Sakofski *et al.*, 1990 *Apidologie* 21: 547), hygienic queens (Spivak, 1996 *Apidologie* 27: 245), and screened hive bottoms (Pettis & Shimanuki, 1999 *Am. Bee J.* 139: 471) in delaying an economic threshold of 60-190 mites on an overnight sticky sheet (Delaplane & Hood, 1999 *Apidologie* 30: 383).

The experiment was set up in June 2001 with 40 package colonies in northeast Georgia. Each colony was randomly assigned to an isolated apiary (no closer than 2 km to a known managed apiary) or a non-isolated apiary (*i.e.* placed within an existing managed apiary). Within each apiary situation each colony randomly received one of the following treatments (1) non-hygienic queen + conventional bottom, (2) non-hygienic + screened, (3) hygienic + conventional, and (4) hygienic + screened. Colonies are monitored *ca.* monthly with overnight sticky sheets to appraise colony mite levels and the onset of economic threshold.

The table presents the results of two sampling dates. On August 9 there appeared to be a average benefit from hygienic queens (0.8 vs. 0.9) and screened bottoms (0.7 vs. 1), but these patterns were reversed on September 4. The data set is too premature to substantiate any conclusions. Plans are to continue monitoring colonies until economic thresholds are achieved and then compare time to onset of economic threshold among treatments.

Table. Effects of apiary isolation, hygienic-selected queens, and screened hive bottoms on number of *V. destructor* retrieved on overnight sticky sheets. Numbers are mean ± standard error. Numbers in parentheses = *n*.

treatment	August 9	September 4
		Isolated
non-hygienic + conventional	1.2 ± 0.4 (5)	1.4 ± 0.2 (5)
non-hygienic + screen	0.6 ± 0.4 (5)	3.0 ± 1.0 (5)
hygienic + conventional	0.8 ± 0.6 (5)	3.4 ± 1.3 (5)
hygienic + screen	0.8 ± 0.4 (5)	2.8 ± 1.2 (5)
		non-isolated
non-hygienic + conventional	1.4 ± 0.9 (5)	1.2 ± 0.4 (5)
non-hygienic + screen	0.5 ± 0.3 (4)	1.8 ± 0.7 (5)
hygienic + conventional	0.6 ± 0.2 (5)	1.8 ± 0.8 (5)
hygienic + screen	0.8 ± 0.4 (5)	1.0 ± 0.6 (5)

9. Fell, R. D.[k] & K. Tignor [l] - MITICIDE EFFECTS ON THE REPRODUCTIVE PHYSIOLOGY OF QUEENS AND DRONES - Beekeeper complaints of queen problems prompted an examination of potential miticide effects on the reproductive physiology of queens and drones. We tested whether miticide use could affect sperm production or viability in drones, or the number or viability of sperm in the spermathecae of queens.

Queens and drones were reared in colonies containing formic acid gel packs, Apistan, or CheckMite (coumaphos) strips. Control queens and drones were reared in untreated colonies. Queens were collected after mating and initial egg-laying; drones were collected before mating. Sperm counts were made by dissecting out a seminal vesicle or spermatheca in modified Kiev's solution and counting the sperm with a hemocytometer and microscope. Sperm viability in the spermatheca was estimated using vital stains (Hoechest 3342, propidium iodide) and a fluorescent microscope. Sperm viability in drone semen was determined after forced ejaculation.

The results indicate that the miticides had no significant effect on queens with regard to the numbers spermatozoa in the spermatheca or to the viability of stored sperm. Mean numbers of sperm in the spermathecae were $4.5 \pm 1.3 \times 10^6$ for control queens, $3.6 \pm 1.4 \times 10^6$ for formic acid queens, $4.9 \pm 1.4 \times 10^6$ for Apistan queens and $6.9 \pm 1.5 \times 10^6$ for coumaphos queens. Percent sperm viability varied from 93.6% and 93.7% in queens from Apistan and coumaphos treated colonies respectively, to 98.9% and 97.4% for queens from formic acid and control colonies.

No significant differences were found in sperm viability between drones raised in formic acid, Apistan or control colonies. However, a significant reduction in sperm number was found in the seminal vesicles of drones reared in Apistan treated colonies (see Table).

The other noticeable effect of miticide treatments occurred with the use of coumaphos. Acceptance of grafted queen cells in treated colonies was less than 5%, versus 95% in control colonies. Coumaphos also reduced queen mating success and drone production in treated colonies. These results suggest that coumaphos should not be used in colonies involved in queen production.

Treatment	Number	Sperm number
Control	8	$4.3 \pm 0.64 \times 10^6$
Formic Acid	10	$3.7 \pm 0.94 \times 10^6$
Apistan	12	$1.9 \pm 0.87 \times 10^6$
Coumaphos	8	$2.6 \pm 0.92 \times 10^6$

Table. Number of sperm in one seminal vesicle of drones reared in miticide treated colonies.

10. Haarmann, T. K.[j], & M. Spivak [j] - THE EFFECTS OF FLUVALINATE AND COUMAPHOS ON QUEEN BEES IN TWO COMMERCIAL QUEEN REARING OPERATIONS - The beekeeping industry has entered a new era with the recent widespread use of miticides to treat the parasitic mite, *Varroa jacobsoni*. For the last ten years, the synthetic pyrethroid, fluvalinate (Apistan) has been used very successfully to treat the mites. However, in the last two years, the mites have developed resistance to this miticide. To relieve the crisis that emerged with the resistant mites, many states obtained Section 18 approval for use of the organophosphate, coumaphos. The coumaphos and fluvalinate impregnated strips used in the U.S. are readily absorbed and accumulated into beeswax, and there is concern about possible ill-effects of miticides to bees. In recent years, some beekeepers have had problems with queen loss and queen supersedure..

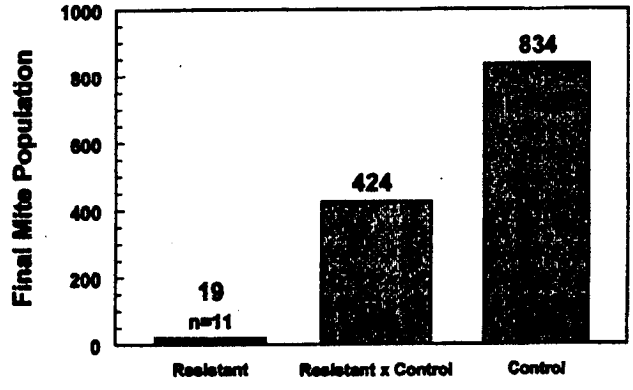
We conducted research on the effects of fluvalinate and coumaphos on queen viability and health to determine if miticides have a negative impact on queen health and viability. Field experiments were conducted in commercial queen rearing operations in both Texas and California. Queens were reared in colonies that had been treated with differing amounts of both fluvalinate and coumaphos. The goal was to explore the extent of miticide accumulation in wax, bee tissue, and queen cells, and to investigate the possible correlation between fluvalinate and coumaphos concentrations and queen development and performance. Pre- and post-treatment samples of both wax and bees were collected from all of the colonies and analyzed for total concentrations of fluvalinate and coumaphos. The queens that were reared during the experiment were sent to the University of Minnesota and were analyzed for queen weight, ovarial weight, and number of sperm in the spermathecae. We measured these characteristics to determine if the treatments affected queen development and mating above the normal variation seen among queens for these characteristics.

The queens treated with high doses of fluvalinate (8 Apistan strips) weighed significantly less than low dose (2 Apistan strip) or control queens, but otherwise appeared to develop normally. The highest fluvalinate concentrations were observed in the wax and queen cells of the high dose group.

The developing queens treated with varying levels of coumaphos suffered a high mortality rate. In general, acceptance of coumaphos exposed queen cells was very low. It was difficult to successfully produce queens when coumaphos was present in the starter colonies for any extended period of time. Many attempts were made to rear queens, using various amounts of coumaphos for varying time periods, before queens could be successfully produced. High mortality of larvae was noted in colonies that contained as little as one CheckMite+^{TM} strip of coumaphos for more than 24 hours. Several of the queens showed sub-lethal effects from the coumaphos including physical abnormalities and atypical behavior. The queens exposed to coumaphos weighed significantly less and had lower ovary weights than the control group queens. The highest coumaphos concentrations were observed in the queen cells and wax of the high dose groups. It is noteworthy that reduced queen and ovary weights occurred at concentrations below the EPA Tolerance Level of 100 parts per million for beeswax.

11. Harbo, J. R.[f] & J. W. Harris [f] – SUPPRESSION OF MITE REPRODUCTION: A CHARACTERISTIC OF HONEY BEES THAT PRODUCES RESISTANCE TO *VARROA DESTRUCTOR* – This test compared the growth of mite populations in colonies of bees that each received one of the following queens: (1) *resistant*, queens selected for suppression of mite reproduction (SMR) and artificially inseminated with drones from similarly selected stocks; (2) *resistant x control*, resistant queens, as above, produced and free mated to unselected drones by one of four commercial queen producers; and (3) *control*, commercial queens chosen by the same 4 commercial queen producers and free mated as above. Each colony started the test with 0.9 kg of bees that were naturally infested with about 650 mites. At the end of the 115-day test period, the total mite populations were measured in each of 57 colonies in the test (see figure).

This study demonstrated that selection of honey bees for a single resistant trait (SMR) can effectively reduce mite populations in a bee colony. Moreover, queens selected for the SMR trait and then free-mated to drones at commercial beekeeping locations can provide a colony with a significant level of resistance to varroa. However, highly resistant queens (SMR queens × SMR drones) are not yet recommended for use in field colonies because of reduced brood production. For details of this research see Harbo & Harris, *J. Econ. Entomol.*, in press.



Average total mite populations (mites on adult bees plus the number of adult mites in brood cells) at the end of the 115-day test period. Standard deviations were high (18, 422, and 755, respectively), yet means were different at the 0.01 level.

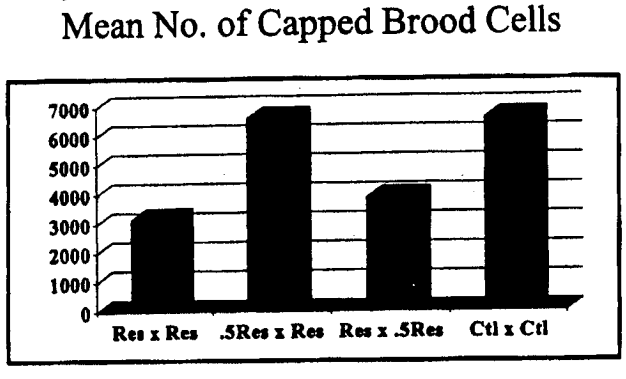
12. Harbo, J. R.[f] – THE RELATIONSHIP BETWEEN NON-REPRODUCTION OF VARROA AND THE QUANTITY OF WORKER BROOD – In an earlier study, Harbo & Harris (*J. Econ. Entomol.*, in press) noticed that colonies produced less brood when they had SMR x SMR queens (queens with genes for suppressing mite reproduction that were artificially inseminated with SMR drones) than when colonies had SMR queens that were free mated with unselected drones.

This experiment used only artificially inseminated queens to compare brood production among colonies that had differing proportions of SMR genes: 100%, 75%, or 0%. Uniform colonies (n = 42) were each given one of 4 different queen types: (1) 100% SMR, SMR queens each mated to a single SMR drone; (2) 75% SMR, heterozygous SMR queens (0.5 SMR) each mated to an SMR drone; (3) 75% SMR, SMR queens mated to a drone from a heterozygous SMR queen (0.5 SMR), and (4) 0 SMR, non-SMR queens each mated to a non-SMR drone. Each colony began with

1 kg of bees, no brood, and a test queen. Brood area and mite reproduction were measured in each colony 7 weeks after the queens were released.

The colonies with 100% SMR had significantly fewer cells of capped brood than the controls (those with 0% SMR). The two groups with 75% SMR were different; group 2 was similar to group 4, group 3 similar to group 1 (see figure). The percentage of reproductive mites was 7, 16, 19, and 65% for groups 1 – 4, respectively (based on sampling 20 single foundress mites per colony).

These results suggest that there is a relationship between the stock that contains our SMR trait and poor brood production. This test does not determine if brood production is always associated with the SMR trait or if this association is coincidental. Pure SMR queens were very good brood producers when they were free-mated (see abstract #11 above), so poor brood production is not solely associated with the queen as data in the figure may suggest. Moreover, group 2 demonstrated that it is possible to assemble genetic combinations of bees that will maintain high levels of both brood production and resistance to varroa.



Colonies in groups 1 and 3 had significantly fewer cells of capped brood than groups 2 and 4 (lsd, $P<0.05$). See text for group descriptions.

13. Hood, W. M. [m] - DEVELOPMENT OF AN INSIDE HIVE TRAP FOR SMALL HIVE BEETLES - The small hive beetle has caused considerable damage to honey bee colonies since its recent introduction into North America, particularly in the southeastern region of the USA (Hood, 2000 *Bee World* 81(3):129-137). Although two pesticide products, Guard Star® and Check Mite +®, are available for small hive beetle control in some states in the USA, there is a great need for other safe and efficient control alternatives for this pest.

Investigations have been conducted to develop traps for the small hive beetle (Elzen *et al.*, 1999 *Am. Bee J.* 139: 934-935; Hood 2000 *Am. Bee J.* 140: 830), but no trap has proven to provide efficient control of this hive pest. An inside-hive trap when filled with beer, 50% ethylene glycol, or mineral oil was tested and found to kill small hive beetle adults (Hood 2000 *Am. Bee J.* 140:830). The trap consisted of a plastic reservoir box (152x80x25 mm) attached to the bottom bar of a hive body frame. The box had nine vents (3x21 mm) on the top surface lid that allowed adult beetle entry.

This research was conducted to further evaluate this inside-hive trapping device using other materials in an attempt to increase the small hive beetle killing efficiency, especially at low infestation levels. The objective of this study was to test raw honey, mineral oil, and cider vinegar in the trap and compare their beetle killing efficiency. Ten small hive beetle infested colonies were used in this study. Two beetle traps with different test materials were placed in each colony with a trap placed in hive-body frame positions #1 and #9. Vinegar and honey were compared from 11 Feb - 5 April 2000 and vinegar and mineral oil were compared from 7 April - 20 Nov. 2000. Dead small hive beetles were removed and counted from traps at 3-4 week intervals during the test period.

The number of small hive beetle adults trapped and killed in vinegar was significantly higher ($P<.05$) than in raw honey or mineral oil. Cider vinegar proved to be more effective in killing adult small hive beetles than the other materials tested, but not at a rate to be considered an efficient pest control alternative at low infestation levels. However, the use of vinegar in the trap to indicate the adult beetle infestation levels may be utilized in the development of a small hive beetle treatment threshold program.

Further investigations may lead to the development of a more efficient inside-hive trapping system which can be placed in a honey bee colony in cooler climates in winter when only small hive beetle adults are present inside the colony (Pettis & Shimanuki 2000 *Am. Bee J.* 140: 152-155).

14. Hood, W. M. [m], J. Evans[n], H. Shimanuki [n], & J. Pettis [n] - INTRODUCTION AND TRACKING OF THE SMALL HIVE BEETLE IN NORTH AMERICA - Small hive beetles (*Aethina tumida* Murray) were first collected in North America from a colony of honey bees in Charleston County, South Carolina in November 1996. A hobbyist beekeeper, who made the discovery, collected and hived a swarm of honey bees from a tree limb in the summer of 1996 in the city of Charleston, which is adjacent to the Port of Charleston, a major eastern US seaport. The beekeeper collected several adult beetles from the colony that fall and submitted them to Clemson University Entomology Department where they remained unidentified past family name until June 1998 when the first small hive beetle identification was made from beetles collected in a Florida apiary. Another unidentified beetle collection was made in South Carolina in the fall of 1997.

Subsequently, pest surveys were conducted in Georgia, Florida, North Carolina and South Carolina and small hive beetles were found in many of the coastal areas of these states by the end of 1998. By 2001, small hive beetles had spread to at least 16 states in the US causing colony losses mostly in the coastal regions of three states, Florida, Georgia and South Carolina.

Mitochondrial DNA analyses of 539 small hive beetles collected from 26 apiaries in Florida, Georgia, North Carolina, and South Carolina showed irregular distribution of two distinct haplotypes. Beetles from the first collections (1996 and 1997) made in South Carolina were of all NA1, which is a haplotype that was generally rare in collections made in Florida, Georgia and North Carolina in 1998 and 1999. In 1998 and 1999, beetle samples from apiaries in South Carolina continued to show a slight bias toward haplotype NA1. Unexpectedly, South Carolina samples collected in 2000 (from a county, Charleston, that had been sampled in each of the previous years) showed a bias toward haplotype NA2, in opposition to earlier samples from this state. Aside from the Charleston County samples, there were no significant changes in haplotype frequency in any of the other three states across time, and the countrywide frequency of the two haplotypes was unchanged. Future small hive beetle collections in the US should reflect current populations in Florida, where the two haplotypes are mixed across all sites, apparently at equal frequencies. However, in the short term it might be possible to infer that newly discovered beetle populations are derived from sources with biased haplotype frequencies (e.g., parts of South Carolina versus Georgia).

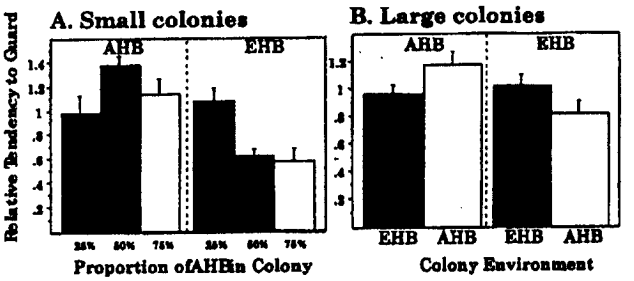
The two distinct haplotypes (NA1 and NA2) found in the US show a close relationship to small hive beetles collected from South Africa (Evans *et al.*, 2000, *Ann. Entomol. Soc. Am.* 93: 415-420.) The two distinct haplotypes found in the US were as different from each other as they were from haplotypes found in South Africa. This result, alongside morphological and field studies (Elzen *et al.*, 2000, *Apidologie* 30: 361-366), provide strong evidence that hive beetles now in the US are derived from southern African populations. The limited number of haplotypes (2) found in the US versus several (12) haplotypes (Evans *et al.*, cited above) found in South Africa suggest that any new small hive beetle introductions into North America may be distinguishable from those beetles currently found in the US. Regulatory agencies may be able to use this information to pinpoint future introductions.

15. Hunt, G. J.[b], E. Guzmán-Novoa [o], J. L. Uribe [o] & D. Preto[o] - OBSERVATIONS ON DEFENSIVE BEHAVIOR IN MIXED AFRICANIZED AND EUROPEAN HONEY BEE COLONIES - It is important to know how Africanized bees and European bees interact to know what levels of Africanization in our hives are tolerable and which bees are the ones pursuing and causing the problems. Studies were conducted in the state of Guerrero, Mexico to study guarding and stinging behavior of co-fostered bees. Six genotypes of bees (from 6 source colonies) were emerged from combs in an incubator, marked with paint to distinguish them, and co-fostered in small colonies. Colonies consisted of 2000 marked bees plus 3000 older bees (with genotypes mixed in the same proportions) to give a "normal" age distribution, plus two frames of brood, and two of honey/pollen. They all contained queens. Three of the source colonies had European queens that were open-mated in areas that do not have Africanized bees (1 from U.S. and 2 from Canada). The other three sources were Africanized queens with African mitochondrial DNA and behavior. Bees were mixed in three proportions: 25% Africanized, 50% Africanized and 75% Africanized, each replicated in two colonies. Colonies were observed six days a week. Each day, we watched guarding behavior for 30 min and at intervals through the day for 21 days. We captured most of the guards and chilled them briefly so we could attach a numbered tag to their thorax.

Overall, European bees were relatively less likely to guard than would be expected from their proportion in the colony. This effect was greatest in colonies containing 75% Africanized bees, suggesting an interaction. But Africanized bees did not seem to change their behavior in different mixtures of genotypes. During observations, 29 marked bees stung the observers. Twenty-six of these were Africanized and 19 (two-thirds) were from a single (the "green") genotype. Five of those had been previously tagged guards. In the 2 colonies with 50% Africanized bees, the Africanized genotypes were more persistent on average. One European genotype had the longest average guarding duration over days, but the green Africanized genotype had individuals that guarded the most during their guarding career. They were observed guarding on 70% of the days and had the highest average number of observed guarding bouts per individual bee (5). We were able to tag more than twice as many guards (37) for this genotype than for any other. Overall, 134 guarding bouts were observed for the green genotype. Europeans genotypes averaged 26-40 bouts and the other Africanized genotypes had 48-50. Africanized bees accounted for 232 of the 328 guarding bouts (70%). These results show that Africanized bees have a much higher tendency to guard and that guards are among the individuals that pursue and sting people.

16. Hunt, G. J.[b], E. Guzmán-Novoa [o], J. L. Uribe [o] and D. Preto [o] - GENOTYPE BY ENVIRONMENT INTERACTIONS IN DEFENSIVE BEHAVIOR OF EUROPEAN AND AFRICANIZED HONEY BEES - In Mexico, the highly defensive behavior of African honey bees is a dominant trait in colonies of hybrid origin (Guzmán-Novoa *et al.*, in Press). We previously reported the results of observations of the guarding behavior and stinging behavior of Africanized honey bees (AHB) and European honey bees (EHB) kept in small colonies (nucs) containing various mixtures of the genotypes. Here we report more analyses and data from these nucs plus data from full-sized colonies. In a stinging assay in the nucs, AHB were highly over-represented during the first 10 seconds of stinging, but not during the next 20 seconds, indicating that the EHB were recruited to sting by AHB, a finding that could explain the genetic dominance of colony stinging behavior. The green genotype was highly over-represented among "first stingers" and pursuers.

The guarding behaviors of about 12,000 marked individuals from four of the six genotypes (2 AHB and 2 EHB) were also observed for several weeks in 4 large colonies (2 AHB and 2 EHB). There was a genotype by environment interaction in guarding tendency. In both the small and large colonies, AHB were more likely to guard in colonies that had more AHB, but EHB were more likely to guard in colonies that had more EHB than in colonies with lower proportions (see figure). But the persistence of individuals in guarding of both EHB and AHB in small and large colonies tended to increase with increasing proportions of AHB. Finally, the guarding and stinging behavior of individuals in mixed colony environments correlated with the defensive-behavior assays of the six colonies that were used as sources of bees in these studies. Eight replicates of 4 different defensive-behavior assays of the source colonies were conducted (D. Preto, unpublished data). The "green" source colony had the highest numbers of stings in assays, the most pursuers, greatest response to alarm pheromone and the highest rating for defensive behavior. AHB source colonies had 17 times as many pursuers and deposited 5 times as many stings as EHB colonies. Among the 3 EHB colonies, the source with the highest guarding tendency and persistence of individuals was the only colony that deposited stings in one assay.



Interaction between genotype and colony environment. A. Small colonies B. Large colonies of either AHB or EHB.

17. Jarolimek, J.P & G.W. Otis P – A COMPARISON OF FITNESS COMPONENTS IN LARGE AND SMALL HONEY-BEE DRONES - Most drones are reared in specially constructed, large drones cells. Small drones are usually produced when workers in a queenless colony lay unfertilized eggs and rear the resulting drones in small worker cells. In one study involving actual mating of queens with large and small drones (Berg *et al.*, 1997. *Apidologie* 28:449-460), large drones had a reproductive advantage over small drones. Previously Berg had reported that the quantity of spermatozoa in small and large drones did not differ (Berg, 1990 *Proc. German Zoological Society 83rd Meeting, Frankfurt am Main*, Gustav Fischer Verlag, Stuttgart). Because honeybees invest energy into building special comb with larger cells in which to rear drones and they preferentially rear large drones, large drones must have a reproductive advantage. We studied several fitness components of regular and small-sized drones to complement Berg's work. As measures of fitness, flight characteristics and longevity of large and small *Apis mellifera* L. drones were quantified. We also verified sperm numbers in drones of different sizes.

We separated newly emerged drones from two queenless colonies into small, intermediate, and large categories, then marked the small and large drones with unique colour-coded numbers for visual identification (177 large drones, 243 small drones). Another 50 drones of a range of sizes were paint-marked. All of the marked drones were placed in one of two queenless colonies. The number-marked drones were observed as they entered and exited one hive during their afternoon flights. Flight times were recorded over several hours on several afternoons to enable us to determine flight duration and frequency. Once per week the surviving drones were recorded to determine longevity. Approximately two weeks after the introduction of the drones into the hive, the seminal vesicles of each surviving paint-marked drone were removed, sperm were counted with a haemocytometer, and the drone (minus his abdomen) dried at 45 C. The sperm counts were correlated with drone dry weight.

None of the attributes of drone flights that we quantified—average flight duration, total flight duration, number of flights per drone per day, and maximum flight duration—were affected by drone size on two days with good flight conditions (e.g., sunny,

>20 C). On a cooler day (~18 C), a higher proportion of large drones (61%) than small drones (7%) took mating flights (P<0.02), but because few if any queens take mating flights at these temperatures, this cannot explain the higher reproductive success of large drones. In one colony the large drones had higher longevity; in the other colony there was no difference, so those results were ambiguous. Most importantly, there was a highly significant positive correlation between number of sperm and drone size (r=0.61, P=0.004), which contradicts the results of Berg (1990). Our results suggest that the explanation for higher fitness of large drones proposed by Berg *et al.*, that large drones achieve higher reproductive success as a result of greater direct competition for mating with queens, may be incorrect. Differential sperm numbers alone may account for the greater reproductive success of large drones.

18. Nasr, M. E. [q], D. Servos [r], R. Bannister [r], & G. Wilson [s] - EFFICACY OF THREE MITICIDES (OXALIC ACID, FORMIC ACID, APILIFE VAR) ON *VARROA DESTRUCTOR* AND *ACARAPIS WOODI* IN HONEY BEE COLONIES IN ONTARIO, CANADA - Oxalic acid, formic acid (MiteAway™ single application pad) and Apilife VAR™ (a product containing thymol) were evaluated and compared with Apistan® against varroa and tracheal mites on honeybees. Honeybee colonies were treated with Formic Acid MiteAway pads (250 ml of 65% formic acid/pad/hive), Apilife VAR, and Apistan® as recommended. Two concentrations of Oxalic acid (dihydrate), 2.8g/l and 3.5g/l in 50:50 (sugar:water) sugar syrup were tested. The amount of Oxalic acid in sugar syrup applied to each bee colony was determined based on the colony strength. Colonies with 4-6 frames covered with bees received 40 ml and colonies with 7-9 frames covered with bees received 50 ml. Oxalic acid in sugar syrup was trickled on honey bees between the frame top bars in the brood chamber. Treatments were applied on colonies near Guelph, Ontario Canada, on the 20th of October, when temperature ranged between 5-11 °C. The number of fallen mites was determined using sticky traps during the treatment period of three weeks. After the treatment period all colonies were treated with Apistan® for another three weeks to determine varroa mites left in treated colonies.

Apistan® was the most effective treatment, with average varroa mortality of 95.21 ± 1.01% (mean ± SE, n = 8). Treatment with formic acid (Mite-Away™) resulted in an average mite mortality of 92.84 ± 2.28% (n = 7). Percentages of varroa mite mortality were 89.42 ± 2.39% and 55.52 ± 6.48% in colonies treated with Oxalic acid concentration 3.5 g/l (n = 7) and 2.8% (n = 8), respectively. Apilife VAR (n = 8) killed an average of 63.0 ± 7.16%. No significant difference (p< 0.05) was found in the efficacies of Apistan®, Formic Acid Mite-Away pads, and 3.5% Oxalic Acid. The ratios of coefficient of variations of varroa mortality percentages in Formic Acid Mite Away and 3.5% Oxalic acid treatments to Apistan® showed tested treatments provided consistent results as Apistan®. Efficacies of Apilife VAR and 2.8% Oxalic Acid were significantly lower than the efficacy of Apistan®, Formic Acid Mite-Away, and 3.5% Oxalic Acid. The number of brood chambers affected the efficacy of Oxalic acid. The efficacy for 3.5% Oxalic Acid was 89.87 ± 2.93% in single brood chamber colonies, 87.21 ± 7.07 in 1.5 and 73.67 ± 18.83% in double brood chamber colonies.

Formic acid showed a significant effect on tracheal mites. Apistan®, Oxalic acid and Apilife Var had no to low effects on tracheal mites. Colonies treated with Formic Acid Mite-Away had 85.7% of colonies which survived winter. This was followed by 3.5% Oxalic acid with 71.4% bee colony survival, Apistan with 62.5% survival, Apilife VAR with 50.0% survival and 2.8% Oxalic acid with only 25% of colonies surviving the winter. These results suggested that formic acid was highly effective in killing both tracheal mites and varroa mites. This may explain the high survivorship of bee colonies treated with formic acid. Bee colonies treated with Apistan®, Apilife VAR and 2.8% and 3.5% Oxalic acid continued to have a high level of tracheal mites.

These results suggest that Formic Acid MiteAway and 3.5% Oxalic acid are effective alternatives to Apistan® as a late fall treatment for varroa mites. Formic acid is the most effective treatment for tracheal mites in the fall.

19. Ostiguy,[a] N., M. Frazier,[a] D. Sammataro [a] & D. Caron [d] - AN UPDATE ON DETERMINING VARROA MITE LEVELS ON ADULT HONEY BEES - Effective varroa management requires an accurate determination of colony infestation level. To achieve this goal, we evaluated the ability of ether roll, powdered sugar roll and stickyboard natural mite fall to predict mite levels in 97 colonies. Ether rolls and powdered sugar rolls, using approximately 500 mites per roll, were performed on the same day as a 3-day natural mite drop stickyboard was inserted into the hive. True mite number was determined by inserting coumaphos or fluvalinate strips and 3-day stickyboards into each colony immediately after 3-day natural mite drop boards were removed. (Unpublished data show that 3-day stickyboards from a miticide-induced mite drop can be used to estimate the total number of mites in a colony.)

Natural mite fall predicts 72% of the true mite number (p = 0.001). Twenty-seven percent and 21% of ether and powdered sugar roll ($\log_{10}$), respectively, can explain true mite number. No significant difference in mite number between ether and powdered sugar rolls (mean number of mites = 27 ± 39.2, 29 ± 41.9, respectively) was observed when the sample size is large (n = 97). When the mean mite number is compared between ether and powdered sugar roll, and the sample size is small (n = 30), there is a significant difference in mite counts (Table 1). Differences between ether and powdered sugar rolls in the mean number of mites/bee and the per bee mean mite number after an alcohol wash are significant. The alcohol wash does not significantly change the number of mites per bee for the powdered sugar roll, but there is a significant change for the ether roll (p<0.001). The differences in mite counts between ether and sugar roll sampling methods may be due to the relatively small number of bees used to obtain a mite count compared to the total number of mites in a colony. It may be possible to improve mite estimates by sampling those bees more likely to have mites, e.g., nurse bees. Preliminary data indicate that estimates of true mite numbers using powdered sugar rolls can be improved by repeating the powdered sugar roll twice. The total mite number obtained from two powdered sugar rolls of the sample bee sample is 75% of the true number rather than 53% of the true mite number when only one powdered sugar roll was performed. We continue to evaluate stickyboard size, sampling procedures and observer variability to improve the predictive ability of the various sampling methods.

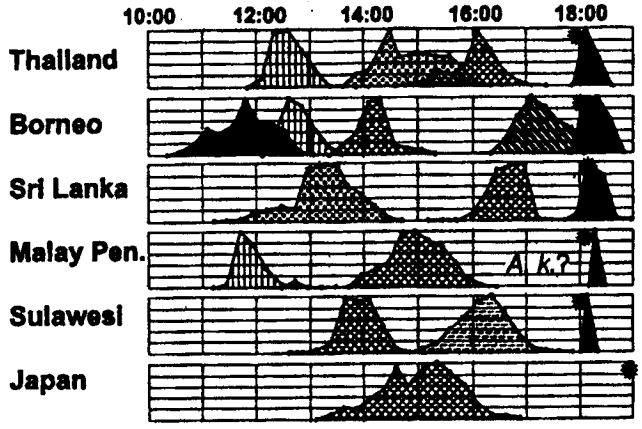
Table	A Comparison of Sampling Methods for Adult Varroa from Adult Honey Bees		
	Ether Roll	Powdered Sugar Roll	p-value
# of mites	20 ±18.7	31±28.8	0.002
# of mites/bee	0.06±0.06	0.1±0.1	0.001
# of mites (roll + alcohol wash) per bee	0.2±0.18	0.1±0.1	<0.001

20. Otis, G. W.[p] – FACILITATED REPRODUCTIVE CHARACTER DISPLACEMENT IN ASIAN HONEY BEES - In Asia, from one to four species of honey bees (genus *Apis*) can live in the same site. Mating flights of different species rarely overlap to any appreciable extent. Only *Apis florea* and *A. cerana* in Thailand are known to have substantially overlapping distributions (*Apis nuluensis* and *A. andreniformis* in Borneo occur at different elevations). There is also great variability in the timing of flights of individual species between sites. For example, the peak of drone flights for *Apis cerana* varies by nearly 3 hours in different localities and seems to be influenced by the assemblage of species present at the locality. Therefore, it appears that the timing

of drone flights is quite plastic, and can be readily molded by selective forces.

The non-overlapping pattern evident in the figure is suggestive of reproductive character displacement (RCD) as defined by Brown & Wilson (1956, *Syst. Zool.* 5:49-64). By their model, selection against the formation of hybrids with reduced fitness (i.e., interspecific matings) would be the selective force that would cause the divergence in reproductive traits, in this case the timing of mating flights. However, by definition, RCD is driven by hybridization, and to date there has not been a single documented natural hybrid between two species of honey bees.

It is more likely that honey bee mating flights are shaped by mating efficiency. Because drones of most (probably all) species are attracted to "queen substance" (9-ODA), interspecific attraction could delay or prevent mating. In the absence of interspecific hybridization as a driving force in honey bees, it is likely that this example constitutes an example of "facilitated RCD" (Howard, 1993. Hybrid Zones and the Evolutionary Process, Oxford Univ. Press), a phenomenon that has been largely overlooked by evolutionary biologists.



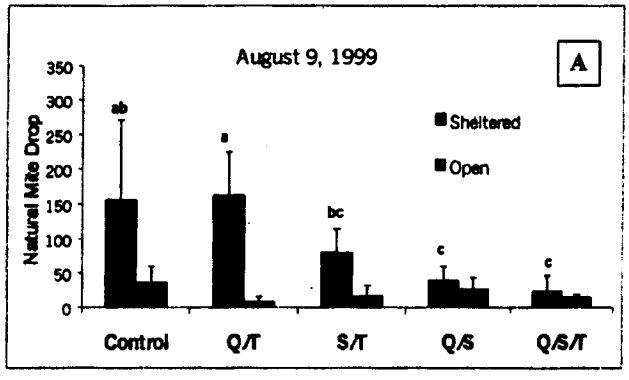
Comparative timing of drone mating flights in six Asian locations (details in Otis *et al.*, in press., Proc. 7th IBRA Internat. Conf. on Apiculture in Tropical Climates). Key to species: *andreniformis*, vertical lines; *florea*, dots; *cerana*, cross hatching; *dorsata*, dark gray; *nuluensis*, light gray; *koschevnikovi*, diagonal lines; *nigrocincta*, horizontal dashes.

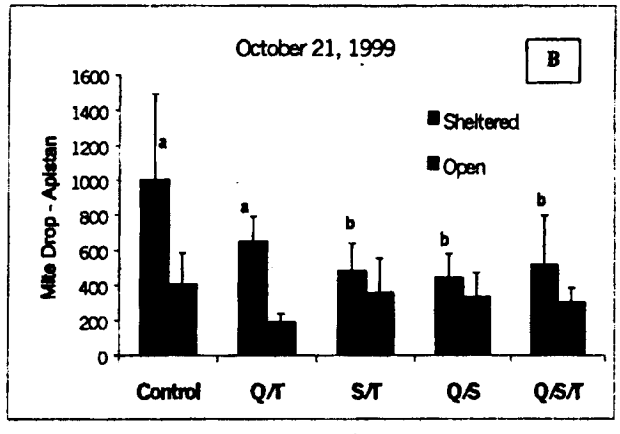
21. Park, A. L.[d], J. S. Pettis[n] & D. M. Caron[d] - MIGRATIONAL CUES OF LARVAL SMALL HIVE BEETLES - The small hive beetle, *Aethina tumida* (Murray), henceforth SHB, is a pest of honey bee colonies in the United States. Both adults and larvae feed on honey, pollen, and brood within colonies. Mature larvae leave colonies to pupate in the surrounding soil, but exhibit an unusual behavior in the process. The majority of larvae in an infested colony typically leave within a short window of time. We have observed this behavior in heavily infested colonies where beetle larvae have massed after feeding. It appears that older mature larvae and larvae that have just attained maturity leave infested colonies at the same time. Anecdotal accounts suggest that rainfall and/or increased relative humidity may be the environmental cue(s) that trigger migration. These are reasonable prospects since larvae are susceptible to desiccation and rain would increase the moisture level of the soil and facilitate burrowing. We decided to test whether SHB larvae would migrate from their feeding site in response to natural fluctuations in rainfall and relative humidity.

Larval migration was examined in the absence of worker bees. Brood frames were removed from colonies, cleared of bees, and placed singly into empty nucleus boxes. Two empty combs were placed on either side of the brood frame to provide additional substrate for beetles and beetle larvae. Ten SHB adults, captured at random from infested colonies, were placed into each nucleus. The entrances were closed with 1/8" screening to prevent scavenging by yellowjackets and bees while allowing movement of SHB larvae. Four such units were created and an additional four were started on each of the following two weeks for a total of 12 units. These units simulated natural conditions where bees have absconded or died and SHB adults scavenge the remains. The units were placed upon bricks within containment moats of detergent that served to prevent ant invasion and which facilitated capture of migrating larvae. Migrating larvae were counted daily and compared to temperature, rainfall, and relative humidity data that were gathered by electronic loggers.

Units were started one week apart to determine whether larvae of different ages would respond to the same migrational cue. This would show that older mature larvae were indeed waiting for a cue and that the synchronous migration was not simply due to mass oviposition by adult females. In the first year that this study was run (August-October, 2000), no appreciable response to rainfall was noticed and little synchrony among migrating larvae was noticed between different weeks. However, synchrony of the four units *within* any given week was very strong. These results were probably due to the lack of any significant amount of precipitation and the low temperatures that arose towards the end of the study. While these were not the results desired, it was clearly demonstrated that larval development is arrested at lower temperatures. Repetition of this experiment (July-September, 2001) revealed little noticeable difference until a major rainstorm in mid-August. The greatest number of larvae throughout the study migrated from 8 units the following day. While data analysis is incomplete, it seems to strongly suggest that either rain and/or the elevated humidity associated with it, was the cue that the SHB larvae could have used to synchronize migration.

22. Sammataro, D.[a], N. Ostiguy,[a] & M. Frazier [a] - RESULTS OF A NOVEL IPM EXPERIMENT TO MANAGE VARROA POPULATIONS IN HONEY BEE COLONIES - A novel combination of IPM techniques were tested to ascertain if *Varroa sp.* populations could be moderated. Fifty colonies in two locations were established in May 1999 in State College, PA. We tested three techniques known to reduce varroa: mite reducing queen stock, screen inserts and T-02® strips (a.i. thymol; G. Wardell, S.A.F.E. Ecological Pest Management, Tucson AZ). The tactics





were combined into five treatment groups (ten colonies each): (1) queens, screens and T-02; (2) queens and T-02; (3) queens and screens; (4) screens and T-02; and (5) control (no treatment).

The screen inserts had a significant independent effect throughout the study. When the T-02 strips were inserted (Day 54), a significant initial knockdown effect was observed in those oil-treated colonies. The queen/screen treatment had a significantly lower mite drop than the other treatment groups by Day 47 and throughout the experiment. There were significant differences between apiary locations (sheltered and open) which may reflect environmental factors. The Open site had smaller mite populations by August 9 than the Sheltered site and no significant treatment effects (see A), even after Apistan drops in October (see B). Mite numbers were lowest in all treatments except the Queen/Thymol strips and Controls at the Sheltered site (A, $p<0.05$). Half the colonies at the Open site faced north, and in August, had a higher mite drop than south facing colonies ($p>0.044$), even after Apistan treatment ($p = 0.042$). Colony health was expressed as number of frames of adult bees. The frame numbers were greater in the S/SW facing hives at the Open site in October ($p=0.002$) and again in April ($p = 0.001$). At the Sheltered site, where mite numbers were higher in all treatment groups, frame numbers in the treatments were higher than the Controls in October 1999, but over winter, the colonies that survived the best were in the Queen/Screen/Thymol group ($p<0.05$).

23. Skinner, J. A.[y], K. Pickens,[y] J. P. Parkman,[y] M. D. Studer[y] & M. T. Windham[y] - A NOVEL APPROACH USING HONEY BEES TO POLLINATE FLOWERING DOGWOODS - The flowering dogwood (*Cornus florida*) is an economically valuable tree for the nursery industry in Tennessee, provides aesthetic beauty for landscapes and serves as a food source to wildlife. Dogwood anthracnose and powdery mildew are two diseases that have caused extensive damage to dogwoods for the past decade. The dogwood cultivar, Cherokee Brave (CB) is resistant to powdery mildew and Appalachian Spring (AS), another cultivar, is resistant to dogwood anthracnose. Cloud Nine (C9) is a dogwood cultivar that is not resistant to either disease. There are no cultivars resistant to both diseases. The purpose of this study was to assist in the production of a resistant hybrid with Appalachian Spring as the female parent and Cherokee Brave as the male parent using honey bees as pollinators.

In Knoxville, TN in spring of 2001, a study was conducted using small colonies of honey bees (*Apis mellifera*) to pollinate three cultivars of flowering dogwood enclosed in screened cages. Ten cages were used to enclose 53 blooming trees of three cultivars as follows: AS X CB, four cages; AS X C9, one cage; CB X C9, 3 cages; C9 alone, 2 cages. Dogwood flowers naturally have very little nectar reward to attract a honey bee. To induce honey bees to visit and reinforce future visits, a "false" nectary was created by adding a droplet of sugar syrup (1.5:1, sugar:water) as a reward to the base of each of the four bracts adjacent to the inflorescence. Each droplet also included 0.003ml queen mandibular pheromone (Fruit Boost, QMP) as an olfactory cue. The flowering period for each cultivar overlapped during the study. Honey bees were observed to move freely between and among flowers of all cultivars in every cage. Appalachian Spring trees in cages containing honey bees and the AS X CB cultivar combination set 175, 201, 90 and 100 fruit (berries), in the four cages, respectively. The AS tree in the cage with bees and the combination AS X C9 produced 137 berries. We feel that the observation of fruit production in the cage that included all Cloud 9 with bees was most likely a result of the accidental entrance of a "foreign bee" that carried pollen from another dogwood cultivar from trees blooming outside the cage. In conclusion, honey bees were successfully utilized to pollinate dogwoods and assist in production of a hybrid dogwood from Appalachian Spring that we hope will be resistant to dogwood anthracnose and powdery mildew.

24. Spivak, M.[j], G. Reuter [j] – COMPARISON OF HYGIENIC, SMR, RUSSIAN AND ITALIAN HONEY BEES IN A COMMERCIAL APIARY– We compared four stocks of honey bees for relative mite resistance and honey production. Hygienic and SMR (Suppression of Mite Reproduction) refer to heritable traits that can be bred into any stock or line of bees. Bees were bred for hygienic behavior at the University of Minnesota from Italian-derived commercial stock. Bees were bred for SMR at the USDA Bee Research lab by Dr. John Harbo from a local stock of commercial bees. Russian bees were imported by the USDA Baton Rouge Laboratory, and are a true line of bees. Italian bees were from commercial stock preferred by some US commercial beekeepers. Queens from each of the sources were reared in March and mated naturally with the same pool of drones in Mississippi. Colonies were transported to Minnesota in May, and comparisons of mite infestations were made in early June and late September 2000. Honey production was measured in late August.

Mites on adult bees were calculated by sampling 600-800 bees in an alcohol-wash. Mites in worker brood were calculated by inspecting 200 cells containing purple-eye pupae. Honey production was measured by weighing supers and subtracting the tare weight.

In June, the number of mites per 100 bees and the percent of mites in worker brood were under 1% in all colonies from all sources, and there were no significant differences between them. In September, the Russian and SMR colonies had significantly fewer mites on adults than the hygienic and Italian colonies (Table). The SMR colonies had significantly fewer mites in worker brood than the hygienic and Italians. The Russian colonies had intermediate levels of mites in brood, and produced significantly less honey than the other colonies (on average, 37 lbs less).

These preliminary results suggest that bees bred for SMR and the Russian line are more mite resistant than bees bred for hygienic behavior and commercial Italian colonies. The hygienic colonies, as in previous published experiments, had the lowest infestation levels only when mite infestation pressure was low. The colonies were wintered in Minnesota, and the experiment will continue in 2001.

Bee Source	Mites per 100 adult bees	% mites in worker brood
Italian	8.8 ± 6.17 a	29.6 ± 19.33 a
Hygienic	8.9 ± 6.11 a	31.3 ± 18.80 a
Russian	4.8 ± 3.61 b	22.9 ± 10.40 ab
SMR	3.2 ± 1.58 b	13.9 ± 4.80 b

Comparison of colonies in late September 2000 in Minnesota. Different letters following means ± standard deviations within columns signify significant differences between bee sources at $P < 0.05$ (two-way ANOVA, comparing effect of bee source and apiary; apiary effect not shown here)

25. Spivak, M.[j], R. Ross,[j] & K. Gramacho [j] – OLFACTORY SENSITIVITY OF HYGIENIC HONEY BEES PERFORMING UNCAPPING AND REMOVAL BEHAVIORS - Hygienic behavior comprises two behavioral components or subtasks: (1) uncapping and (2) removal of diseased and *Varroa*-infested brood. In a previous study (Arathi & Spivak, 2001 *Anim. Behav.* 62: 57-66) in which colonies were composed of 50% and 100% hygienic bees, some bees performed the subtask of uncapping cells at higher frequencies than the subtask of removing cell contents. We hypothesize that bees which tend to specialize on uncapping may have greater olfactory sensitivity (and lower response thresholds) than bees which display a higher frequency of removal behavior. Bees with greater olfactory sensitivity would detect and then uncap diseased or parasitized brood more readily than bees with lower sensitivity.

From two observation hives containing different colonies selected for hygienic behavior, we collected individual bees either uncapping (poking a hole through a cell capping) or removing (tugging a dead pupae from the cell) freeze-killed brood. Collected bees were tested first for their ability to discriminate between the odor of chalkbrood and healthy pupae using proboscis-extension

reflex (PER) conditioning. The bees were next tested for their ability to perceive different concentrations of the odor of chalkbrood extracted in hexane by electroantennogram (EAG) recordings. The results of the PER experiment indicated that both the uncappers and removers learned to discriminate between the odors by the eighth presentation of each odor, but the removers made significantly more mistakes (generalized between the odors) in the first several presentations by extending their proboscises to both rewarded and punished odors. The uncappers did not generalize as much between the odors. The EAG recordings demonstrated that uncappers had significantly greater olfactory responses (greater sensitivity) than the removers to the concentrations of 0.1 and 0.5 chalkbrood equivalents.

In addition, we conducted a pin-killed brood assay for hygienic behavior in the same colonies by poking a #1 insect pin through the cell capping and piercing the pupa through to the bottom of the cell. We then collected bees that were first to expand the hole in the cell capping made by the pin. EAG recordings of these bees indicated that they had significantly lower olfactory sensitivity than the uncappers of freeze-killed brood, but similar sensitivity as the removers. The data will be published elsewhere, but the practical application may be that selection of hygienic bees using the pin-killed brood assay may not select for the bees with greatest olfactory sensitivity. We recommend that the pin-killed brood assay be used only to initially screen colonies for hygienic behavior. A freeze-killed brood assay can then be used to select among colonies with the fastest removal rates.

26. Webster, T. C.[t], F. V. Vorisek[t] & E. M. Thacker [t] – DO FERAL HONEY BEES CARRY TRAITS FOR VARROA MITE RESISTANCE? - Honey bee colonies which had been surviving in Kentucky for at least three years without acaricide treatments were collected in 2000 and 2001, with the hope that they would carry heritable resistance to the mite *Varroa destructor*. The hives and the periods in which they did not receive acaricides were reported by cooperating beekeepers. The colonies had been living in buildings, trees, logs, and unmanaged hives. They were transferred to standard Langstroth equipment before evaluations. These candidate colonies were compared to colonies derived from bees bred by commercial queen producers in the U.S.

Each colony was evaluated according to two criteria that may indicate heritable resistance to the mite. The first was the proportion of mites fallen to the bottom board which were mutilated. This was determined by an examination of mites adhered to a sticky board after it had been placed on the hive bottom board for seven days. Mites with damaged legs or carapace were easily seen with a microscope at 10X.

The second criteria was the proportion of female mites in bee brood cells which were not reproducing. Capped worker pupae at the pink-eye stage of development or older were removed from their cells. Mature mites without offspring in those cells were considered non-reproductive.

Compared to the commercial stock bees, the candidate colonies tended to have more mutilated mites on the bottom board and more female mites which are non-reproductive in brood cells, although the data collected to date do not allow conclusive statistical tests. The proportion of mutilated mites from the candidate hives ranged from 10.9% to 31.1% (n = 6 hives). Mutilated mites fallen from the commercial stock colonies ranged 1.5% to 16.7% (n = 7). Female mites not reproducing in brood cells ranged from 33.3% to 93.3% (n = 4) in the candidate hives. In the commercial stock, mites not reproducing ranged from 22.6% to 40.0% (n = 3). If further data show statistically significant differences between candidate colonies and commercial stock we may consider a breeding program devoted to mite resistance and other desirable traits.

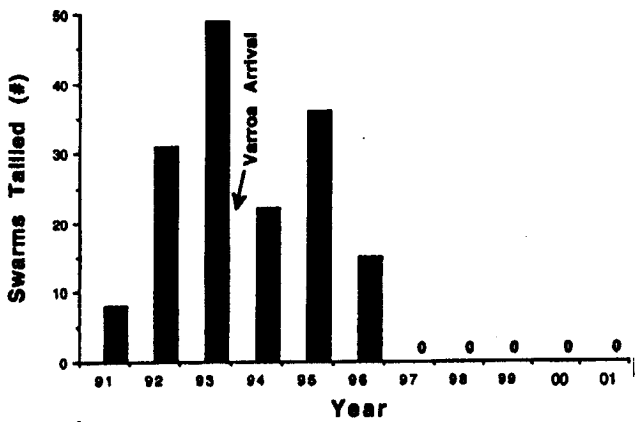
27. Wenner, A.[u] & R. W. Thorp[x] – VARROA IMPACT ON FERAL COLONIES: ISLAND *VS.* MAINLAND – In Santa Barbara County, within and near vast wilderness and national forest areas where no beekeeping occurs, the resurgence of feral honey bee colonies after *Varroa* arrival has been quite dramatic. Documented cases of several-year survival of feral colonies now exist, and swarming incidence has increased each year in that region (e.g., Wenner, 1999, *Am. Bee J.* 139:658).

The western Cosumnes River area and Mt. Diablo State Park in central California have also experienced repopulation by feral colonies, where none could be found earlier (Wenner & Thorp, 2000 *Am. Bee J.* 140: 746). Others who have studied feral bee colonies near remote areas have reported a similar increase in colony persistence (e.g. Loper, 2000 *Am. Bee J.* 140: 744).

A deliberate introduction of varroa mites in mid-winter of 1993-94 into the eastern half of an isolated 25,000 hectare ecosystem (Santa Cruz Island, California) permitted study of elapsed time before feral colonies collapsed. None of 117 monitored colonies perished from mite infestation within two years after inoculation, but did succumb during the following two years (with only about a 6-month delay for colony demise in the western half of the island – where mites had not been introduced).

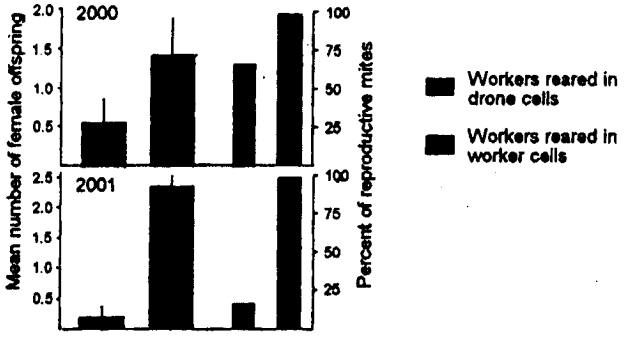
Nearly eight years after varroa onset, apparently only two unmonitored island colonies survive. We caught swarms on the island during three seasons after varroa introduction, but no swarms subsequently (see figure).

The difference in mainland and island feral colony survival after varroa onset appears significant. Whereas remote areas on the nearby mainland have feral colonies with mixed parentages, island bees possess a narrow genome (R. Page, personal communication). Colonies of varied genetic mixture thus might prove more promising candidates than more uniform lines in efforts to find varroa resistant stock. Accordingly, in late June of 2001, P. Cronshaw and A. M. Wenner shipped 10 queens from never treated feral colonies to J. Tew and G. Otis to be included in a study of how well bees from those queens could fare in a different part of the U.S.



Lack of feral swarm capture following impact of varroa mites on the island.

28. Zhou, T.[v], J. Yao[v], S. X. Huang[v], & Z. Y. Huang[w] - LARGER CELL SIZE REDUCES VARROA MITE REPRODUCTION - In a study trying to determine the mechanisms of why varroa mites do not reproduce on worker brood of *Apis cerana* (the Asian hive bee), we accidentally discovered that both *Apis cerana* and *Apis mellifera* queens lay worker eggs in drone cells in the fall. We took advantage of this and compared the reproductive output of mites on two hosts: workers reared in worker-cells (WW) or workers reared in drone-cells (WD). We selected recently sealed (within 6 hours) brood cells as transfer hosts. We obtained phoretic mites from adult workers and transferred them into brood cells with a paint brush after each cell was opened with a small pin. The opening was immediately sealed with melted beeswax after mite introduction. The brood frames were incubated at 35°C for 9 days after which each cell was opened and mite progeny scored. In 2000, mites introduced into WD showed differences to WW in both the percentage of mites that reproduced and the average number of offspring, but the differences were not statistically significant (mean offspring number:



Mean number of female offspring and percent of mites that reproduced on worker brood reared in different cell types. Experiments were conducted in 2000 and 2001 in Beijing, China.

t = 1.6, P = 0.12, % reproduction: X^2 = 3.59, P = 0.058). This is most likely due to the small sample sizes (N=7 and 13 for WW and WD, respectively). In 2001, with larger samples sizes (N = 47 and 29), we found that only 17% (5 out of 29) of mites reproduced on WD, while 100% (47 out of 47) of them reproduced on WW (X^2 = 45.7, P < 0.0001). Among these mites that reproduced, they also had less reproductive output: the mean number of female mite offspring was 0.20 ± 0.2 (mean ± SE) for WD, but 2.38 ± 0.2 for WW (t = 3.87, P < 0.001).

To our knowledge, this is the first study to show that varroa mite reproduction can be affected by cell size of the host. It is not clear why mites would reproduce less on identical hosts that are housed in larger cells. One possibility is that workers reared in drone cells are fed a different diet by nurses. A second, more plausible, mechanism is that workers spin larger cocoons in drone cells and mites detect this "geographic" change and somehow change their reproductive behavior accordingly.

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