Special Feature

Proceedings of the American Bee Research Conference

The 1989 American Bee Research Conference was held at the Hoblitzelle Center of the Texas A & M Agricultural Experiment Station in Weslaco, Texas on October 3 and 4, 1989. A Tracheal Mite Symposium was held at the same place on October 1 and 2, and a few of the participants in that symposium chose to publish their abstracts with those of the research conference. Abstracts from the mite symposium are noted as such. Complete articles from the Tracheal Mite Symposium will be published by OneBetter Publishing (watch for details in bee journals).

The fifth American Bee Research Conference will be held in Tucson on October 1 and 2, 1990. The following are abstracts from the 1989 conference.

1. Clark, K. J.[a] D. L. Nelson,[b] and D. McKenna[c] – EFFECT OF MENTHOL ON QUEEN REARING – The effect of three dosages of menthol was investigated during the rearing of queens in northern Alberta in June and July, 1989. Twenty-five mating nucs in each of four treatments received 0, 10, 20 and 40 grams of menthol pellets in 15 cm. square, 2 mm. nylon mesh packets placed on their top bars. Queen cells within 2 days of emergence were installed in the center of each nuc at the same time that menthol was applied. The menthol packets were weighed 3 times over a treatment period of 17 days. During the first week, the weight of menthol which evaporated in each treatment was approximately 2.7, 3.8 and 5.3 grams, respectively. Following the treatment, the queens were tagged, caged and introduced into small colonies which were allowed to build up for the rest of the summer.

The table shows the number of queens surviving each treatment as recorded on day 7 (emergence in mating nuc), day 16 (egg laying in mating nuc), day 26 (acceptance in small colony) and day 33 (egg laying in small colony). In the group which received no menthol, queens emerged from 80% of the cells, and the number of queens accepted and laying after transfer to the small colonies was 76% of the number of installed cells. In the three menthol treatment groups, live queens emerged and were present at day 7 in only 48 to 56% of the nucs, while the number of queens accepted and laying in the small colonies was between 48 and 28% of the number of installed cells (see Table).

Most of the reduction in success occurred in the first week after installation of the cells, and appeared to result from lack of emergence from the cell or destruction of the cell by worker bees. Bees involved in the study had no tracheal mites. (Presented at the mite symposium)

Table – Number of queens surviving from 25 cells installed per treatment.

Dose (gm. Menthol)	Day 7	Day 16	Day 26	Day 33
0	20	19	19	19
10	13	10	8	7
20	14	13	12	12
40	12	7	7	7

2. Coelho, J. R.[d] – THE EFFECT OF THORAX TEMPERATURE AND BODY SIZE ON FLIGHT SPEED IN HONEY BEE DRONES – The temperature of the thorax (T_{th}) of a flying insect is believed to be an important determinant of its flight performance because increases in T_{th} improve flight muscle function (up to a limit). However, few studies have actually demonstrated the effect of T_{th} on any aspect of flight performance itself. Honey bee drones consistently have a T_{th} ~3°C higher than workers; therefore, it might be concluded that this results in faster, more agile flight, which would presumably result in greater success during aerial mating attempts. Drones are approximately twice the size of workers, which should also result in more rapid flight for drones. This study examined the effects of T_{th} and size on flight velocity (V) in an attempt to test these ideas.

Individual drones were captured while returning to the hive, taken ~30 m from the hive, marked with paint and

released. One investigator measured V with the use of K-ban Doppler radar, while another captured the drone as it again returned to the hive, measured T_{th} with a digital thermocouple thermometer, and stored the drone for later morphometric analyses. The percentage of drones which returned to the hive was improved from 23% to 80% when a single drone was captured, quickly taken away from the hive, marked and released, as opposed to capturing many drones, taking them away and releasing them one at a time. Rapid recovery of drones was improved when the first investigator held the drone aloft, rotated it slowly in a complete circle, then released it in the direction of the hive. Data were taken only for drones that returned within ~15 s in order to minimize potential changes in T_{th} which may have occurred since V was measured.

V (meters per second) was related to T_{th} (°C) according to the following equation: $V = 0.287(T_{th}) - 5.86$, $r^2 = 0.21$. V was also related to thorax mass (M_{th}), an index of body size: $V = 61.7\ (M_{th}) + 0.367$, $r^2 = 0.15$. However, M_{th} and T_{th} were correlated ($T_{th} = 101\ (M_{th}) + 31.4$, $r^2 = 0.16$). Multiple regression analysis including all three variables showed that T_{th} was significantly related to V ($P < .05$), but M_{th} was not ($P > .05$): $V = 38.8\ (M_{th}) + 0.226\ (T_{th}) - 6.7$, $r^2 = 0.26$. V was not related to wing loading ($r^2 = 0.05$). Mean V for drones (5.37 ± 0.93 m/s, n = 184) was significantly different from that of workers (5.90 ± 0.57 m/s, n = 130, $p < 0.0001$). Mean V of nectar-loaded workers was 5.58 ± 1.22 m/s (n = 48), while that for unloaded workers was significantly higher, 6.08 ± 0.95 m/s ($n = 82$, $p < 0.0001$).

The rate of physiological processes generally increases two- to three-fold with a 10 degree Celsius increase in temperature (Q_{10} = 2 to 3). Since metabolic rate in drones increases with T_{th} with a Q_{10} of 2.1, one might expect a similar effect on V. The Q_{10} of V in drones in this study was 1.69. The increased aerodynamic drag associated with higher V may result in a lower Q_{10}. In free-flying sheep blowflies, V increased linearly with T_{th}, and Q_{10} ranges from 1.23 to 1.33 (Yurkiewicz & Smyth, *J. Insect Physiol.* 12:189-194), perhaps indicating that V in endothermic insects (such as bees) is more temperature sensitive than in ectothermic insects (such as flies).

T_{th} has a positive effect on V of drones, at least within the range of T_{th} observed. Furthermore, T_{th} is more important than size in determining V. These results underscore the significance of thermoregulatory strategy in establishing flight performance. Greater size only augments V because it results in higher T_{th}. Thus, because drones have greater size and T_{th}, one would predict that they should be faster flyers than workers. The fact that this is not the case indicates that drones have not evolved large body size and high T_{th} as a means of improving V. It is possible that T_{th} is still important for competition among drones during mating attempts. A hotter drone may reach the queen faster than a cooler drone by virtue of more rapid flight and, consequently, succeed in copulation.

3. Collins, A. M.[e] — CONSIDERATIONS ON BREEDING HONEY BEES RESISTANT TO MITES — Chemical treatments for tracheal mites are being developed and used with some success (Cox *et al.*, *Amer. Bee J.* 129:129-131; Burgett and Stringer; *Gleanings* 117:522-524). However, the likelihood is that mites will be able to develop resistance to these materials. In the long term, the most effective solution for mite control is to breed honey bees that are resistant to tracheal mites.

For any success with a breeding program, it is first necessary to have variation, phenotypic as well as genotypic, and to be able to measure it in a biologically significant way. Gary and Page (*Exper. & Applied Acarol.* 3:291-305) reported that phenotypic variation in the level of infestation of tracheae does exist. They went on to select for resistance in their highly variable population and quickly (in few generations) achieved some success (personal communication), not an unusual occurrence in honey bee selection. Certainly the reports from beekeepers of the variable effects in individual apiaries, where some colonies will be devastated and others only mildly infested by mites, show that sufficient stock differences do occur in the U.S. To look at the results of this past year's heavy losses in a positive light, we can say that surviving colonies represent a pool of naturally selected raw material readily available to us for further controlled selection.

Additionally, scientists are in the process of importing resistant stocks from abroad to take advantage of selection that has already been done. These include stocks from Great Britain where long exposure to the parasites may have selected phenotypes that are resistant (Morse, personal communication). Rinderer and Kulincevic have done several generations of controlled selection for *Varroa* resistance and are now transferring stocks from Yugoslavia (Kulincevic, *et al.*, *Apidologie* in press).

As with any honey bee selection program, care must be taken to begin with populations of sufficient variation that inbreeding depression does not counteract any selection success. The closed population breeding system of Page *et al.* (*Amer. Bee J.* 122:350-355; see also Lawrence and Coby, *Amer. Bee J.* 25:687-688, Severson *et al.*, *Amer. Bee J.* 126:93-94) is a good model for any prospective breeders. Also line breeding as used in the past (Mackenson and Nye, *J. Apic. Res.*, 5:79-86; Nye and Mackenson, *J. Apic. Res.* 9:61:64) will continue to prove effective, but requires more care and skill. The emerging field of biotechnology deserves some mention also. It has the potential for allowing us to tap the resistance mechanisms present in the original host species of honey bees and transfer them to *Apis mellifera*.

The ease with which any such selection program can be done is also dependent on the ways in which the level of resistance is measured. At the current time, we are still limited to dissection and visual counting of dead or living mites for clear indications of infestations. This is extremely labor intensive and limits our efforts. I am pleased to see that some of the papers to be presented in this symposium are addressing potential assay techniques to speed our characterization of colony status. (Presented at the mite symposium)

4. Collins, A. M.,[e] H. V. Daly,[f] T. E. Rinderer,[g] and J. R. Harbo[g] — CORRELATIONS BETWEEN IDENTIFICATION AND DEFENSIVE BEHAVIOR TRAITS — Because of difficulties in visually identifying Africanized honey bees in the field, the suggestion has been made that defensive behavior might be a suitable character for preliminary identification (Spivak *et al.*, in "Africanized Honey Bees and Bee Mites," Needham *et al.*, eds., pp. 313-324). Currently the only widely accepted identification of Africanized bees is the body measurement (morphometric) system of Daly and Balling, *J. Kansas Entomol. Soc.* 51:857-869. If significant correlations exist between these measures and defensive behavior, the vigor with which a colony defends itself would be an appropriate way to choose possible Africanized colonies.

In 1979 two populations of honey bees, 150 colonies in Louisiana, USA, and 148 colonies in Monagas, Venezuela, were measured for defensive behavior (Collins and Kubasek, *Ann. Entomol. Soc. Amer.* 75:383-387), alarm pheromones (Collins *et al.*, *J. Chem. Ecol.* 15:1747-1756), and body size (morphometric identification). Using a refined morphometrics database (Buco, unpublished), the Louisiana bees were identified as all European and the Venezuelan bees were identified as European, Africanized, hybrid and questionable. Using the pooled values from both populations, correlations were determined for the 25 morphometric measures

with the 7 defense measures and the 12 pheromones. Values in the table indicate that some defensive behavior traits had significant correlations with the morphometric measurements and could usefully be employed for quick identification purposes.

Table – Correlation Coefficients Between Various Traits[1]

	Morphometric Measures				
	Forewing Length	Forewing Width	Hindwing Length	Femur Length	Angle 34
Defense measures					
No. stings	-0.393	-0.548	-0.186	-0.308	-0.318
Time to react to:					
pheromone	0.376	0.448	0.188	0.387	0.219
target	0.318	0.492	0.128*	0.324	0.230
No. of bees at 60 s	-0.201	-0.310	-0.106ns	-0.200	-0.179
Pheromone production level					
hexyl acetate	-0.647	-0.619	-0.485	-0.595	-0.177
heptanol	-0.553	-0.506	-0.421	-0.538	-0.072ns
nonanol	-0.573	-0.552	-0.442	-0.532	-0.127*
isopentyl acetate	-0.024ns	-0.010ns	0.018ns	-0.014ns	0.078ns
2 heptanone	-0.001ns	0.104ns	-0.058ns	0.043ns	-0.017ns

1 – All traits except isopentyl acetate and 2 heptanone production were significantly different for the two populations.

All values are significant at $P < .01$ except * = $P < .05$ and ns = not significant.

5. Danka, R. G.,[g] J. L. Williams,[g] and T. E. Rinderer[g] – ACEPHATE BAITING TECHNOLOGY: REFINEMENTS AND PRELIMINARY FIELD TESTS[ff] – Initial development of a baiting system designed to eradicate undesirable honey bee colonies was reported previously (Williams *et al.*, *Apidologie* 20: 175-179). In initial tests colonies were destroyed after foragers collected sucrose-honey solution containing 250 ppm acephate from feeding stations located 10 m away. More recent tests examined the effect of treatment distance, levels of insecticide residues in treated colonies, and practical aspects of eradicating populations of feral colonies.

Each of 12 colonies treated from 500 m with 500 ppm acephate were successfully destroyed (see Table). In addition, treatment distances of up to ca. 700 m did not limit successful treatments during field tests (see Table).

Residues of acephate and its metabolite methamidophos were quantified by gas chromatogaphy after extraction from dead bees and food-storage comb (*i.e.*, a mixture of wax and honey, syrup or nectar). Samples were collected from 5 combs in each of 5 colonies treated with 500 ppm acephate at 500 m. In dead bees, acephate peaked at ca. 10 ppm 1 day after treatment and dropped to 2 ppm after 10 weeks. Methamidophos levels were at 2-3 ppm throughout the sampling period. In comb samples, acephate peaked at ca. 1.2 ppm and methamidophos at ca. 0.1 ppm; ca. 50% of these levels were present after 10 weeks. The low residue levels probably represent a minimal environmental hazard, especially since an average of only 31 mg of acephate was collected during all treatments (see Table.)

Four sequential replications of a simulated eradication program were conducted on an isolated barrier island (Grand Terre) in southeast Louisiana. Simulated feral colonies were moved into randomized positions on the island; densities ranged from ca. 1-7 colonies/sq km. Baits typically were placed at 500-m-grid intersections, and treatments with 500 ppm acephate ranged from 88 to 691 m. During the first three replications, 12 of the 13 colonies that were treated (and 12 of 14 overall) were eradicated (see Table). In the fourth replication, colony density was high, leading at times to multiple colonies per feeder; there was also an increasing nectar flow. At least 7 colonies were known to be treated, and 9 died (9 of 15 overall). The system thus was almost always successful when treatments were made to individual colonies that were foraging actively. Difficulties arose, however, when foraging activity at bait stations was inadequate because of a nectar flow, poor flight conditions, or multiple colonies per feeder.

Table – Responses of honey bee colonies treated with 500 ppm acephate. Colonies were baited to feeding stations that delivered sucrose-honey syrup. When foraging activity was acceptably high (>100 bees per feeder), plain syrup was replaced with acephate-treated syrup. Tests at Baton Rouge, Louisiana, evaluated baiting performance at a longer distance than was previously attempted. Tests on Grand Terre Island, Louisiana, simulated an eradication program.

Site	Rep.	Treatment distance, m (x̄, range)	Total No.	No. treated	No. died	Max. no. foragers on feeder during treatment	ml treated syrup collected	mg acephate collected
Baton Rouge		500	12	12	12	205 ± 67	67 ± 23	34 ± 12
Grand Terre	I	154 (88-198)	6	5	5	152 ± 90	37 ± 24	19 ± 12
	II	132 (88-198)	5	5	5	178 ± 84	54 ± 10	27 ± 5
	III	477 (265-691)	3	3	2	165 ± 44	63 ± 17	32 ± 9
	IV	376 (182-566)	15	>7[1]	9	173 ± 56[2]	56 ± 16[2]	28 ± 8

1. Multiple colonies were known to be treated from one feeder.
2. n = 3

6. Davidson, F.,[h] T. Udagawa,[i] N. Lakey[h] and G. B. Kitto[h] – HONEY BEE IDENTIFICATION: CHARACTERIZATION OF THREE PROTEINS SPECIFIC TO AFRICANIZED BEES – The arrival of africanized honeybees (AHB) in the U. S. will necessitate rapid, economical methods for their detection. Antibodies, directed against AHB-specific proteins, can be used in ELISA and dipstick-type immunological assays and provide a rapid, highly specific and economical methodology. We describe here the characterization of three proteins which appear to be unique to africanized honeybees.

Using pH 5-7 non-denaturing isoelectric focusing in polyacrylamide gels, we have identified three proteins (A1, A2 and B1) which are restricted to AHB. Proteins A1 and A2 have isoelectric points (pI) of 5.51 and 5.49, respectively. Protein B1 has a pI = 5.11 while protein B2 (found in all european honeybees and in ca. 50% of all AHB) has a pI (5.09) very similar to but distinct from that of B1. We have screened 16 different sources of AHB from Mexico, Honduras, Costa Rica, Venezuela and Brazil; A1 is found in 60% of the populations, A2 in 24% and B1 in 45%. Seven per cent of the AHB populations have neither A1 nor A2 nor B1. We have not found AHB-specific proteins in any of eight european bee samples we have tested. Using antibodies directed against the three AHB-specific proteins, we anticipate we will be able to detect africanized honey bees in approximately 93% of bonafide AHB samples.

The AHB specific proteins are found in the thoraces of africanized honey bees but not in the abdomens or heads. To further characterize the subcellular distribution of AHB-specific proteins, 50 AHB thoraces were pooled and the mitochondria isolated by differential centrifugation: the AHB-specific proteins were found only in the cytoplasmic and not the mitochondrial fraction. Since several proteins can have the same isoelectric point, IEF gels of AHB samples were stained with Coomassie Blue and the A1, A2 and B1 bands cut out. The excised bands were then electrophoresed on slab 10% SDS-PAGE gels. A single major band corresponding to each of the AHB-specific proteins was found on

the SDS-PAGE gel. The molecular weights of A1 and A2 appear to be identical (ca. 43,000 daltons). The molecular weights of B1 and B2 are also identical and are also approximately 43,000 daltons. We are currently doing amino acid composition and sequence studies to further elucidate structural relationships among the four proteins. We are also raising antibodies against each of the four proteins for use in ELISA immunoassays.

7. Dawicke, B. L.,[j] G. W. Otis,[j] and C. D. Scott-Dupree[j] — PREDICTING TRACHEAL MITE INFESTATIONS AND EFFECTS ON COLONIES — Economic damage from the honey bee tracheal mite in the form of decreased honey yields (Eischen, F. *et al., Apidology* 20:1-8) and increased winter mortality (Otis, G. W. *et al.*, 1986, Proceedings of the Honey Bee Tracheal Mite Scientific Symposium, St. Paul, MN) has forced beekeepers to seek out methods of control against the mite. The expense of these treatments necessitates knowledge of optimum treatment times which could be based on trends of mite prevalence within colonies.

Using data from four different years (1985-1989) and four different locations we examined correlations of mite prevalence from one month to the mite prevalence of another month within that same year. We also correlated the mite prevalence of a given colony for a given month to the brood area of that colony found in the spring (May).

Beginning with the month to month correlations from the four years of data, we noted general trends. These trends are represented by the data of 1988-1989. The August 1988 mite prevalence was highly correlated with that of September, ($r = .90$, $p < .001$) and November 1988, ($r = .78$, $p < .001$). However, the correlations with the subsequent months tended to decline and became negligible by May 1989. The month of January appeared to be a crucial one in that it correlated with all of the months, including the previous summer and the following spring. The May 1989 correlations re-emphasized a lack of relationship between summer or late summer mite prevalence levels with mite prevalence levels of the following spring.

The correlations indicate that the mite prevalence of months which closely follow each other are highly significant but the mite prevalence correlations decline as the span between months increases. This decline prevents the use of summer mite prevalence values as predictors of spring trends.

The trends for the correlations of mite prevalence to spring brood area were not consistent from year to year. In 1985-1986 we found that no relationship of mite prevalence with brood area existed until into the winter months; January, February, March and April. For 1986-1987 the pattern of correlations was different. The significant relations began earlier and continued into the winter; October, December and March. The data for 1987-1988 was similar to the previous year with October, November and March mite prevalence values being significant with the spring brood area.

The mite prevalence values when correlated to spring brood area indicate that a relationship exists between mite prevalence of colonies in the fall and winter with spring brood area. However, the correlation trends for this method are not consistent from year to year and the low correlation values suggest other variables are more important for influencing spring brood.

In conclusion, the magnitude of changes of mite infestation which occur during the fall and winter make summer and early fall measurements not useful as predictors for treatment. (Presented at the mite symposium)

8. Delfinado-Baker, M.[k] — INTRODUCING OTHER TRACHEAL MITES: *LOCUSTACARUS BUCHNERI, L. TRACHEALIS* AND *L. MASONI* (PODAPOLIPIDAE: ACARI) — This is a review of parasitic mites related to Tarsonemidae *(Acarapis)* that infest the tracheal system of insects other than honey bees *(Apis)*. Within the superfamily Tarsonemoidea are two families, Tarsonemidae and Podapolipidae, that contain true parasites of insects having a great diversity in living habits and of structure of the body, legs and mouthparts. Included in this group are tracheal parasites of the genera *Acarapis* (Tarsonemidae) and *Locustacarus* (Podapolipidae). They live and multiply in the tracheal system, injuring their hosts by piercing the walls of tracheae to feed on haemolymph and hindering respiration by blocking the tracheae. The importance of these mites is related to agriculture. Among the tarsonemids, only the honey bee tracheal mite, *Acarapis woodi*, is considered of importance economically. This species infests adult honey bees, *Apis cerana* and *A. dorsata* in Asia, and *A. mellifera* worldwide. The possible importance of podapolipids is largely unknown. All podapolipids are internal or external parasites of several insect groups. They occur in the vaginal membranes and oviduct sacs of beetles, in the tracheal air sacs of bees and orthopterans, under the elytra of beetles, and on the wings and bodies of locusts, grasshoppers and cockroaches. The tracheal parasites *Locustacarus buchneri* infest bees of the genera *Bombus* and *Psithyrus* in Europe and North America, while *L. trachealis* and *L. masoni* infest acridiids (locusts and grasshoppers) in the U.S., Africa, New Zealand and Australia. They are considered to be more harmful to the host than are external parasites. Mites may be recovered in the tracheal air sacs in the abdomen of the host by making an incision between two sternites (IIS-IIIS) and pulling them apart to expose the sacs. Infested air sacs appear dark brown. Frequently mites are found in the posterior section of the air sacs. (Presented at the mite symposium)

9. Diaz-y-de-la-Garza, C.,[l] W. L. Rubink,[e] and W. T. Wilson[e] — FOLKLORE AND MAGIC IN RUSTIC APICULTURE OF TAMAULIPAS, MEXICO — The majority of the rustic beekeepers are primarily uneducated senior citizens in rural areas where the per capital income is low. Their knowledge of the biological processes of honey bees is small to nonexistent. Family tradition has preserved extensive and varied folklore and superstition regarding honey bees and their husbandry. Also, honey is used in combination with herbs for traditional magic healing medicines sold in "Yerberias" (herb apothecary) in Texas along the Rio Grande Border and throughout most of Mexico. These medicines are used by "Curanderos" and "Shamans" (good witch doctors). A good example of medicinal herbs is "Ajo" (garlic) mixed with honey. It is used for dandruff and applied to scorpion bites.

Some of the rustic beekeepers believe that bees are endowed with senses much keener than man and also with human intelligence. Because bees were felt to be in sympathy with a family, it is a custom for them to tell the bees about marriages, births, and deaths that were taking place in the home.

Since none of the beekeepers surveyed owned veils or tools, a widely believed superstition, and one that will be rather dangerous with the incoming Africanized bees, is that bees will not sting a person if he holds his breath or clenches his fist (the fact that a bee may not sting at times under such a circumstance is probably because a person is apt to be more quiet and less disturbing to the bee). Most other superstitions compiled along that line can be easily explained the same way (de Lys, *Philosophical Library*, 45-50).

The following beliefs are stranger and not so easily explained:

- A girl that is a virgin can go through a swarm of bees without being stung.

- Bees flying into a house means a visitor is coming.
- When bees swarm on a dead tree there will be a death in the family.

Since capturing swarms represents the principal means of establishing new colonies, myths also surround that practice:

- To make a flying swarm settle down, make loud noise by beating pans or ringing cow bells.
- Urinating in a rustic bee hive (box or hollowed out tree trunk) will attract bees.

Most of the superstitions and myths regarding beekeeping in Tamaulipas are not from literature or native to Mexico. They are of European origin, part of the legacy that Spaniards brought to the new world when they also introduced the European honey bee *(Apis mellifera)* in the 16th century.

Native Mexican indians had extensive folklore associated with stingless bees (*Melipona beecheii* and *M. favosa*), the Toltecs and Aztecs, mainly in the central plateau of Mexico (Byer, *University of Texas Press*, 280-283), and the Mayas in the Yucatan peninsula and Central America (Sepulveda, *Editorial Everest*, 26-28).

10. Dietz, A.,[m] J. F. Leitner,[n] C. Vergara,[m] and M. Mejia[m] – EFFECT OF PROLONGED CONFINEMENT IN A REFRIGERATION CHAMBER ON THE SURVIVAL OF AFRICANIZED AND EUROPEAN HONEY BEE COLONIES – A comparative study was conducted from June 9 to Sept. 9, 1985 in Nogoyá, Entre Rios, Argentina to determine the survival of 6 Africanized and 6 European honey bee colonies under constant low-temperature conditions. The method employed was similar to the one used in our 1984 survival study (Dietz *et al.* pp. 237-42, In: Needham *et al.* eds, 1988, AHB and Bee Mites). The only modification was the replacement of the glass inner cover with a masonite board. This modification prevented condensation inside of the colonies. The board was replaced temporarily with a piece of glass during the regular inspection periods.

The results showed that there was no significant difference between the rates of survival of confined Africanized and European honey bee colonies. A total of four colonies, two Africanized and two European, died during the 91 day study period. One colony each of the Africanized and the European honey bees was found dead on day 56. The other two, one Africanized and one European colony, died on days 70 and 77, respectively. The remaining four Africanized and four European colonies survived the entire 91-day period.

Although the colonies of Africanized honey bees died sooner than colonies of European bees in our 1984 study (cited above), this was not the case in the present study. Not only was there an identical number of Africanized and European colonies surviving, but additionally both groups of honey bees were confined about 2 weeks longer than those colonies tested in our 1984 refrigeration chamber study. A possible explanation for the increase in the colony survival rate has been a concerted effort to reduce colony disturbances as much as possible during our routine inspection periods.

Based on the present results, and our previous studies on overwintering in Cordoba (Krell *et al. Apidologie* 16:109-118, 1985), San Juan (Dietz *et al. Proc. AHB Symp., Atlanta* 87-91, 1986), and the discovery of Africanized honey bees near the 39°S in the province of Rio Negro, Argentina (Dietz *et al. Apidologie* 16:99-108, 1985), it is clear that the ability to survive low temperatures for extended periods of time is not the main factor in limiting the distribution of Africanized honey bees in Argentina, or in most areas of the United States where the Italian honey bee, *Apis mellifera ligustica* is kept throughout the year. Food sources and nesting sites, which ultimately are influenced by weather conditions, *i.e.*, temperature and rain, appear much more limiting than long periods of confinement and low temperature conditions.

The evidence from this study, combined with other cold survival studies (Krell *et al. Apidologie* 16:109-118, 1985; Dietz *et al. Proc. AHB Symp., Atlanta* 87-91, 1986; Dietz *et al.* pp. 237-42, In: Needham *et al.* eds, *AHB and Bee Mites*, 1988; Spivak, *Am. Bee J.* 126:834, 1986; Villa, *Am. Bee J.* 128:835, 1986) demonstrates that the distribution and the survival of Africanized honey bees in Argentina, and probably most areas of the U.S., is not limited mainly by temperature conditions. These findings also do not support the contention of Taylor (*Bull. Ent. Soc.*, 31:14-24) that 60°F is the overwintering limit temperature for Africanized honey colonies in the U.S. Our conclusions are consistent with data that reveal the past existence in Europe of a group of bees now confined to the tropics and subtropics (Culliney, *Bee Wld.* 64:29-38).

A recent comparative overwintering experiment in Germany again demonstrated that there were essentially no differences in survival between Africanized and Italian honey bees (Rinderer, personal communication). Since numerous German beekeepers and beekeeping organizations were opposed to the presence of Africanized honey bees in Germany, the experiment was unfortunately terminated in February, 1989.

11. Harbo, J. R.[g] – EFFECTS OF PLASTIC COMBS ON HONEY BEE POPULATIONS[gg] – Plastic combs produced in Germany (ANP combs) may affect *Varroa* mites. The explanation was that workers produced in plastic combs had a shorter development time and consequently provided less time for *Varroa* mites to reproduce in capped brood (Posern, *Am. Bee J.* 128:698-702). Although I had no *Varroa* in Louisiana to test this hypothesis, I did measure how these plastic combs affected the development time of workers as well as other characteristics (see table).

The development time of brood was measured with known aged larvae compared side by side in plastic or wax cells in the same colony. No significant differences were found (see table).

Population growth and honey production were evaluated in 7 colonies with plastic combs and in 10 colonies with wax combs. Colonies were established on April 21 with 5141 ± 139 (mean ± SD) worker bees, sister queens, and 5 Zander frames (8¾ X 16½ inches) in each colony. A queen excluder and a super of 5 wax combs were added on May 24. Colonies were evaluated June 14. Methods for establishing and evaluating colonies were described by Harbo, *J. Apic. Res.* 25:22-29. These colonies provided all the data in the table except for worker development time.

Table – Characteristics of bees reared in wax or plastic cells.

	Wax combs	Plastic combs	Probability that means are equal
Worker development time:[1]			
Uncapped larva	4d & 19h	4d & 23h	ns[2]
Capped cell	11d & 19h	11d & 18h	ns
Total dev.[3]	19d & 13h	19d & 16h	ns
Wt of emerging adult worker	107 ± 8mg (40)[4]	131 ± 11mg (36)	<0.01
No. of ovarioles per worker	9.4 ± 4.4 (48)	8.9 ± 3.0 (37)	ns
Estimated adult longevity	26.5 ± 3.7 d	30.6 ± 2.3 d	<0.05
Colony evaluations:			
Adult population	13554 ± 2149	10206 ± 1880	<0.01
Cells of brood	13927 ± 1970	10007 ± 1683	<0.01
Grams of honey	4004 ± 1413	3261 ± 496	ns

1. Mean times for 3 different stocks (38 bees)
2. ns = not significantly different at the 0.05 level
3. Assuming 71 hours for egg development
4. These and following data are in mean ± SD (number measured)

There was no indication that plastic combs shortened worker development time in Louisiana. In colonies starting with 5000 bees, plastic combs retarded the first 8 weeks of population growth. However, workers produced in plastic cells were larger and may have lived longer as adults. I emphasize that I do not know if plastic combs can reduce populations of *Varroa* mites, but if they do, it is probably not because of a shorter development time of worker bees.

12. Harris, J. W.[o] and J. R. Harbo[g] — OVARY DEVELOPMENT OF WORKER BEES WHEN CAGED WITH WORKERS FROM DIFFERENT STOCKS — Three stocks that differed in time required for workers to lay eggs were evaluated. Stocks A and B became laying workers rapidly (*ca.* 9 days), and stock C was slow (*ca.* 25 days). To test effects that mixing different stocks might have on the ovary development of worker bees, 5 "select" workers from stock A were marked and placed into each of 40 incubator cages. Each cage was given an additional 25 workers (attendants) from stock A (10 cages), from stock B (15 cages) or from stock C (15 cages). All bees were less than 24 hours old at the start of the experiment. Each cage received a section of drone comb and honey, water and fresh pollen *ad libitum*. All cages were maintained through 10 days in an incubator (34°C; 50% RH). On the 10th day all select workers were removed from their cages, and the attendants were maintained for another 24 hours to check for egg production from them. Ovary development in the select workers was evaluated using a ranking system described by Velthuis (*Ent. Exp. & Appl.* 13:377-394; Class I — resting or inactive; Class II — early stages of development when eggs appear round to bean shaped; and Class III — fully mature ovaries having sausage-shaped eggs). Means reported for number of ovarioles per ovary were weighted by the number of select workers examined for each cage.

Select workers in cages containing stock C attendants required two extra days to begin laying when compared to the control group (stock A attendants). Since none of the cages containing stock C attendants produced eggs after removal of the select workers, egg production in that group was probably from select workers only. The percentage of select workers with developed ovaries was highest when attended by their supersisters and lowest when attended by stock B workers.

Table — Ovary development of "select" workers and overall egg production in cages. Groups of 5 select workers from stock A were caged with 25 attendants from their own or from other stocks.

	Attendant Stock (mean ± SD)[1]			Probability of no differences
	Stock C (n = 15)	Stock B (n = 15)	Stock A (n = 10)	
Oviposition onset in days	10.3 ± 0.5, b	8.0 ± 0.4, a	8.2 ± 0.4, a	<0.01
No. of eggs after 10 days	6 ± 6, b	28 ± 17, a	32 ± 24, a	<0.01
No. of eggs after removal of select workers	0 ± 0, b	11 ± 12, a	12 ± 8, a	<0.01
Ovaries/ovary for select workers	4.0 ± 1.2	3.9 ± 0.8	3.7 ± 0.9	n.s.
Percentage of select workers with developed ovaries[2]	47 ± 22, a,b	34 ± 19, b	56 ± 15, a	<0.03

1. Means in the same row having the same letter do not differ using the LSD multiple comparison.
2. Class II or Class III ovaries (see text).

13. Ibay, L. A.[o] and D. M. Burgett — BIOLOGY OF THE TWO EXTERNAL ACARAPIS SPECIES OF HONEY BEES: ACARAPIS DORSALIS MORGENTHALER AND ACARAPIS EXTERNUS MORGENTHALER — The tracheal mite, *Acarapis woodi* Rennie, is the only *Acarapis* species for which detailed biological studies have been carried out. Although the two external *Acarapis*, *A. dorsalis* and *A. externus*, are known to be hemophagic parasites (Orosi-Pal, *Bee World* 15: 93-94), they have been largely ignored since they are considered to be harmless to honey bees. No studies have been done to determine the impact of these *Acarapis* species on the colony health. For this reason, biological studies were undertaken in order to better understand the interaction of these two external *Acarapis* species and their honey bee hosts.

Both external *Acarapis* species were observed to have *ca.* 8-9 days total developmental period. *A. dorsalis* required four days for embryogenesis and 4-5 days more before the emergence of new adults. For *A. externus*, the egg incubation period took only three days, but the immature stages lasted for 5-6 days. In both species, males emerged earlier than females.

Variations in mite load and percent infestation of both *Acarapis* mites were monitored on marked bees as the bees became older. Decreases in mite load and infestation rate of *A. dorsalis* were observed as the bees aged. However, *A. externus* seemed to maintain its population on older bees.

The seasonal population fluctuations of both species were also monitored. *A. dorsalis* had the highest infestation levels recorded in spring months (March to June) when suitable hosts were emerging, and during mid-late summer (August and September) when brood rearing started to decline. For *A. externus*, infestation was highest in the fall (October and November), which was coincidental with the decreasing brood rearing activities inside the colonies. During this period there was a higher proportion of older bees, which may indicate that the age of bee hosts has little effect on *A. externus* population.

The lowest infestation rates of *A. dorsalis* were recorded in January when no suitable hosts for the mites were available and in July, which coincided with the peak bee emergence inside the colonies. This drop in July could indicate the dilution of mite populations due to more new, uninfested bees emerging during this month. Percent infestation by *A. externus* was also lowest in July.

Both species were observed to reproduce year round as shown by the constant presence of immatures throughout the sampling period. However, fecundity decreased during the winter months (December and January). The average female: male ratios were established at 1.9:1 for *A. dorsalis* and 2.07:1 for *A. externus*. (Presented at the mite symposium)

14. Kitto, G. B.,[h] E. Broussard,[h] J. Lemburg,[h] L. Davidson,[h] F. Davidson,[h] W. Rubink[e] and O. Taylor[q] — MALATE DEHYDROGENASE (MDH) PROFILES OF MEXICAN TRAPLINE HONEY BEES PRIOR TO AFRICANIZATION — The establishment of honey bee traplines in Mexico and South Texas and the collection of samples from these areas prior to Africanization, by the USDA/ARS personnel at Weslaco, Texas, provides an exceptional opportunity to examine in detail what happens to local European bee populations as the African bees move through these locations. In addition to providing essential and specific information about the temporal changes that occur as ingression by the Africanized bees proceeds, studies of trapline samples can also prove useful, in a more general sense, by providing data for the development of models for the dynamic interaction of two competing insect populations.

Previous studies have established that malate dehydrogenase (MDH) is one of the few polymorphic enzymes found in honey bees, with three allelic forms occurring in most European strains. Samples of *A.m. scutellata* from Africa are essentially monomorphic for one of these allelic forms (Nunamaker *et al.*, *J. Kans. Entomol. Soc.* 57(4):622-631). We have determined MDH allele frequencies for both

Mexican and South Texas trapline samples by starch gel electrophoresis at pH 8. The information obtained, to date, is presented in the Table. While the Mexican trapline samples show both general constancy from site to site and a relatively low frequency of the MDH-5 allele, this is in marked contrast to the South Texas trapline samples where a significantly higher frequency of the MDH-5 allele was observed.

Such site specific variation in MDH allele frequencies have also been observed in previous studies (Nunamaker *op cit.* and Gartside, *Experientia* 36:649-650) and these findings highlight the need for long-term evaluation at specific trapline sites prior to and throughout the time of the Africanized bee ingression.

Determination of MDH allele frequencies provides just one measure with which to evaluate the changes which will occur as the Africanized bees pass through the trapline areas. We anticipate that, through collaborative interactions, this picture will be enhanced by additional information such as that for morphological variation, mitochondrial DNA analyses and cell size assessment.

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Table – MDH Allozyme Frequencies for Mexican and South Texas Honey Bee Trapline Samples.

LOCATION	# BEES	MDH ALLELE FREQUENCIES 1	3	5
Mexico				
Bait hives	257	34%	54%	12%
Rustic Hives or Feral Colonies	134	30%	58%	12%
Modern Managed Colonies	10	25%	65%	10%
South Texas Trapline				
Bait Hive Transect	102	40%	29%	33%

15. Labougle, J. M.,[r] M. Mancera,[r] and O. R. Taylor[q] – A MORPHOMETRIC AND ELECTROPHORETIC STUDY OF THE AFRICAN HONEY BEE IN SOUTHERN MEXICO – African honey bees (AHB) entered México in September 1986. The feral African bee population spread rapidly along both coasts, advancing approximately 240 miles per year (if measured from Tapachula, Chiapas to González, Tamaulipas). In this study we attempted to determine whether the feral population entering Mexico differed from other feral neotropical African bee populations and from European bees used by beekeepers. Because of numerous speculations that African bees would be modified through hybridization with large European bee populations in Mexico, we utilized morphometrics and allozymes to determine whether such modifications have occurred. Samples were obtained from feral swarms collected in Guatemala (n = 31) during 1986, from managed hives along the coastal area of Chiapas state in Mexico (n = 471) during 1986 and from 80 feral swarms as AHB moved into the region in 1987. In addition, 18 feral European swarms were collected in Oaxaca in 1986. These were compared with 44 feral swarms collected in this region in 1987 and 165 collected in 1988. Another 68 feral swarms were obtained from Las Choapas in Veracruz.

The electrophoretic data are based on collections made during 1987 and 1988 at Tapachula, Chiapas, and in January 1989 at Tapanatepec, Oaxaca, and Las Choapas, Veracruz. Two allozymes, malate dehydrogenase (MDH) and hexokinase (HK), which differ markedly in frequencies between African and European bees, were used to characterize the samples from feral and managed colonies.

Our data indicate that migratory swarms are African bees which do not differ substantially in phenotypic or genotypic characteristics from African bee populations in Central and South America. Non-parametric tests and discriminant analyses of morphometric data clearly distinguish European and African honey bees. The frequency data for MDH and HK alleles support the morphometric data. The morphometrics and allelic frequencies of the feral population during the first year of migration suggest that modifications due to hybridization with European bees were minimal. Frequencies for the MDH fast allele were 0.81 at Tapanatepec and 0.86 at Las Choapas. These values are higher and more "African-like" than obtained at Tapachula, Chiapas indicating that the feral African population is not becoming "Europeanized " as it advances further into Mexico.

Both the morphometric and allozyme data indicate that the migrating feral bees found along the Gulf Coast are more similar to the AHB type than those found on the Pacific coastal region, however, the differences are small. At this time there is no clear explanation for these differences. The morphometric data are particularly difficult to interpret because of possible environmental interactions and, as has been reported by Boreham and Roubik (*Bull. Ent. Soc. Am.* 1986) in Panama, there is a trend toward smaller size the longer the feral African bees are resident in a given area.

16. Labougle, J. M.,[r] E. Yarce,[r] and O. R. Taylor[q] – SWARM CHARACTERISTICS AND BAIT HIVE SELECTION BY AFRICAN HONEY BEES IN SOUTHERN MEXICO – Migratory swarms of African honey bees (AHB) entered Mexico in September of 1986. They reached the southern portion of the Isthmus of Tehuantepec in May and the northern part in September of 1987. Our study on the swarming biology of AHB was conducted at Tapanatepec, Oaxaca, and Las Choapas, Veracruz. In July of 1988 we established a bait hive line 20 kms long at each area. Our objectives were to test two types of bait hives: 1) a pressboard bait hive, and 2) a cardboard bait hive. We also tested the efficiency of a synthetic Nasanov pheromone lure. The null hypotheses tested in this study were that characteristics of swarms did not differ 1) between areas, 2) at different seasons within these areas, 3) throughout the transect and 4) did not differ among bait hive types.

The swarming biology and the characteristics of swarms were very different between areas. In a six month period we captured 178 swarms at Tapanatepec and 70 at Las Choapas. Pressboard bait hives were preferred over cardboard ones at both study sites. Most swarms were captured in bait hives containing Nasanov lures. The average swarm weight for Tapanatepec was 616.2 gr., for Las Choapas 2087.35 gr. Another important difference between the two areas was the number and distribution of drones; there was a significant number of swarms (n = 66) with drones at Tapanatepec and almost none in Las Choapas (n = 4). At Tapanatepec there was a distinct drone season. Few drones were found in swarms from July-Sept. However, a large proportion of the swarms collected from October to January contained drones. At Las Choapas we found that most swarms were captured from bait stations 46 to 70 (from a total of 80). This relatively small portion of the transect seemed to represent a migratory pathway. Swarms in and out of the pathway were statistically different.

In each case the null hypotheses were rejected. The characteristics of swarms differed between areas, seasons, locations on the transect and other factors such as type of bait hive and the presence or absence of the Nasanov lure. The pressboard bait hive is strongly recommended over the cardboard bait hive. Not only does it capture more swarms but larger ones. These larger swarms are more likely to survive and reproduce than the smaller swarms attracted to cardboard bait hives. The pressboard bait hive has the additional advantages of minimal preparation time and great durability. Depending on the environment and the number of colonizing swarms, the half-life of pressboard

bait hives is usually more than two years. Cardboard bait hives seldom last more than a few months.

17. Loper, G. M.[s] and R. K. Smith[t] – CUTICULAR OLEFIN ASSAY OF AFRICAN HONEY BEES VISITING COTTON FLOWERS – The African honey bee *(Apis mellifera scutellata)* has variously been described in the popular literature (especially newspaper articles) as being either less-efficient than managed honey bees or just as good, if not better. Most of the confusion comes from a lack of definitive research studies, but a lot also comes from the bias of the particular reporter.

In one study, I (GML) used individual colonies inside screen cages (3m x 6m x 2m) and observed their behavior including visitation to flowers of cotton. The study was conducted near Tapachula, Chiapas, Mexico. I had 3 cages of AHB and 3 cages of EHB in each of 2 years, 1987 and 1988. The AHB colonies were obtained from the La Norteña Apiary maintained by the Mexican Agricultural Agency (SARH) under the direction of Ing. Meliton Fierro ('87) or Lic. Francisco Choy ('88). The colonies originated from AHB swarms caught by SARH personnel in the local area. The EHB colonies were headed by queens purchased in California as "Ultra-lite" yellow Italians. All colonies were fed sugar water and water, and they all had pollen stores at the beginning of the experiment. The colonies were placed in the cages when at least 20% of the cotton plants had flowers.

In general, the AHB colonies attempted to abscond; most of the foragers went to the upper corners of the cages and formed large groups hanging on the screen. Most of them returned to the hive in late afternoon. Additionally, they exhibited considerable aggressive behavior for at least the first several days. Also, the AHB generally ignored both the cotton flowers and the extra floral nectaries. The EHB colonies also exhibited some of the "escape" behavior, hanging in much smaller numbers on the screen, but very quickly many foragers began visiting the flowers and nectaries, collecting both pollen and nectar.

In all the cages, we caught individual foragers at the cotton flowers and from the groups of bees hanging on the screen. These samples were air-dried (at room temperature) and olefin assay performed (by RKS) for determination of AHB or EHB. This technique is able to discriminate subgroups within both population types (Smith and Lavine, *Proc. Am. Bee Res. Conf.* 1987).

The olefin profiles of all EHB samples from this study were identical, but the AHB foragers that did visit the cotton flowers – in both years – had unique hydrocarbon characteristics placing them in a separate sub-group from the rest of the workers in the same cage (total of 4 reps, 4 floral visitors/rep).

We are not sure how to interpret these results. The AHB workers that did visit the flowers may represent a separate patri-line within the colony – one more genetically inclined to forage under these confined conditions than were their half-sisters which were distinguished by a detectably unique olefin profile.

18. Lozano de Haces, L.,[u] W. L. Rubink,[e] W. T. Wilson,[e] and M. Guillen-M.[u] – NOSEMA AND HONEY BEE TRACHEAL MITE INTERACTION IN SWARMS FROM NORTHEASTERN MEXICO – Recent studies have suggested that the role of *Acarapis woodi* in causing decreases in colony production may be related to mite-associated diseases, and not the tracheal mite itself (Gary and Page, *J. Econ Entomol.* 82:734-9). *Nosema apis* is a disease which has been shown to have a significantly greater effect on honey bee longevity when tracheal mites are present (Bailey, L. *Parasitology* 48:493-506). We evaluated the relationship between levels of *Acarapis woodi* infestations and Nosema in 181 honey bee swarms captured over a one year period in central Tamaulipas state, Mexico. This study is part of an ongoing characterization (Rubink *et al.*, *Amer. Bee J.* 128:807-8, Rubink *et al.*, *J. Kans. Entomol. Soc.*, in press) of honey bees of the region prior to their Africanization. The captured swarms probably had primarily feral and rustic-colony origins.

Standard methods were used for the *Nosema* detection and spore counts (Cantwell, *Amer. Bee J.* 110:222-3). Tracheal mite infestation levels, measured in terms of the number of adult mites per worker bee, were evaluated by simple dissection. Ten bees per swarm were examined.

Nosema levels were generally light, ranging from 2.5 x 10^5 to 4.8 x 10^6 spores per bee. Of 181 swarms (1810 bees) examined, a total of 21 bees from 13 swarms were found to be infected. Swarms were infested at rates from 10% to 30%. *A woodi* was present in 300 bees from 76 of the swarms; infestation rates ranged from 10 to 100% per swarm. Comparisons of mite-infested/non-infested and *Nosema*-infected/non-infected bees and swarms in 2x2 contingency tables showed no statistically significant association between the presence of the two diseases. The presence of tracheal mites is not associated with increased susceptibility to *Nosema* in swarms of probable feral origin.

Nosema levels were higher in early (spring) season, and lower in late season samples. Tracheal mite levels followed a similar trend. Interestingly, *Nosema* levels showed remarkable geographic variation. Swarms captured in the Coastal plain region on the Gulf of Mexico coast were more frequently infected than those from farther inland. The drier inland climatic conditions may play a role here, or the observed results may be a result of the introductions of more susceptible bees in the last two decades of increased modern apicultural practices in the coastal region.

19. Mohamed, M. A.,[g] H. A. Sylvester,[g] B. P. Oldroyd,[g] and J. A. Stelzer[g] – AN EFFICIENT METHOD FOR THE ISOLATION OF MITOCHONDRIAL AND NUCLEAR DNA FROM SINGLE BEES[gg] – Our protocol capitalizes on the direct invagination of the tracheoles into insect cells. Flash freezing the entire organism followed by lyophilization dehydrates the bee and allows controlled access to organelles, mitigating premature cellular disruption prior to homogenization. Additionally, a substantial proportion of hemolymph contents such as lipids and proteins are removed prior to cellular disruption.

The method is as follows: single bees are flash frozen in liquid nitrogen, transferred to 1.5 ml Eppendorf tubes, and lyophilized overnight in a vacuum desiccator over a bed of Drierite. Each dried sample is cut longitudinally. The tissue is washed by gentle vortexing in 0.5 ml of ice-cold buffer 1 (composed of 50 mM Tris, pH 7.4, 250 mM sucrose, 1 mM EDTA, 2 mM $CaCl_2$ and 5 mM $MgCl_2$). The buffer is discarded and the tissue is resuspended in buffer 1, homogenized with a Tissuemizer, and then centrifuged at 1000g at 4°C for 10 minutes. The supernatant is carefully removed and discarded. The pellet is resuspended in 0.5 ml buffer 1 supplemented with Triton X – 100 (0.01% or 0.025% for the larvae or adults, respectively) and then dounced with a loose fitting pestle. Prior to this douncing, for the efficient recovery of mt DNA, the adult homogenate should be filtered to remove chitinous debris (a glasswool pad in a perforated lower half of an Eppendorf tube is used to filter the homogenate into an intact tube by a 1000g/2 min. spin in a centrifuge). The sample is then centrifuged at 1000 g at 4°C for 10 minutes to pellet nuclei and intact cells. The pellet can be processed once again to enrich the cytosolic organelle fraction. The supernatant is pooled and centrifuged at 15,000 g at 4°C for 30 minutes to recover the mitochondria. The nuclear and mitochondrial pellets are lysed in 0.3 ml of a second buffer (10 mM Tris, pH 8.0, 0.5 mM sodium acetate, 10 mM EDTA, 5 mM $MgCl_2$, 10 µl Proteinase K (10 mg/ml), 5 µl RNAase A (DNAase free, 10 mg/ml), and 0.5% N-lauryl sarcosine), at 42°C for 30 minutes.