Changes in Honey Bee (Hymenoptera: Apidae) Colony Swarming and Survival Pre- and Postarrival of *Varroa destructor* (Mesostigmata: Varroidae) in Louisiana

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ABSTRACT   The impact of *Varroa destructor* Anderson & Trueman (Mesostigmata: Varroidae) on colonies of *Apis mellifera* L. (Hymenoptera: Apidae) in southern Louisiana was evaluated by analyzing changes in swarming and longevity of colonies for 17 yr. Swarming rates were calculated from yearly captures of swarms in bait hives placed in five areas of Louisiana from 1991 to 2006. Colony longevity was monitored in 104 swarms established from 1990 to 2000 and followed until 2004. In the first years, before *V. destructor*, average swarm capture rates ranged from 0.85 to 0.95 swarms per bait hive-year, and survival of colonies established from swarms averaged 14 mo. In years immediately after the arrival of *V. destructor* (1993–1996), swarming rates and colony longevity decreased to 0.36–0.60 swarms per bait hive-year and 10 mo, respectively. After ≈5 yr in the presence of *V. destructor*, both rates recovered to levels at least as high as those seen before varroa arrived; swarm capture rates were 0.75–1.04 swarms per bait hive-year and average longevity was 26 mo. Analysis of varroa infestations in three colonies established from swarms in 1997 showed the presence of varroa at oscillating densities for 5 to 8 yr. Possible causes for this apparent recovery are natural selection for resistance in honey bees, introgression of selected resistant genetic material or reduced virulence of the mites.

KEY WORDS   *Apis mellifera*, *Varroa destructor*, survival, swarming, recurrent event

When populations of honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), first encounter parasitic varroa mites, *Varroa destructor* Anderson & Trueman (Mesostigmata: Varroidae), the mites spread rapidly between colonies and reach high levels of infestation (Ritter et al. 1984, Harbo and Zuhlde 1988, Kraus and Page 1995a), which typically kill untreated colonies (Kraus and Page 1995b, Fries et al. 2006). These observations have lead to the widespread notion that feral bees are eliminated, and only colonies under the care of beekeepers are able to survive. However, a stable coexistence between parasite and host may be expected to develop through time (Oldroyd 1999), particularly if the parasite is transmitted from parent to offspring colonies (Fries and Camazine 2001). Stable situations of coexistence between *A. mellifera* and *V. destuctor* are reported in widely different climates and with different honey bee and mite genotypes

(Eguaras et al. 1995, Moretto et al. 1995, DeJong & Soares 1997, Vandame et al. 2000, Fries et al. 2006, Seeley 2007, Le Conte et al. 2007). There are also anecdotal reports from beekeepers indicating elimination of the need for acaricides after a number of years of propagating new colonies from survivors.

In the southern United States, a threat in addition to varroa parasitism is the Africanization of honey bees. State regulators are interested in monitoring the spread of Africanized bees to address concerns of both beekeepers and the general public. In Louisiana, an extensive monitoring program for Africanized bees presented an unplanned opportunity to measure effects of varroa parasitism on swarm production and survival of colonies begun from swarms. We used data from the monitoring program and from other collected swarms to explore the impact of initial and early stages of parasitism by *V. destructor* on swarming and longevity of feral colonies, especially with regard to changes in the host–parasite relationship through time.

Materials and Methods

Data on swarm capture rates and survival of colonies were collected for 17 yr in Louisiana. Swarming rates in different areas of Louisiana were obtained from a program by the Louisiana Department of Agriculture and Forestry monitoring for Africanized bees. Survival times in months were determined for

colonies initiated from swarms during nine swarming seasons beginning in 1990–2000 and followed until 2004. The parasitic mite *V. destructor* was first detected in study areas in 1992 and spread rapidly to feral and managed colonies.

Swarming Rates. Wood-pulp bait hives (Schmidt and Thoenes 1987) (157–254 hives per year) were placed to monitor for the presence of Africanized bees at five geographic areas in Louisiana. A line of traps along the Mississippi river from south of New Orleans to Baton Rouge was maintained during 1991–2006. Trapping near deep water ports in Lake Charles and New Iberia began in 1991 and was expanded in 1995 to cover the area between Lake Charles and Toledo Bend. Trapping in northwestern Louisiana (Toledo Bend Lake to Shreveport) began in 1999. Captured swarms usually were sampled and removed within 1 mo, and the bait hive was re-placed. Swarm capture rates per year were calcu-lated by dividing total swarms by number of avail-able bait hives. From 1991 to 1994, samples were checked for the presence of varroa mites by using ether rolls in the field (Dietz and Hermann 1988). From 1995 to 2006 (except for 1996 and 1999), sam-ples from most swarms were washed in alcohol to remove varroa mites and the percentage of swarms positive for mites from each area was calculated. All swarms were screened initially for Africanization by using average forewing length (Rinderer et al. 1987). Samples with average lengths below 9.01 mm were subjected to multivariate discriminant analy-ses of 23 morphological characters (Rinderer et al. 1993).

Swarm Survival. Swarms ($n = 104$) were captured in bait hives at other locations during nine seasons (1990–1994 and 1997–2000; $n = 10$, 12, 9, 21, 24, 14, 7, 5, and 2, respectively). Before 1992, swarms pre-sumably originated from feral and managed colonies in East Baton Rouge and Iberville parishes. After the arrival of varroa mites at the end of 1992, swarms probably tended to originate from managed bees that were protected with miticides. During 1997–2000, an effort was made to avoid swarms from colonies artificially selected for resistance to *V. de-structor* by collecting swarms in areas with little or no beekeeping in St. Tammany and Tangipahoa par-ishes.

Swarms were either kept where captured or relo-cated within a week. Most were maintained in the hives in which they were captured. All colonies from swarms were checked every 1 to 3 mo to see whether they were still alive. Thirteen swarms in 1993 and seven in 1997 were transferred to standard hives. Hived colonies received no management aside from the addition of comb foundation in additional hive boxes. Ten swarms in 1993 and five in 1997 that were still alive in the autumn of each year had 100 cells of sealed brood sampled to measure the initial level of varroa. Three of the 1997 swarms alive in the winter of 1999 were sampled three times per year until 2006 or until they died. In these colonies, 100–150 g of adult
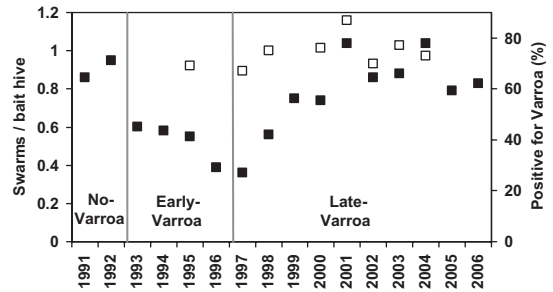

Fig. 1. Yearly capture rates of swarms in Louisiana (■, left axis) from 1991 to 2006, and percentage of swarms positive for varroa (□, right axis) in 1995, 1997, 1998, and 2000–2004. Bait hives ($n = 157$–254) were established every spring in five areas and monitored for occupation with swarms monthly. LSDs between years for the swarm capture data range from 0.33 to 0.35 swarms/bait hive. The percentage of swarms positive for varroa did not differ significantly between years.

bees were collected, and mites were washed from bees in alcohol to determine mites per gram of bees.

Statistical Analyses. Swarm capture rates per year (total swarms captured per bait hive in place) and percentage of swarms positive for varroa for each area were analyzed as a randomized block design with year as a fixed effect and area as a random effect by using the Mixed procedure (SAS Institute 2000). Least square means for rates each year were compared by calculating a least significant difference (LSD).

The death rates and survival times of 104 colonies were estimated using statistical methods for recurrent events (Nelson 1998; Meeker and Escobar 1998) with modifications for events observed in windows of time (Zuo et al. 2008). Colony establishment and death times were placed on a common time scale in months starting March 1990 and ending October 2004. To make the data compatible with the selected statistical methods, each colony was considered as an indepen-dent unit that was observed in a single window of time of length equal to its life time. For 97 of the colonies only one event (death) occurred at the end of its observation window. The remaining seven colonies were right censored and did not experience an event during the study.

The approximate time of detection of *V. destructor* in the area was used to cluster time periods into dif-ferent groups: 1) No-Varroa, the time period between 1990 and 1992; 2) Early-Varroa, the period from initial detection to early spread of mites (1993–1996); 3) No + Early-Varroa, which grouped the two previous time periods; and 4) Late-Varroa, with colonies es-tablished between 1997 and 2000 and followed until 2004. These time periods were used to test whether the death rates and survival times were homoge-neous through time.

A recurrence event parametric model, the homo-geneous Poisson process (HPP) (Meeker and Escobar 1998, Nelson 1998, Zuo et al. 2008), was used to model the number of deaths per month per colony. The HPP is characterized by a constant (homogenous) recur-rence rate parameter, $1/\eta$. The maximum likelihood
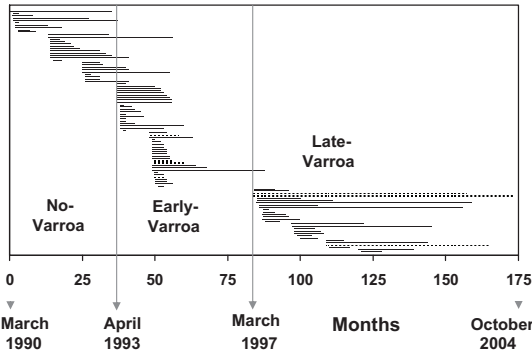
Fig. 2. Event plot of longevity of 104 colonies. Each horizontal line in the plot represents a colony. The left end of a line indicates the date when the colony was established. The right end of the line is the date of death or of censoring of the colony. Seven colonies with right-censored observations are indicated with dashed lines.

parameter estimate for the recurrence rate was calculated as the inverse of

$$\hat{\eta} = \frac{\sum_{i=1}^{n} t_{Ui} - \sum_{i=1}^{n} t_{Li}}{\sum_{i=1}^{n} r_i}$$

where n was the total number of colonies, the summation of r_i was the total number of deaths, and t_{Ui} and t_{Li} are the beginning and ending time, respectively, of a colony. In accordance with the theory of recurrent events analysis when the data are compatible with a HPP, the inter-recurrence times are independent and exponentially distributed with mean equal to η (Meeker and Escobar 1998). In the present case η represents the mean time to death of the colonies and its standard error was approximated using the variance for an exponential distribution.

Three HPP models were fitted using log-likelihood methods. Model M1 had a single recurrence rate fitted to the entire data set with the hypothesis that all colonies experienced a similar death rate throughout the study. Model M2 had two separate HPP to fit the data, one for the No + Early-Varroa group and another for the Late-Varroa group. This model is based on the hypothesis that the colonies that were alive between 1990 and 1994 experienced different death rates than those colonies established 5 yr after the arrival of the mite. Finally, model M3 with three separate HPP for the No-Varroa, Early-Varroa, and Late-Varroa groups, is based on the hypothesis that the death rates for colonies established in

those three periods were different. The models were tested against each other using log-likelihood ratio tests to determine which hypothesis could be rejected. The log-likelihood of each model was calculated as the sum of the log-likelihoods coming from the fit of the corresponding HPP components. Analyses were performed in S-PLUS (Insightful, Seattle, WA) and SPLIDA (Meeker 2008).

Results

Swarm capture rate was affected by year ($P = 0.009$; Fig. 1) and geographic area (maximum likelihood estimate for area = two thirds of residual), but there was no interaction between year and area. Before the arrival of varroa mites (1991–1992), average swarm capture rate was 0.85–0.95 swarms per bait-hive year (Fig. 1). Swarms positive for varroa were first found from 1993 to 1995 in each of the four areas monitored at that time. For the 6 yr after the first discovery (1993–1998), there was a decrease in capture rates (yearly averages 0.36–0.60 swarms per bait-hive year). From 1999 to 2006, capture rates returned to levels similar to those before the arrival of mites (0.75–1.04 swarms per bait-hive year). In 2005, four swarms (three southwest of Lake Charles and one northwest of Shreveport) were the first identified as Africanized.

Survival times of monitored colonies varied significantly through time (Fig. 2). Before the arrival of varroa mites, the estimate for colony survival was 14 mo (No-Varroa, Table 1). Mortality rates increased starting in 1993 (after the detection of varroa mites in the immediate area in 1992) and the survival decreased to ≈10 mo (Early-Varroa, Table 1). The colonies within the period 1997–2005 had an estimated survival of 27 mo (Late-Varroa, Table 1).

Mite infestations of captured swarms and from some colonies monitored for survival matched the assumptions for the three time periods. Ether rolls detected the first infested captured swarms in one of the areas in spring 1993, the beginning of the early varroa period. Ten hived swarms captured in spring 1993 and used in the survival study had brood infestations ranging from 2 to 43% in the autumn. By the middle of the early-varroa period (1995), mites were found in a high proportion (69%) of captured swarms (Fig. 1). At the beginning of the late-varroa period, five of seven swarms hived in the spring of 1997 survived until the fall and had 28–65% of brood cells infested. Three of these colonies alive at the end of 1999 showed varroa mites at oscillating densities

Table 1.　Statistical parameters estimated from observations of 104 colonies through different time periods from 1990 to 2004

Group	Log-likelihood	$1/\hat{\eta}$ Death rate (in deaths per month per colony at risk)	$\hat{\eta}$ Survival time (mo)	SE for $\hat{\eta}$
Entire data	−362.36	0.065	15.42	
No-Varroa	−112.60	0.072	13.91	2.49
Early-Varroa	−134.09	0.103	9.68	1.50
No + Early-Varroa	−247.87	0.087	11.50	1.35
Late-Varroa	−107.12	0.037	26.70	5.32

V. destructor was first detected in the area in the autumn of 1992. No-Varroa, 1990–1992; Early-Varroa, 1993–1996; and Late Varroa, 1997–2004.
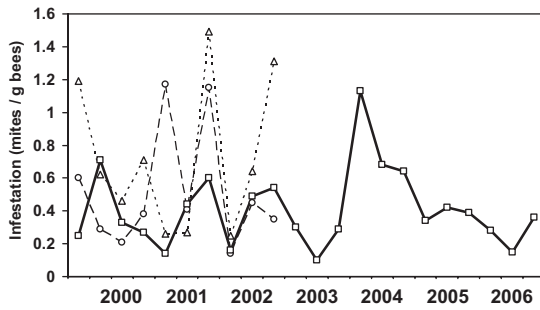
Fig. 3. Infestation with varroa mites in three colonies established from swarms in 1997 and sampled three times per year starting at the end of 1999. Initial infestation with varroa (49–65% of brood infested) was confirmed in November 1997. Two of the three colonies died during winter 2002.

until either the colonies died (two died late in 2002) or sampling ceased at the end of 2006 (Fig. 3). Percentage of swarms positive for varroa seemed to be uniformly high (67–87% of captured swarms) once varroa became established (Fig. 1).

The three hypotheses about the effects of the varroa mites on the death rates and survival of honey bee colonies were compared using likelihood ratio tests and the results indicated that M2 and M3 fitted the data better than M1, and that M2 fitted the data better than M3 (Table 2). This indicates that during the 175 mo of this study, the death rates of colonies immediately before and for the first few years after the arrival of varroa mites were much higher than those experienced by colonies in the last 7 years of the study.

Discussion

Swarm captures and colony longevity of feral honey bees in southern Louisiana decreased for ≈5 yr after first exposure to *V. destructor*, but then rebounded to prevarroa levels. The initial decline in apparent colony health coinciding with the onset of varroa parasitism is well documented (e.g., Kraus and Page 1995b, Fries et al. 2006). More notable in our data is the apparent recovery in both swarming and longevity. We did not expect the emergence of a more stable situation so rapidly because such a trend had not been reported when we were collecting these data. Since then, however, similar patterns in rates of colony swarming and mortality have been found in an isolated population of colonies and mites in Sweden (Fries et al. 2006). Apparent recoveries in survival of untreated honey bee

colonies have also been observed in France (Le Conte et al., 2007), and in feral populations in the northeastern United States (Seeley 2007) and in Arizona (G. Loper, personal communication).

Increased tolerance (resistance) of honey bees to mites, decreased virulence of mites through time, or both have been hypothesized as possible explanations (Fries et al. 2006, Seeley 2007). Reduced mite virulence was suggested in one study (Seeley 2007). In contrast, when effects from potentially avirulent mites were compared with those from virulent mites, increased tolerance seemed to explain the host–parasite coexistence (Fries and Bommarco 2007). In our situation, increased resistance to mite infestation may have been produced by natural selection on the resident population, or by introgression of other honey bees into the feral population. Natural selection seems likely given that large numbers of the swarms were captured much longer distances from known beekeeping than the average swarm movement distance of 3 km recorded for Louisiana (Villa 2004). Introgression from Africanized bees did not contribute to these changes given that the first detections occurred in 2005. However, we cannot rule out that some varroa resistant bees from the USDA laboratory in Baton Rouge may have been represented. The "late-varroa" rebound of swarming and longevity of feral bees coincided with the period of initial selection and breeding for varroa resistance in two types of bees at the laboratory (Harbo and Hoopingarner 1997, Rinderer et al. 1997). A decrease in the virulence of mites seems unlikely in our situation because of a high potential for horizontal transmission of mites in our longevity study where colonies were maintained in proximity to each other.

Other possibilities exist for the observed changes in colony swarming and survival time. First, the changes could occur if beekeepers were unsuccessful initially and more successful later at treating their bees against varroa. The fairly constant level of varroa mites in swarms once mites were established suggests that this was not the case. Furthermore, most locations of swarm monitoring are thought to be far from significant beekeeping activity. Second, environmental factors may have directly affected colony performance. Slow growth of varroa mite populations in short tests coincided with high temperatures and low humidity in Louisiana (Harris et al. 2003). However, there were not extended periods with temperatures or rainfall deviating consistently from long-term averages during the time we saw a decline and recovery in swarming rate and longevity.

The recovery trends raise the possibility of obtaining varroa resistant germplasm from feral honey bee popu-

Table 2.   Results of pairwise likelihood ratio tests comparing the three models (hypotheses) concerning the changes of mortality rates in association with the arrival time of *V. destructor*

H_0 log-likelihood	H_a log-likelihood	$-2[\log(H_0) - \log(H_a)]$	df	$\chi^2_{df, 0.95}$	Conclusion
M1–362.36	M2–354.98	14.75	1	3.84	Reject M1 $P = 0.00012$
M1–362.36	M3–353.81	17.37	2	5.99	Reject M1 $P = 0.00017$
M2–354.98	M3–353.81	2.63	1	3.84	Fail to reject M2 $P = 0.10$

M1 was the model fitted to the entire data set (one death rate). M2 was fitted to No + Early-Varroa and Late-Varroa data sets separately (two death rates). M3 was fitted to the No-, Early-, and Late-Varroa data sets (three death rates).

lations. Such an attempt was made earlier, when we evaluated local feral material and bees from elsewhere thought to be surviving without management for varroa (Danka et al. 1997). We did not find significant resistance. The current study now classifies this effort as having occurred during the "early-varroa" period, and resistance may have increased subsequently. Broad feral populations may offer novel recombinations of honey bee genetics that could prove useful for breeding programs aimed at varroa-resistant bees.

We conclude that predictable changes in the host-parasite relationship between honey bees and varroa mites (Oldroyd 1999) are the most likely explanation for our observations. Immediately after the arrival of varroa mites there was a strong impact on colony survival and reproduction. After a period of 5 to 10 yr, there has been an improvement and currently colonies seem to be reproducing and surviving as well as in prevarroa days. Others have reported similar trends, and it is possible that this may be a generalized situation in a number of areas.

Acknowledgments

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