Krispn Given and the Breeding of the Indiana Mite-biters

Part 1

Aning Westfall

by MARY AND BILL WEAVER

Purdue's College of Agriculture recognized Krispn Given in 2021 with its prestigious "Behind the Research" award for his work in breeding the Purdue Mite-biter honey bee strain, in addition to his other valuable contributions through Extension, teaching, and innovative honey bee research in his position as Purdue's Apicultural Specialist.

Purdue's mite-biters are a unique solution to the havoc wreaked on the beekeeping world by the parasitic varroa mite since its arrival in the U.S. in 1987. Mite-biter bees are genetically bred to literally do direct combat with the mites, removing them from their bodies and biting them, causing mortality. Most often, bees bite the mites' legs off. Some bees also bite the mites' shells.

Old-timers (including the authors) can remember the golden days of beekeeping before the arrival of the mites. Most hives overwintered successfully, perishing mainly if the cluster ran out of food. Splits thrived. Queens lived long, productive lives.

Then the mites arrived. The varroa mite, because of the viruses it injects, has been the biggest killer of U.S. honey bee colonies for decades. Winter colony losses are frequently heartbreakingly high, despite use of miticides. New beekeepers begin with enthusiasm, but frequently drop out in discouragement a year or two later.

Into this dismal situation stepped Dr. Greg Hunt, hired by Purdue in 1996 just after he completed his Ph.D. in behavioral genetics at UC Davis. A brilliant researcher who became world famous, Hunt also had a practical side.

"I found the first varroa mite ever in my county in CA, in MY research hives," he explained. Once he moved to Purdue, Hunt determined he would try to help the beleaguered beekeepers.

Dr. Hunt's original plan was to try to breed resistance to varroa by simply breeding from the healthiest and best of a few colonies he'd found that were somehow able to survive without varroa treatment.

Hunt started his breeding with a hive his brother Vince had collected as a swarm and kept without treatments in Ohio. He'd noticed the hive had survived for 10 years with no treatment. "The colony had swarmed repeatedly, but had never died out," he commented.

The hive itself was decrepit and slowly collapsing. A side panel was missing, and the bees had that area all propolized. The bees inside the hive, though, big, gray Carnica/Caucasian-looking bees, carried genes that were pure gold.

Hunt then added to his breeding stock a hive headed by a VSH queen from John Harbo, and two hives headed by Russian queens from Tom Glenn. Some Indiana-adapted "survivor" bees from commercial beekeepers Don Shenefield and his son Dave completed the gene pool. Hunt later added other valuable genetics from time to time, particularly additional locally-adapted bees from the Shenefields, who have for decades reared all their own queens without purchasing outside stock.

Other beekeepers and micro-breeders also sometimes brought stock to

Krispn Given

Ten-year-old Krispn. Krispn took care of the hives and sold the comb honey, earning money for his first car.

Hunt that had worked out very well for them. After testing, some of it was included in the program.

In 2003, the second key player in the mite-biter story, Krispn Given, arrived at Purdue to ask Hunt about employment. Krispn had been working in the culinary arts, but had long been an avid beekeeper. He was hoping to combine earning a living with working with bees. Hunt hired Krispn on a provisional basis as his apiary research assistant, caring for the project's 100 or so hives. Krispn's employment did not remain provisional for long.

Krispn turned out to be ideal hire for Hunt's breeding project. Hard-working and detail-oriented, he had developed a deep curiosity about the social insect world as a child, catching or digging up one of his favorites to observe the *Tetramorioum* (pavement ants) ant queens as they returned from their nuptial flights and using them to create homemade ant farms. "At the time I didn't realize it, but it was the social behavior I was interested in," Krispn said. "Ants were my first love."

His father was a hobby beekeeper for many years. When Krispn turned 8, he began to learn beekeeping. Later, he produced comb honey, which he sold in the family's antique store. By graduation, Krispn had worked his way up to 50 colonies. After graduation, he continued keeping bees, raising his own locally-adapted queens.

All of this turned out to be excellent preparation for his new job with Dr. Hunt. Krispn had learned to be observant and to take note of details that others might have dismissed as unimportant.

Counting mites

In addition to breeding, Krispn's job involved counting the number of groomed-off mites that fell onto the sticky boards placed under each hive twice a year. "This was the way mite populations were monitored in the beginning."

In 2007, as he was proceeding with the tedious job of mite-counting, Krispn noticed that some of the mites had legs that had been bitten off. Rather than idly noticing this and promptly forgetting about it, he mentioned it to Hunt. After doing some checking, they determined that some of Purdue's current hives, selected over the past ten years for good survival without mite treatment, had about 3% of their fallen mites that had been bitten.

Hunt had read about mite biting in the bee literature. He recognized that it was likely a grooming-related trait, and grooming was recognized as an effective way bees could fight back against varroa. Hunt made the decision in 2007 to make a sharp turn in the breeding program, and to breed specifically for bees that bit a high proportion of the mites that fell onto the sticky board.

Bee scientists had been remarking on mites with body parts bitten by bees since the early 1990s. Some Europeans had even recognized a probable connection between grooming and mite biting and suggested that breeding for mite biting could be valuable.[1] But nobody had started a breeding program for mite-biting bees before.

"I think," commented Hunt, "that the reason nobody had started a mite-biter breeding program, although others had considered it, was because it was too much work and there was no glory in it. You couldn't write a whole bunch of articles for publication about it." But fortunately for the beekeeping community, despite his relatively young age, Hunt already had a lot of publications under his belt from his work making the first genetic map of the bee and mapping genes that influence bee behaviors. He decided that he was willing to take on this challenge.

Breeding for the behavioral trait of mite biting created new challenges for Krispn's work. Now he didn't just have to investigate mites. He also had to rate every mite that was bitten on a scale of 1-4, indicating how badly it was bitten. Krispn personally did all the rating from 2007 until 2014. "Then we decided to hire an assistant to take over that part of the job." To be certain the results are reliable, each year all of the rating has always been done by a single individual.

Hunt gradually became Krispn's great friend and mentor. "I'm only where I am today because of him," noted Krispn. "Dr. Hunt gave me the opportunity to join him on this amazing scientific journey."

Learning and teaching instrumental insemination

Instrumental insemination (II) is an essential skill for bee breeders, necessary for controlling the drone side of the genetics. Dr. Hunt had been taught II by the "Father of Honey Bee Genetics" at UC Davis, Harry Laidlaw, Jr. Recognizing the outstanding qualities of his new research assistant, Hunt then taught Krispn. One of Krispn's first II projects was producing inseminated queens to make specific crosses needed for two Purdue students' projects. "Greg was going to Mexico for a couple weeks to work with Ernesto Guzman," Krispn recalls. "I remember he patted me on the shoulder as he left and said, 'You'll do just fine.'

"But at first, I was only 'OK' at instrumental insemination," Krispn ex-

Ernesto Guzman

Gary Reuter

(L) *Krispn was an invited participant to teach II for EAS in Ontario, 2015. In center of photo is Paul Kelly, YouTube celebrity for his how-to videos on bees, from the University of Guelph.* **(R)** *Krispn and Dr. Marla Spivak with U of MN II students. Krispn is holding a queen bank with the newly inseminated queens. Young bees beneath the queens will feed them in the lab.*

Krispn Given

II queens being gassed (narcotized) the second time in Krispn's lab. Queens are gassed to anesthetize them for the II procedure. They are frequently gassed a second time so that they start laying more promptly.

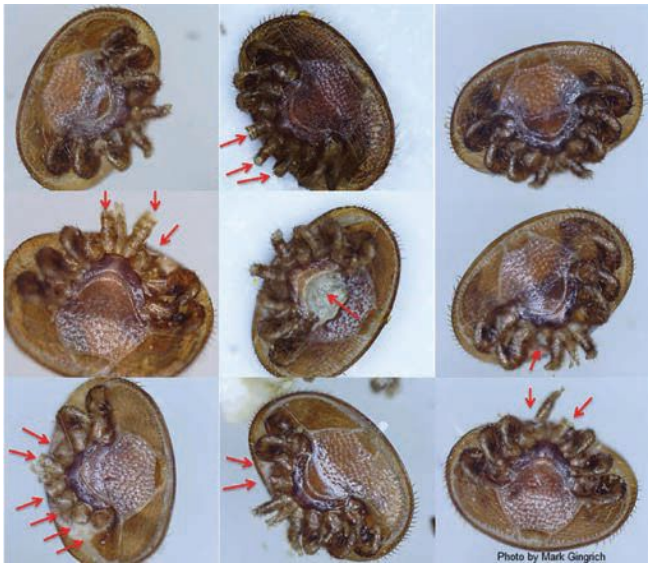
plains. "I stayed at the lab working a few nights until 9 p.m. I knew the two students were relying on me to successfully inseminate their queens. Because of my inexperience, I worked through what, at times, felt like agony."

To make a long story short, Krispn was ultimately successful, and today is recognized as one of the preeminent authorities in the field of instrumental insemination and honey bee breeding. Because of this reputation, when Dr. Keith Delaplane of the University of Georgia needed someone to inseminate 120 queens for a polyandry research project, he chose Krispn as his insemination consultant.

Delaplane says today, "I've worked with Krispn in a research capacity and also in a continuing education capacity. He is an effective speaker and he relates well to beekeepers, plus he has the technical skills to teach II. That's a hard combination to find. Trainers in II are a pretty elite fraternity, and Krispn's right up there with the best. He's a great ambassador for using genetics to improve honey bee health and for delivering that message to the beekeeping community."

At the University of Minnesota, Dr. Marla Spivak has also arranged for Krispn come to the campus to teach II to her students. Krispn has also been an invited participant on II at Eastern Apicultural Society and the Heartland Apiculture Society conferences, introducing the skill to hundreds of beekeepers.

Mites showing typical legs bitten off and ventral shield bitten



Mark Gingrich

Mite biting in the darkness of the hive

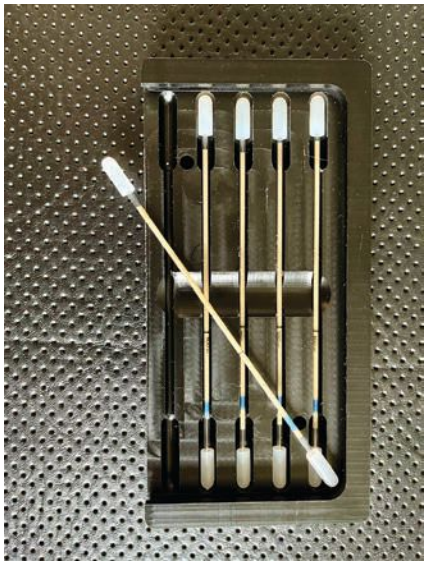
Dr. Hunt had wondered just why it was that after the bees groomed the mites off each other, some of those bees then bit the mites. "But then I found," he said, "that some Italian researchers had shown that there is a chemical that dribbles down on the bees' mandibles called 2-heptanone. This chemical paralyzes invertebrates like wax moths and mites. This means that for a period of time after the mites are bitten, they are paralyzed and not able to climb back up on the bees."

And it gets better. When mites are bitten, they're "like a hydraulic system," explained Krispn. When a mite is bitten, it simply dessicates and dies. Thus, biting is a very effective method the bees use to control the population growth of varroa mites in the hive.

"I like to see mites with bites on the idiosoma [shield of the mite]," continued Krispn. "That indicates the highest degree of aggression toward the mites, and perhaps the most response to selection, as well as an uptick in the trait."

In the darkness of a hive that is infested with mites, the bees become agitated. Individual bees will do what Krispn calls a "grooming dance" to request grooming from their sisters. "We call this 'allogrooming,'" explained Krispn. "The bee will kind of waggle around. A bee will also express 'autogrooming,' moving its front legs in a windshield wiper movement over the thorax. Bees will groom the mites off themselves successfully and bite them."

Krispn Given



Krispn Given

(L) *Five straws (glass capillary tubes) of newly collected mite-biter germplasm to be inseminated in selected virgin queens.* **(R)** *Paint-marked mite-biter drones ensure age and patriline. We typically collect the marked drones when retuning to the colony.*

Greg Hunt

Three-year-old instrumentally inseminated mite-biter breeder queen

Mite biting is a heritable trait

Purdue's work has proven that mite grooming and biting are heritable, and also that colonies that have a high proportion of chewed mites have smaller mite populations.

"We had a research project with our Mexican colleagues," explained Dr. Hunt, "that mapped several locations in honey bee chromosomes that influenced the grooming behavior of individual bees in response to having a mite on their back. There were about 30 genes in those regions. We were able to obtain evidence that the Neurexin gene (AmNrx-1) is expressed at a higher level in colonies that have higher levels of mite biting. Neurexin gene produces a protein that is found in the synapse of nerves and regulates synapse formation and function."

Mite grooming and biting appear to be what are called "additive traits," with multiple genes working together in concert.

"We wondered," said Krispn, "what the mechanism was that the bees were responding to that initiated mite biting. Perhaps the bees are responding to the movement of mites in the darkness of the hive, but there is much we still don't know."

Mite biting is "hard-wired" in honey bees

The mite-biting trait that Krispn noticed and Hunt decided to start breeding for is not something new. Mite biting is a trait that is present at a low level in most diverse populations of *Apis m.* (The Asian honey bee, *Apis cerana*, has over the centuries developed grooming and mite biting to a high degree to survive with varroa mites.) "Russian bees have a naturally higher expression of grooming, partly because they have been dealing with the mites longer than other ecotypes. Italian bees have a naturally very low expression of the trait," Krispn explained.

Purdue has been selecting their best mite-biting hives and crossing those bees to increase expression of the trait. After 16 years as a honey bee breeder and researcher, Krispn is finding much higher levels of mite biting, as well as lower number of mites overall, than were found in Purdue's hives in 2007 when the trait was first noticed. "We had about 5% mite-biters in the beginning and have achieved upwards of 50% overall. The mite-biter hives, in previous studies comparing them with other commercially available stocks used as controls, also produce more honey (because dead hives do not produce honey!) and have better winter survival than the controls."

To treat or not to treat

Purdue's hives require minimal miticide treatment. Krispn treats about 5-12% of Purdue's 150 hives a year for varroa mites. "I only treat," Krispn explained, "when we see Deformed Wing Virus symptoms, or sick crawling bees in front of the hive with twisted wings.

"Even great genetics for fighting varroa can be overwhelmed by high mite loads and virus pressures." The problems usually arise from nearby hives with queens that lack the genetics for mite biting and grooming, or even VSH. Varroa mites are parasites that "hitchhike" on the bodies of robbing bees that carry them from the dying, infested hive to strong healthy hives.

Sometimes these mite loads can be more than the mite-biters can handle. Besides the mite loads, the mites vector viruses. "The mite's bite is like a dirty hypodermic needle," explained Krispn. "When it feeds, the mite introduces viruses like DWV that eventually compromise the bees' immune system. The viruses get in the hemolymph (bloodstream) and replicate and are passed from bee to bee. The viruses can eventually compromise the immunity of the entire superorganism. I've seen a colony have all the perfect traits, yet be overwhelmed with mites and virus. "Many hobbyists don't like to treat, but I don't believe in 'letting nature take its course' and simply allowing mite-infested hives to die. We treat pets and farm animals, for example, when they get sick. Our bees should be no different."

Breeding the bee that bites the mite

"Breeding for the mite-biting phenotype is not as simple and straightforward as it may sound," Krispn explained. "Factors other than genetics can influence the number of bitten mites in the hive, and the beekeeper must take into consideration the history of the colony."

If the colony swarmed in the spring, for example, this could have reduced the mite population because swarming produces a break in brood rearing. Did you split the colony? Was the queen superseded? That could produce the same effect. The breeder must keep good records.

Mite biting is also influenced by environmental factors, like the time of year. "If there's a large population, there will be an uptick in mite biting. In the spring, if a lot of young bees are emerging that have not yet become foragers, you'll have more chewing. In the fall there can suddenly be an uptick. Hives chewing fewer mites the first time we check them can later have a higher proportion of bitten mites." That's why it's important to check the proportion of chewed mites at least three times each season.

"I handle most of the breeding myself," Krispn explained, "with the help of an undergrad. I maintain the population and do the crosses, keeping good records on the colonies and selecting 'best of the best.'" The colonies with the most chewed mites are chosen from the previous year's data.

Medhat Nasr

Kirsten Traynor

(L) *Krispn with three great friends and pioneer honey bee breeders/researchers. From left, Dr. Greg Hunt, Krispn, Dr. Rob Page, Jr., and Dr. Ernesto Guzman in Ontario, Canada.* **(R)** *Krispn and Dr. Keith Delaplane at the University of Georgia Bee Lab. Krispn, as insemination consultant, inseminated 120 queens for Dr. Delaplane's polyandry project.*

"We don't have a long enough breeding period in the north to pick them based on the current year's data.

"This is a hierarchical breeding program with grooming and mite biting at top, but we also select for other characters at the colony level," explained Krispn, "such as gentleness, disease resistance, overwintering ability and comb temperament. Our breeder queen's colonies must also pass the freeze-killed brood assay. Within 24 hours we like to see 98% removed. We have eliminated chalkbrood by selecting it out. If I have two hives with great genetics, and one hive is dark bees and the other is light, I'll select from the darker phenotypes because they seem to survive better in the North.

SDI

Krispn advises that you can accelerate trait selection with Single Drone Insemination (SDI), inseminating the queen with the semen from one drone. "This can really amplify a trait quickly because the colony is comprised of one subfamily — we call them 'supersisters'— expressing all the behaviors determined by their genes.

"The downside of SDIs, however, is that the bees don't perceive the SDI queen as a well-mated queen, and will often try to supersede her in the beginning. Or she may turn into a drone layer within six months. That's because she will run out of semen more quickly than a Multiple Drone Inseminated (MDI) queen. For an MDI queen, Krispn uses the semen of 8 to 10 drones.These queens can last 2 to 5 years if managed well. But SDI is a great tool for trait selection in a modern beekeeping paradigm because you're able to increase the expression of a particular trait of interest."

Bee breeding is a road that never ends

Bee breeding is a process, not a goal. "We can never say, because we've achieved 50% mite-biters, for example, that we've 'arrived.'" Because queens naturally mate with multiple drones in the drone stream, many of which would have no mite-biter genetics, it's a constant battle to keep breeding for the desired traits so they're not diluted and lost. You also have to be alert for, and breed out, undesirable deleterious alleles that can pop up in trait selection, like chalkbrood or aggressiveness. "It's like walking a tightrope. Some traits are easier to select for than others. Mite biting is a very complex trait. The mites are also adapting to behavioral shifts in the nest."

Needed: More bee breeders

"What we really need," stated Krispn, "are more bee breeders who are seriously selecting for heritable traits. We have plenty of queen rearers. Sometimes the best bees are in our backyards — locally adapted bees we can use to select for high levels of certain desirable traits."

One of the great challenges for breeders is **controlled mating**. Unless you have an isolated island of your own, or are in an extremely isolated area, you will need to do instrumental insemination if you are going to control the drone side of the genetics. Otherwise, you may have a great queen, but if she goes out and mates with drones carrying unknown genetics or alleles, any good traits she may have had to pass on to her progeny will be seriously diluted.

The procedure for doing II is exacting. "You need a good eye and a steady hand," explained Krispn, "and you need to be able to concentrate for long periods. Sterile technique is a must. But the procedure is one that most tenacious people who apply themselves could learn."

The expense of specialized equipment for doing II is also a limiting factor for some. That, however, is changing for the better. Apis Engineering, a company that mechanical engineer and beekeeper Dale McMahan started with Krispn as his business partner, has already produced two models, the innovative Queen Station and the MicroStation, and is planning to put on the market a more economical model soon.

Beekeepers interested in becoming honey bee breeders can check out Krispn's top-notch PowerPoint, which could well be titled "Everything you ever wanted to know about breeding for varroa resistance," to find out how to get started. You can download it from the Heartland Honey Bee Breeders Coop website, **https://hhbbc.org/**.

Current genetic detective work by Dr. Brock Harpur

Dr. Brock Harpur, hired by Purdue in 2019 to replace Dr. Hunt after his retirement, is hard at work developing a practical, in-the-beeyard test to help beekeepers identify hives with mite-biting potential. "One of our goals with the mite-biting project is to develop some kind of predictive technology for beekeepers so they can look at the stocks in a bee yard and say, 'Based on their genetics, this colony is more likely to

produce mite-biters than that colony over there.'

"I'm sequencing all the colonies In Purdue's bee yards," Harpur continued. "I can compare them to other colonies around the world and find differences, but because the trait is so nuanced — difficult to measure and difficult to work with — it will take a little time to do the genomics and to be sure we have identified the mutations associated with mite biting."

Dr. Harpur, frequently described as a "world-class population geneticist," has inspired hope in the beekeepers who have met him. Pennsylvania beekeeper Jeff Berta, for example, who has worked hard to make the mite-biter genetics available to PA beekeepers, said, "Now that Dr. Brock Harpur is at Purdue, he's coming at the problems from so many different angles, and he has so many great ideas, I know in another couple of years we're going to be several steps beyond what we can imagine now."

References

1 Moosbeckhofer, R. Beobachtungen zum Auftreten beschädigter Varroamilben im natürlichen Totenfall bei Volkern von *Apis mellifera carnica* (Observations on the occurrence of damaged Varroa mites in natural deaths in *Apis mellifera carnica* colonies). *Apidologie* (1992) 23 (6):529-530.

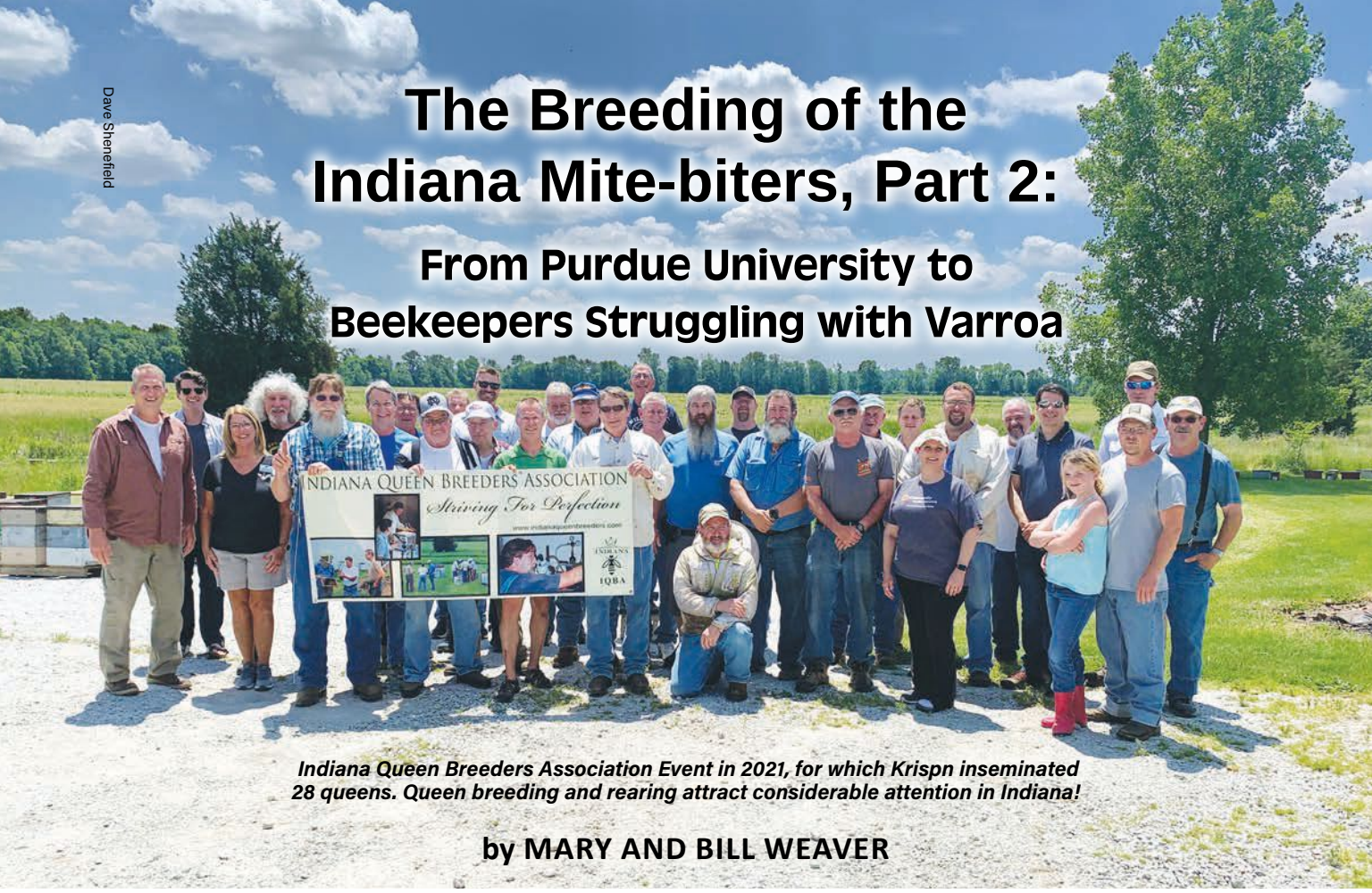
After a decade of high school teaching, **Mary and Bill** spent 15 happy years running a wholesale vegetable growing operation. The beekeeper they hired to pollinate their squash seemed to be having such a good time working his bees that Bill said, "Why should he have all the fun? Let's get ourselves some bees." Several years later, the Weavers had built up to 600 hives, moving them for spring and summer pollination and bottling all their own honey, in addition to many hundreds of barrels purchased from Midwestern beekeepers.

Mary has been writing for beekeeping and vegetable growing publications for 40+ years, with Bill as photographer.

The Breeding of the Indiana Mite-biters, Part 2:

From Purdue University to Beekeepers Struggling with Varroa

Dave Shenefield



Indiana Queen Breeders Association Event in 2021, for which Krispn inseminated 28 queens. Queen breeding and rearing attract considerable attention in Indiana!

by MARY AND BILL WEAVER

Over the past quarter century, Dr. Greg Hunt and Krispn Given at Purdue have done the laborious work of breeding a line of "Mite-biter" bees. Mite biting is a genetically transmitted behavioral trait. Bees with this trait do battle with varroa mite intruders in their hives, biting their legs and bodies, disabling and killing them. As U.S. honey bees are gradually developing resistance to that "workhorse" of miticides, amitraz, this trait is becoming increasingly valuable.

Getting the mite-biting genetics from Purdue to the beekeepers who are struggling to keep their hives alive, however, has not been easy. Unfortunately, the mite-biting trait is not easy to test for or to breed for. Consequently, most U.S. queen rearers don't include this trait. The skilled labor needed for selecting, mating (including Instrumental Insemination, or II), maintaining, and monitoring the purity of the line would be prohibitively costly for a private bee breeder.

Dwight Wells

Dr. Greg Hunt and Krispn Given with a double nuc owned by Dwight Wells in Logan County, Ohio containing two II Purdue queens

Fortunately, the connection between Purdue's breeding program and grassroots beekeepers has been made, since 2013, by an astonishingly dedicated, I would even say heroic, group of beekeepers who have given up their free time and, in many cases, run themselves ragged to make this connection because they believe in the value of Purdue's Mite-biters. Helping with Insemination Fests at Purdue from 2013 to 2019 (until the pandemic), these beekeepers have purchased pricey II breeder queens with Mite-biter genetics, taken them to their home states and produced as many locally-adapted daughter queens as they possibly could to sell to interested beekeepers.

The demand for Mite-biter queens is there. "I could sell many, many times the number I can produce," stated Meghan Milbrath, owner of The Sand Hill Apiary and assistant professor of entomology at Michigan State University. "There is a lot of interest in Indiana Mite-biter genetics in Michigan."

This article will tell the stories of four of these exceptional beekeepers who brought Mite-biter genetics

Larry Connor

Queen rearing class at Purdue

from Purdue to beekeepers in their home states, and will end with a look at what's ahead for Purdue genetics research under Dr. Brock Harpur. (My apologies to the many other beekeepers who have also worked long and hard on this project whose stories I am not able to include.)

Dave Shenefield and the Indiana Queen Breeders Association

The Indiana Queen Breeders Association (IQBA) in Purdue's home state was the first of many queen improvement associations that grew out of Purdue's breeding work.

Commercial beekeeper Dave Shenefield recalls the early days. "I was President of Indiana Beekeepers Association," he explained. "Dr. Hunt gave me my very first II queen. She lived for four years. Dr. Hunt wrote a two-year grant for us to finance the start of the IQBA. I recognized the importance for our own operation of raising our own queens from locally-adapted stock. We tried to encourage other beekeepers to do this. We raised queens to distribute and held queen rearing classes.

"I asked around to find out why so many queen-improvement projects don't last. The answer was simple: They ran out of money. So, we made our group a 501c3 so we could accept donations. We drew it up so that whenever a member sold a daughter from an II queen, they added $5 to the price of the queen, then donated that $5 to the group. So that's how we are funded, and we're very financially sound." The group even owns three [pricey!] devices for doing II. "We use them for training, and they're always available for beekeepers to take home.

"The IQBA has an Insemination event each year the first weekend in June," Shenefield continued. "We teach II as well as queen rearing. We

Dave Shenefield

IIndiana beekeeper Dave Shenefield, at right, in California almond pollination with sons Derek, left, and Cory. Dave had a prize Purdue II queen that survived and thrived throughout five trips to CA and back!

Krisspen Given

Everted mite-biter drone ready for semen collection

Gladys Andino

Krispn teaching II class in 2015. Front to back: Marlyn Miller, Mike Risk, Bernie Svidergol, John Wilson, and Dwight Wells.

inseminate about 50 queens from our selected stock. These are then given to queen breeders in the group. They are free. We just ask for a donation." Shenefield also brings to the event some of his operation's own II queens that have done well. "We let people graft from them, and they can take the cells home."

Mite-biters in a large commercial operation

Shenefield is a successful second-generation commercial beekeeper, running 3,000 colonies. "We truck 2,000 to CA almonds every year, and keep the other 1000 on bottom boards in permanent locations in Indiana," he explained.

"To keep our Mite-biter Indiana survivor stock going, we haven't brought in any other genetics besides ours and Purdue's for 20 years. We never buy queens or bees. When we did, our mite counts and winter losses always went up because those bees weren't suited for our Indiana conditions.

"We haven't been to Texas since 2001. We raise all our queens in Indiana, raising nucs through July and queens through the whole season." It helps that Shenefield's operation doesn't requeen all its hives every year. "Instead, we breed for queen longevity. I think selecting for longevity is a big thing we do that has helped make us successful. When I get a good, productive queen, I like to keep her around. We had one II queen that lasted for five years, including yearly trips to CA, before she finally went missing."

Shenefield's son Derek and employee Matt Evans raise all the queens for the operation. "We don't have time to check for mite-biting or do alcohol washes, but we do use sticky boards, usually when the hives should be low on mites, to make sure everything is under control," noted Evans.

"The last genetics we got from Purdue was in 2019," Evans continued. "Dr. Harpur brought a nuc with a single-drone-inseminated (SDI) queen Krispn did that was really, really good for mite chewing. Dr. Harpur explained to us that when you find a really good mite-biter hive, SDI is a good way to fix that trait. Krispn had inseminated the queen with a single brother.

"Dr. Harpur gave us permission to graft from her. We raised 18 queens, labeled SDI-1 to SDI-18. We are still using SDI-1 and 2. Those queens have also gone to CA every year. Whenever we put in a cell, we mark the side of the hive with the name of the breeder queen. That way when we see a hive that's really doing well, we can check its genetics."

Tom Campbell, Purdue

Krispn Given heads a beekeeping workshop at Purdue in 2016 as part of his Extension work.

"Because we go to CA," added Shenefield, "we routinely treat all our hives, even though we use Mite-biter genetics. But when our bees are not around a lot of mite-infested, susceptible bees, they do very well. There is no breed that will never need to be treated." Even the best genetics can eventually be overwhelmed by large mite loads and high virus levels, caused by robbing out of dying infested hives, for example, and will need treatment.

When eight of Shenefield's hives were tested for the Aphis Apiary Health Assessment on October 4, 2021, mite levels were found to be 1.1 mites per 100 bees and the nosema load was 0.1, both well below the recommended treatment thresholds. There were no deformed wing symptoms, and virus loads were low.

Shenefield remarked, "Each year, we select queens from a couple of our best hives, and Krispn inseminates those. Then we graft from them. Because we don't buy outside bees or queens, the Mite-biter gene pool among our drones in the hives that stay in IN is really good."

Ohio beekeeper Dwight Wells

Dwight Wells, a retired project engineer at General Motors whose job was selling ideas to plant managers and vice-presidents, had learned beekeeping in 4-H as a young teenager. Then a very busy life intervened. But when Wells was retired and newly single after the death of his wife, he decided to reconnect with the beekeeping community.

Larry Connor

Inaugural HHBBC event at Purdue University Honey Bee Lab with Dave Shenefield holding the banner. First row Marlyn Miller and Ginger Davidson; second row Jeff Berta, Dwight Wells, Dave Shenefield, Krispn Given; back rows Joe Kovaleski, Jeff Dittemore, Dan O'Hanlon, Sammy Moehlet, Dr. Greg Hunt, John Wilson.

Krispn Given

Dwight Wells of Ohio holds a queen cage with a Mite-biter queen.

"I saw my first varroa mite under a microscope at the Heartland Apicultural Society (HAS) in 2011," he recalled. "I crossed paths there with Dr. Greg Hunt and Krispn. Later I got better acquainted when several of us were helping them with a queen rearing course they were giving at Purdue in 2013.

"I was astounded that Greg and Krispn weren't spreading the word about their work in breeding Indiana leg-chewers. A group of us helping with the course went to a restaurant for supper at the end of the day. I said, 'I think the Heartland states should come together and exchange genetics and publicize what Greg and Krispn are doing and see to it their breeding material is distributed widely.' Judge O'Hanlon was sitting beside me, and he said, 'Dwight, I think that is an excellent idea.' We were determined that the whole world would know about the important work they were doing at Purdue."

Krispn Given

Megan Milbrath hard at work for a custom advanced II course taught by Krispn at Purdue

Wells got together with several members of HAS before their 2013 annual meeting to broach the idea, which was well received. As a result, the Heartland Honey Bee Breeders Cooperative (HHBBC) was officially voted into existence at that summer's HAS meeting.

"That winter," continued Wells, "Judge [Dan] O'Hanlon wrote the legal documents to establish the group as a 501c3 nonprofit." Indiana, Ohio, and West Virginia immediately joined. Pennsylvania followed later, but PA beekeepers were a part of the organization from its beginning.

Wells was a tireless promoter for the new group. "I put 25,000 miles on my pickup in short order, giving presentations to beekeeper groups all across the Midwest. It's easier now," he added. "I do it by Zoom."

Wells was also a 'heavy lifter' at the annual Insemination Fests the group held each summer at Purdue. "There were so many details to organize so the inseminations would run smoothly," explained Wells. "I'd spend several days in a motel working to get drone collections organized and training helpers to be sure we had enough people to get the job done. We'd go out to the hives about 1:30 p.m. to collect the previously marked drones returning to the hives for a meal of honey. We grabbed them with our fingers from the queen excluders in front of the hives. When we had collected a container full of about 150 drones we took it back to the lab, laid a paper towel soaked with honey across it so the drones could eat, and then put them in the incubator. Later we would harvest the semen from them.

"Once the queens were inseminated, we made sure people were waiting to put them in queen cages and then into traveling nucs. The queen is very fragile for a while. If the bees don't take care of her, she will die. Everything has to be timed precisely."

Meghan Milbrath recalls, "Every summer the Purdue building was taken over by lots of super-eager beekeepers who felt a lot of ownership and investment in the program. I'm sure it was very disruptive and took a lot of planning from Greg and Krispn, but they were great!"

Krispn remembers those long, hectic days well. "I got so that it didn't bother me when I was trying to inseminate queens with six people looking over my shoulder and talking to me and snapping pictures. It made me better in the long run," he chuckles. In the midst of all this enthusiastic activity, Wells' organizational abilities were very valuable!

Through the years, Wells has also encouraged and mentored new beekeepers across Ohio, teaching them queen rearing in addition to basic beekeeping. His schedule of speaking, teaching, swarm trapping, and working with his own hives would be daunting to a man 30 years younger!

West Virginia beekeeper Dan O'Hanlon

Judge Dan O'Hanlon has been another key player in getting the Mite-biting genetics from Purdue to the beekeepers struggling with varroa. Beekeeping has long held an important place in O'Hanlon's life. His bees have been a valuable escape from the pressures of the courtroom. When varroa mites started ravaging his hives, O'Hanlon said, "I wanted to fight this thing that was killing the bees.

"Our West Virginia ag commissioner, who has always been very supportive of beekeepers, got together $100,000 to 'do something for the beekeepers,'" related O'Hanlon. "He had $90,000 of the spending figured out. I said, 'If you end up having $10,000 left over, I'd like to start a new queen producing group here to improve WV queens.'

"We ended up getting that $10,000," O'Hanlon continued. "I took queen rearing courses for two summers

Dr. Kathy O'Hanlon

Dan O'Hanlon, West Virginia bee breeder and first chair of HHBBC, holds 10 queens from Purdue that Krispn has inseminated. He'll use these to raise daughter queens to sell to WV beekeepers.

with Sue Cobey, who emphasized the importance of using locally-adapted queens. We started looking to see where we could get the best queens for our state. That's when we found out what Dr. Hunt and Krispn were working on."

O'Hanlon was elected as the first chair of HHBBC, and the group hired Krispn for marathon sessions of instrumentally inseminating queens for the members. "Those queens were both very precious and very expensive," stated O'Hanlon. "The participants took these latest and greatest queens back to their home states. There queen rearers grafted from them to produce locally-adapted daughter queens with the top Purdue genetics to sell. We did this every summer from 2013 to 2019, until the pandemic.

"Dr. Greg Hunt and Krispn were our heroes," continued O'Hanlon. "They had done the science. They helped us, and they allowed us to come in and get breeder queens with the top genetics every year. It worked extremely well primarily because of Krispn. Krispn is a guy that everybody likes. He worked hard to inseminate all our queens. I can't say enough good things about Dr. Greg Hunt and Krispn."

To spread the word about the project, O'Hanlon became a traveling ambassador for Purdue's Mite-biter genetics. "I was asked to speak all over the country — as far away as California, down in Alabama, over in upstate New York, in many, many states," he continued. "Whenever I went to speak, I always took some of the Purdue daughter queens I had raised with me so that if a new state was interested, I could make sure they got 25 or 30 queens to get them started. Because of Purdue, I was able to give them hope that we were making some progress against the varroa."

In 2014, while employed full-time as a judge, O'Hanlon singlehandedly raised an incredible 1200 daughter queens to sell to beekeepers across WV. "We tried to get these genes out as fast as we could, all across the country. That was our goal."

A group of WV beekeepers organized the Mountain State Queen Producers. O'Hanlon turned out to be a very effective writer of grants. The grant money paid for a succession of II classes, both beginner and advanced. In one class alone, for example, 10 WV beekeepers and six from other states learned the art of II. Grant money also

Krispn Given

Beekeepers learn the fine art of queen rearing at HAS. Krispn designed the popular course, which is offered annually to 40 students.

paid to send an outstanding WV bee breeder, Jason Bragg, to Washington State University to take an advanced II course from Sue Cobey. While there, Bragg also learned to cryopreserve honey bee sperm. He has since set up his own cryopreservation system, probably the only such private system for honey bee semen in the U.S.

"Breeders can make a lot of money producing queens if they use II and can guarantee there are Mite-biter genetics on both sides," noted O'Hanlon. "Using II is the only way to control both sides of the equation, like race horse breeders do, in breeding honey bees."

Jeff Berta

Jeff Berta, former president of HHBBC, grafts in one of his Pennsylvania bee yards.

Pennsylvania beekeeper Jeff Berta

Jeff Berta, a vegetable grower, got hooked on bees after helping a beekeeper providing pollination services for his farm to unload a batch of hives that weighed over 200 pounds. "By the time we were done unloading the hives and I'd watched the beekeeper checking that they were queenright, I was excited and captivated," he recalled. "When it was time to move them away, I just bought them."

Pennsylania joined the HHBBC as their 4th state. "One of the restrictions to joining was that you had to have a state queen improvement program in place so the breeder queens from Purdue could go to beekeepers who were actually producing queens and could disseminate the genetics. We set up the PA Queen Improvement Project (**https://psqueenimprovementproject.com**) and put together a structured approach to improving, particularly, the winter hardiness and varroa resistance of our PA bees," he explained. "Our PA State Beekeepers Assn. President Charlie Vorisek appointed Mark Gingrich and me as co-chairs.

"Dr. Hunt got each of the HHBBC states a USDA SARE grant for $4,000 for four years. That money paid for

about half of the expenses to distribute mite-biter queens across the state. With the help of that grant, PA went on to produce more Mite-biter germplasm — II queens, virgins, open-mated queens and queen cells — than any other state. A lot of that was because our state association newsletter supports us and gave us publicity."

Berta was also successful in personally applying for SARE grants. "I was awarded seven grants up to 2018, and those grants paid for a lot of the queen development in PA," he recounted. "We checked mite levels with sticky boards, and we hired a Penn State student to check for the number of bitten mites and keep records. Sometimes these Mite-biters can surprise you. We do everything by 300 bees. One hive went quickly from 6 mites to 50, even though those bees had been chewing 50% of the mites. That's crazy high. It should have been below 6. I thought, 'That's a pretty crappy queen.' This was in October when mite levels tend to spike. But two weeks later, we did another alcohol wash, and those bees had the mite count down to 6 again. Any other hive would have been crushed by a mite count like that! But if we hadn't been paying attention, we wouldn't have noticed it."

During the pandemic when Purdue was closed, the PA group didn't miss a beat. "We'd get together at someone's place and bring virgins and drone colonies and nucs and do our own insemination events. We did this four times in 2020 and two or three times in 2021.

"You can never stop selecting for Mite-biter genetics, or you will lose your trait," Berta continued. "We looked at this problem while doing one of our SARE grants. The first-generation daughters of our Mite-biter breeder queens were good. The granddaughters were good. But with the great-granddaughters, we could see a drop-off in the Mite-biter trait. This can happen quickly if a hive swarms a couple of times."

Berta summed up progress on disseminating the Mite-biter trait this way: "We didn't develop any of this. We're standing on the shoulders of giants and are privileged to walk in their footsteps. This whole thing could die unless people step up and keep doing it. We're carrying a torch. Passing it on is how we pay our debt to our forebears."

Future genetic research at Purdue under Dr. Brock Harpur

Dr. Brock Harpur, a geneticist hired by Purdue in 2019, is very hopeful about the future of honey bee breeding. "With cattle and pigs," he explained, "it's possible to use genomic information to predict how much meat or milk they will produce or how good they'll be at producing offspring. All their heritable traits can be predicted from genetic sequencing.

"Honey bees have a very different biology from cattle and pigs. We can't control their mating as easily. But it's reasonable to predict that within a decade, if the industry starts looking at sequencing for ancestry identity, honey bee breeding can be like the other animal industries in predicting traits from the genomes of honey bees. We need to look at sequencing the mutations in the whole genome."

Harpur is looking to sequence the genomes of as many colonies as possible so he can make correlations between the genetic mutations and the corresponding traits of the bees. "We'll be able to predict that, given these mutations, we're likely to see this trait or that trait," he explained.

The downside of sequencing a lot of colonies is that it's expensive. But it's far less expensive than it used to be. "Ten years ago, when I started my doctorate, the cost to sequence a single bee was $100,000," he explained. "It was obscene!"

Today Harpur's group can sequence a bee for less than $100. Much of that cost, according to Harpur, is for enzymes and chemicals needed to treat the DNA extracted from the bees so it can be sequenced. "You can't just sequence raw DNA," he explained. Those chemicals are likely to remain a large part of the fixed costs in the future.

Harpur continued, "If we can get the cost low enough and I can sequence a large percentage of the colonies in this country, I think we can have some really good predictive values. This is something we're striving for in my lab. We're offering sequencing services to individuals. In 2021 we received samples from 300 colonies. Working with IQBA and HHBBC, we're hoping to start sequencing every colony we breed. It'll be a big step forward!"

Harpur is a very approachable academic who enjoys working with beekeepers. "The beekeepers inspire me and ground me. I get the most wonderful questions in my inbox. Sometimes these have even led to projects in the yard," he commented.

Larry Connor

Krispn answers questions from students at a queen rearing course at Indiana Summer Field Day at Purdue Bee Lab.

After a decade of high school teaching, **Mary and Bill** spent 15 happy years running a wholesale vegetable growing operation. The beekeeper they hired to pollinate their squash seemed to be having such a good time working his bees that Bill said, "Why should he have all the fun? Let's get ourselves some bees." Several years later, the Weavers had built up to 600 hives, moving them for spring and summer pollination and bottling all their own honey, in addition to many hundreds of barrels purchased from Midwestern beekeepers.
Mary has been writing for beekeeping and vegetable growing publications for 40+ years, with Bill as photographer.