Breeding *Varroa*-Tolerant Honeybees: Examining, Demonstrating, and Disseminating a Flexible Selection Method

Principal Investigator

Scott A. J. Johnson, Ph.D.

Director	11927 West State Road 59	scott@lowtechinstitute.org
Low Technology Institute	Evansville, Wisconsin 53536	608-886-9584

Bio: Johnson comes to this project with a background in evolutionary biology (Anthropology Ph.D., Tulane, 2012) and treatment-free beekeeping on the small scale. His academic work has provided him with experience using data, statistics, and the scientific method to explore research questions. In 2017, he was awarded two grants from the United States Department of Agriculture. One funded a pilot bee-breeding study. The second grant supported a controlled study of potato-growing methods with market gardeners. He has also received grants from the National Science Foundation, National Geographic Society, American Philosophical Society, and others.

He is the founder and director of the Low Technology Institute, a 501(c)(3) nonprofit research organization. The premise of the institute is that when fossil fuels are no longer available, we should have developed strategies for individuals, households, and communities to be self-sustaining using local resources. Bees are essential in this future, as they not only provide calories but also make other food-production efforts more efficient. Without fossil fuels, it is unclear what miticides will be available and therefore breeding locally adapted, mite-tolerant bees is the institute's primary avenue of bee research.

Johnson has experience communicating scientific concepts to nonprofessional audiences. In addition to teaching at the university level, he has written two books, both praised for their lucid explanation of complex topics in easy-to-digest language. He speaks regularly on bees, the history of societal collapse, and human adaptation to changing environments.

Cooperating Investigator

Paul Zelenski

	W3508 Exeter Crossing Rd	paulzelenski@gmail.com
	Belleville, WI 53508	608-957-6858

Bio: Paul Zelenski began his interest in bees as a kid when his dad kept a few hives in the back yard. Once he purchased his own house, he was able to get a couple hives and begin the beekeeping tradition again. Despite dire warnings that first-year hives never make it through winter, those hives survived and were split into more. Those hives were subsequently split into more hives until Paul quit his job to focus on keeping bees. He has been keeping bees now for over ten years with a focus on the local community. He takes joy in educating new beekeepers and giving them the knowledge they need to make informed decisions about treatment levels and different management techniques. He also works to raise locally adapted bees and produce locally mated nucs without sending his bees south for the winter. He is excited to work on this project as a way to offer more sustainable options to local beekeepers who wish to get off the package and heavy treatment treadmills.

Date and Duration of Proposed Study

The study will begin in the spring of 2020 and continue for at least three years. We are requesting funds to cover the major expenses associated with start-up costs in the first year. We will be able to provide a final report of activities supported by PAm funding in the spring of 2021 and can provide updates as the long-term study continues.

Problem and Significance

No one wants to treat honeybees against *Varroa destructor*, but commercial, side-line, and hobbyist beekeepers spend considerable time and money to prevent high mite levels through chemical and behavioral means. The solution is a European honeybee that does not need treatment to survive with mites but will still produce a viable amount of daughter colonies and honey. Creating such a bee is not the problem—it has been done independently in at least three locations outside of North America. The two impediments to wide-scale implementation are lack of knowledge and/or concerns about financial feasibility. This study plans to 1) replicate and document the method to breed mite-tolerant bees in North America and 2) develop and share resources for beekeepers of all scales to adopt this system.

Beekeepers find themselves on a "treatment treadmill" that locks them into repeated applications of mildly to fairly toxic miticides and the use of behavioral methods to increase their winter survival rates. *V. destructor* quickly spread from its first occurrence in the US in 1987 to every state, and beekeepers scrambled to find a solution to their high rate of winter losses. The answer came in the form of chemical treatments, but the well-worn observation that it is "hard to kill a bug on another bug" is proving prescient. The first chemicals interfered with mite (and to a lesser extent bee) nerve function but left residues in wax and honey. Later, organic acids and other less toxic compounds were introduced. Additionally, beekeepers began to employ behavioral methods to control mites (e.g., culling brood comb, brood breaks, small-cell comb, and screened bottom boards). Regardless of treatment method, however, the symptoms are addressed, not the root cause. Beekeepers must continue to use chemicals and/or behavioral methods to treat their colonies because the bees are not allowed to develop tolerance, and prophylactic treatment masks any naturally occurring resistance.

In Asia, *Apis cerena* has developed behavioral tolerance to *V. destructor* (which originated in this region) and similar adaptations have occurred in isolated *Apis mellifera* populations. When *Varroa* emerged over a century ago in Asia, the local beekeepers had no treatments available and the local bees went through a genetic bottleneck, allowing only bees with mite-tolerant behavior to survive. More recently, researchers in Wales, Sweden, and Germany have been recreating this process through selective breeding of *A. mellifera*. Others have noticed feral honeybees that have developed mite tolerance in France and Africa. In all of these instances, bees have been artificially or naturally selected for mite tolerance by letting the nontolerant colonies die.

The process is counterintuitive: stop treating for mites to breed mite-tolerant bees. Without treatment, most colonies succumb to mite loads in a season or two, but a few will survive. Queens are raised from the strongest surviving colonies. Because queens mate with fifteen to twenty drones, genetic segregation can be challenging. By using many colonies and isolating them, the local gene pool will be flooded by the survivors' genes. Previous studies suggest an initial heavy die off and gradual improvement of winter survival until a new, stable population of mite-tolerant bees has been established. This study will demonstrate whether or not this breeding strategy is successful in a North American context. Importantly, the project will use simple beekeeping techniques available to every beekeeper to carry out the task. Finally, a series of resources will be produced to assist other beekeepers in converting their treated colonies into mite-tolerant ones.

Beekeepers agree that mite-tolerant European honeybees are the long-term solution to the *Varroa* problem. While others have shown that selective or natural selection can produce *A. mellifera* that can survive with mites in the Old World, this solution is not adopted in the New World because it is either unknown or considered economically unviable. This study will test the feasibility of this program on a medium scale in North America. The data from this study will be used to create resources (online, in print, and in person) to attack the lack of knowledge surrounding this solution. The data will also inform a plan for larger scale producers to transition away from treatment-dependent bees, making this a more economically attractive proposition. The successful demonstration of this breeding strategy and targeted information sharing may make a difference in the systemic challenged posed by *V. destructor*.

Objectives

This project has long-term and short-term objectives. As is explained in the previous and following sections, this study will attempt to breed mite-tolerant honeybees through natural selection and expanding survivor colonies. If the study bees follow the same trajectory of similar breeding programs in other parts of the world, the following milestones are expected: 1) Significant die-off in the first one to three years, with 50–90 percent attrition. 2) Survivors' hardiness will improve between years two and five; mite levels should be observed at a steady, low level. 3) Colony populations should stabilize and evince better-than-average survival in spite of the presence of a steady, low mite level. 4) Once a stable population is recognized, colonies will be selected for honey production and workability. 5) Throughout this process, a growing number of beekeepers will be reached through hands-on workshops, literature, presentations, online resources, and community building.

Funds from PAm are requested to fulfill our short-term objectives, which set up the study and allow it to run out its long-term goals. The first year of the full-scale project is the most labor and cost intensive. The apiary must be established and populated (Objective 1). The hives must be divided and managed (Objective 2). Nontolerant hives will be allowed to die out and survivors will be split (Objective 3). The process will be repeated until surviorship stabilizes (Objective 4). Finally, tools for beekeepers of all scales must be created and shared (Objective 5). All of these tasks incur costs that cannot be made up by honey sales or other revenue because its success depends on concentrating all the bees' efforts on repopulating their colonies each year instead of producing honey. These objectives are described in detail in the following section.

Experimental Design, Materials, and Methods

Honeybees in North America and globally face the same issues: *Varroa* mites, diseases, viruses, fluctuating agricultural resources, and pesticides. Together these problems contribute to what is known as colony collapse disorder. *Varroa* mites, however, are the leading cause of colony mortality as they destroy the brood (reducing a colony's reproductive success) and transmit viruses (causing early winter colony collapse) (see summary in Rosenkranz et al. 2009).

Currently, beekeepers must treat their hives with mild to fairly toxic miticides, severely modify the brood pattern, or both in order to keep *Varroa* levels low. These are only short-term solutions, however. Most beekeepers agree that the long-term solution is to breed bees with mite tolerance (ability to live with mites) or resistance (ability to reduce mite loads). As with any attributes we want in domestic animals, we must select for the trait by only breeding those that have those genes. For beekeepers focused on honey production or pollination, however, culling a majority of their hives every year is not an acceptable rate of loss. This project, therefore, proposes to keep hives specifically for developing mite-surviving bees. As these bees will not pay for themselves with honey production, pollination service fees, and the sale of daughter colonies, they need external funding for start-up costs.

Relevance to Beekeeping Industry

The mites were first recognized in the United States in 1987 and spread out quickly thereafter (Wenner and Bushing 1996). Since 2007, the Bee Informed Partnership (2019) has conducted an annual survey of beekeepers in the United States. While this is self-reported data and the results must be taken in that context, a worrying trend is visible, with winter losses rising from a quarter to a third of hives a decade ago to over half in 2018. Although it is difficult to say in every case why a colony has died out, beekeepers perceive *Varroa* as a growing cause (37 percent in 2007–8 to 61 percent in 2017–18). An increasing number of beekeepers have begun treating for *Varroa* (from 28 percent in 2010–11 to 44 percent in 2017–18), yet survival rates for those who use treatment are only 10 to 13 percent better than those who do not throughout this same period. More formally, the USDA survey of operations with five or more colonies (USDA 2019) shows *Varroa* as present as a stressor in between 41 and 56 percent of colonies at any given quarter in 2018—approximately as much as all other stressors combined.

While tolerant and resistant bees have been in development in the United States, these generally involve selection and breeding methods unavailable to the backyard, sideline, and even commercial beekeeper. The Russian hybrid, *Varroa* sensitive hygenic, and Minnesota hygenic bees have all been developed to exhibit tolerance and/or resistance to mites. Research has suggested that many genes control the behaviors conducive to this survivability (Boutin et al. 2015; Kefus et al. 1996; Lapidge et al. 2002; Tsuruda et al. 2012).

Honeybees have developed a variety of defenses against *Varroa* mites. Boecking and Spivak (1999) summarize what have been recognized as the main biological mechanisms of defense. Hygenic behavior entails the entombing or removal of infected brood, first recognized against American Foulbrood and chalkbrood. This behavior is also applicable to combating *Varroa*. Although estimated to occur in lest than 10 percent of hives, hygenic behavior can be selected for by allowing the mites to remain present in the population (Boecking and

Drescher 1999; Spivak 1996). Bees also use grooming to remove *V. jacobsoni* (Boecking and Ritter 1993) and *V. destructor* (Szabo et al. 1996) mites from infected sisters.

These targeted breeding programs have the benefit of professional researchers and specialists with funding and resources to focus solely on this problem. Most backyard, sideline, and even commercial beekeepers do not have the time or monetary resources to undertake similar efforts. This project, therefore, follows the suggestions of Blacquière and colleagues (2019: 2519), who "call for local groups of beekeepers and scientists to join a novel natural selection program," in what they call the "Darwin's Black Bee Box" breeding program. While it is important—vital in fact—for researchers to continue to develop and understand the relationship between *V. destructor* and *A. Mellifera*, this method allows nonspecialists to steer genetic selection towards greater survivorship.

The first scientific case study of this method was carried out on the island of Gotlund in the Baltic Sea (Fries et al. 2006). A hundred and fifty colonies with low level *Varroa* infestations were established on this island. The colonies were left untreated and allowed to swarm. Initial winter mortality was high (76 and 57 percent in years three and four), but quickly stabilized at a low level (13 and 19 percent in years five and six). Mite infestations fell from 40 mites per 100 bees to about half of that during this time. The researchers found a decreased winter mortality rate, increased swarming rate, and constant but low level of mite infestation (Fries et al. 2006: 568).

The West Wales Breeding Project was established in 2011 and continues to move regional beekeepers towards colonies with greater survivorship. After suffering unusually high losses, beekeepers began to repopulate their apiaries with walk-away splits rather than buying in colonies or queens to replace dead outs (Williams 2013: 20–22). This effort is ongoing and producing positive results with anecdotal suggestions of increased grooming behavior (Dylan Elen, personal communication 2019).

In Tunisia, resistant colonies were identified and their queens helped impart some benefit to European hives when exported (Ritter 1990). Anecdotally it has been noted that African beekeepers who did not have araricides available to them for logistical or financial reasons inadvertently selected for mite resistance and tolerance in their surviving colonies after mites reached the continent.

In the US, one study (Kefuss et al. 2016) has carried out a commercial-scale breeding study similar to the one proposed here with positive results. Seely (2007) has noted feral colonies surviving in the Arnot Forest, and Villa and colleagues (2008) report self-selected survivorship in feral colonies in Louisiana. Our study seeks to recreate these, and other international projects focused on a simple selection process available to every scale of beekeeper as well as to create materials to help others implement and test this strategy.

Aims and Objectives

This study is not comparative—it either succeeds or fails to achieve its result. In effect, it attempts to accept or reject the following null hypothesis:

> an apiary of mite-tolerant European honeybees can be bred *in situ* by allowing intolerant colonies to die and heavily increasing the survivor population over five to ten years.

Accordingly, this study has no control group, but survival statistics can be compared to state and national information from the Bee Informed Partnership and USDA surveys.

Objective 1:	Build, situate, and populate thirty hives at Agrecol, a prairie-seed company in an isolated location with rich resources in southern Wisconsin.
Objective 2:	Monitor hives with regular inspections. Aggressively split hives to fill sixty hives by mid-summer.
Objective 3:	Allow nontolerant colonies to die out during the winter and split the surviving hives aggressively.
Objective 4:	Repeat these steps to cull nontolerant genes out of the population and increase survivorship.
Objective 5:	Create resources for our fellow beekeepers. We will use our data and results to write articles for general and technical audiences and publications. This information will also be shared through presentations to interested groups, media, and an online network.

a) For the hobbyist beekeeper: written, video, audio, and presentation materials to describe our methods and how s/he can work to improve mite-tolerance in his/her own hives.

b) For the sideline beekeeper: written and presentation materials with strategies to breed mite-tolerant populations in their own apiary(s) in a cost-effective way.

c) For the commercial beekeeper: a written strategy to create an isolated “breeding apiary” of mite-tolerant bees to feed their traveling apiaries in a cost-effective way.

d) For the researcher: a complete record of our efforts, methods, data, results, and analysis for comparative study.

Experimental Plan

This study will follow the procedures that produced mite-tolerant honeybee populations on the Island of Gotlund, in Wales, and in Africa. The basic steps are to 1) establish colonies, 2) make increases, 3) allow nontolerant colonies to die off over winter, and 4) repeat steps 2–4 until population stabilizes. While this results in large losses in the first three to five years, the population should stabilize and increase without treatment after this “selection period.”

1) Establish Colonies

In early 2020 we will work with Agrecol—a local prairie-seed producer with 80 ac of grasses, flowers, and forests—to establish sixty colonies. In previous years, Agrecol has had a commercial beekeeper providing pollinator services, but the business is committed to advancing honeybee research and has agreed to let us use their space for this study. They will pay us $3,000 per year for pollinator services (of course as we cannot sell honey or nucleous colonies in the first few years to recoup startup cost). We are working with Agrecol staff to place our colonies near species of plants pollinated by honeybees rather than scattershot across their property, as has been the case in the past. Agrecol is located in the country and is fairly isolated from other beekeepers.

Enough hives must be built to house these colonies. Johnson has been building hives for years and maintains a fully appointed woodshop. In the spring of 2020, he will build fifty-five colonies to add to the existing five from a pilot study of this project. Each hive will include a bottom board, two deep hivebodies, an inner and outer cover, and ratchet strap, as well as 20 frames at cost of $155 per colony. Ten hive stands built of landscape timbers and cinder blocks will be built on site before the spring thaw’s muddy conditions. Eight hive stands will be placed around the periphery of the Agrecol property and two hive stands will be placed in the center of the space for breeding colonies, as shown in Figure 1.

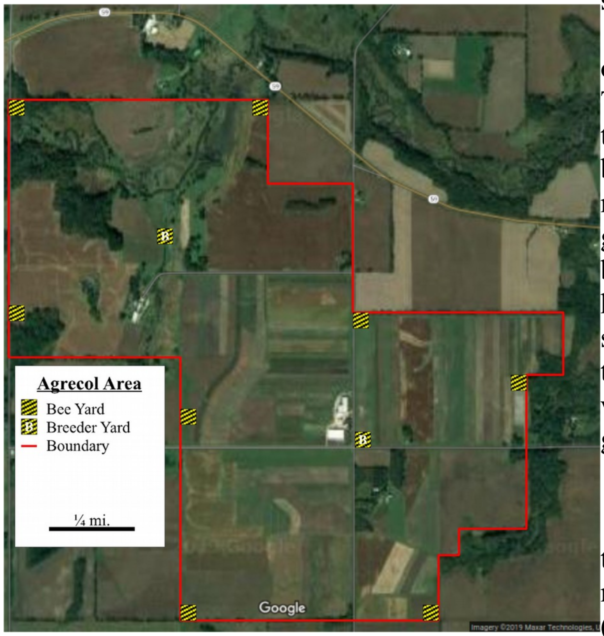

Figure 1: Agrecol property boundary and hypothetical location of bee yards and breeder yards.

As soon as is feasible in the spring of 2020, thirty nucleous (or larger) colonies of bees will be brought to Agrecol. These will be installed in hives on the peripheral hive stands: three or four hives on each of the eight stands. All hives will be south-facing and entrance reducers will be applied and removed as colony strength dictates. Each hive will be held together and to the stand by a strap. Initial recordkeeping will begin at this point, recording the relative strength of each hive, queenrightness, and mite count (see stratified random sample below). Monitoring of the hives will take place every two weeks. Volunteers are likely to assist the project staff with this effort: providing educational opportunities for beginning beekeepers and others who are interested.

2) Make Increases

As the summer progresses, colonies will be divided with the goal of creating sixty total hives. To be split, colonies must meet the following criteria by July 15: have at least six (preferably eight) frames of brood, no obvious signs of disease or distress, and not been split already in that year. Splitting will be accomplished by removing four to six frames of brood (with at least two frames containing larvae less than 36 hr old; drone comb will be left in the original colony), two frames of honey, one of honey, and empty frames and placing them in a single deep box. This new colony will be moved to the central hive stands. The original queen will be left in the original colony with at least two frames of brood, the remaining stores, and enough empty frames to replace those removed.

The now-queenless split will raise its own queen from the less-than-36-hr-old larvae. Three to seven days after the split, staff will check each new colony to insure that a queen cell is present. If it is not, an extra queen cell from a neighboring central hive will be brought in by swapping frames, or, if no extra cells are available, brood with young larvae will be put in the hive without a queen cell. When this queen emerges, she will fly out to mate with local drones—by placing the splits in the center of a ring of our own colonies, she is more likely to mate with a drone within our genetic pool.

As the new colony becomes established with a mated and laying queen, it will be moved out to the periphery to make room for new splits coming to the center. To avoid the loss of field bees, the entrance of the hives will be covered with vegetable matter such as leaves or sticks to cause workers to reorient themselves when leaving the new location.

As the season progresses, a second deep hive body with frames will be added to colonies as they begin to fill the first deep box. If a particularly strong hive fills both deep hive bodies before the fall, its resources will be shared with weaker hives on its same hive stand. After the first year and most of the frames are drawn, this practice may be discontinued, as we are not interested in artificially supporting weak colonies.

The reverse split process reduces the likelihood of swarming. If swarm cells are built and capped, however, the hive will be split, as long as it meets the first two above splitting criteria by the middle of July; if a hive has already been split and still builds up to be strong enough to swarm, those are genetics we should preferentially preserve and expand.

Throughout the season, we will perform both “quick” and “thorough inspections.” All hives will get a “quick inspection” (QI) each month. A QI involves counting the approximate number of “frames of bees” as well as frames with honey, pollen, and brood. Additionally, evidence of queenrightness (visual identification of queen or at least presence of eggs) and lack of obvious illness will be noted.

A “thorough inspection” (TI) will be carried out on one hive per stand throughout the entire season. This hive will be selected through the roll of a six-sided die (each stand can hold six hives) during the first inspection of the season. This stratified random sample ensures that we will have a deeper look into a variety of colonies. A monthly TI involves all of the data of a QI plus a mite count (powder-sugar roll method), hive weight, as well as sending off samples of bees to the University of Maryland’s bee lab to test for diseases at the end of the spring and fall.

3) Allow Nontolerant Colonies to Die Off Over Winter

In the fall we will prepare hives for the winter. Entrance reducers will be put on early in the fall, as the robbing season begins. Near the end of the season, we will carry out a final inspection and consolidation of frames: brood frames moved to the center of the bottom box and honey frames to the center of the top box.

We will not “equalize” hives, but we will distribute the resources of dead-outs. In the fall, many beekeepers take extra honey from a strong hive and give it to a weaker hive to increase its chances of living through the winter. We will not do this because we are attempting to cull the colonies of weaker hives. When a hive has died out in the winter, it is often still full of honey and pollen. In this case, the resources will be distributed proportionally to other hives on the same stand: stronger hives get more of the resources and weaker hives get less. This regressive distribution will exaggerate the underlying natural selection going on throughout the winter die-off.

We will check in on the hives as the weather allows throughout the winter (>55°F, little to no wind). Dead-outs’ resources will be distributed and empty boxes will be collected, cleaned, and returned. We will record the point at which each hive died out.

The winter is also a time for us to consolidate our notes into a database and run any applicable analyses. In the first year we may not have data enough to establish patterns, but in future years, we may be able to predict which hives are likely to die out based on mite counts, hive weight, frame counts, or other data. We will also create our outreach materials during the winter (see *Plans to Disseminate Information*, below).

4) Repeat Steps 2–4 until Population Stabilizes

As each spring approaches, we will begin the cycle again. As the spring sets in, we will assess which hives survived the winter and their relative strength. They will the be redistributed evenly around the peripheral hive stands. We will then proceed through the year as outlined in steps 2 and 3, above. In three to five years—if previous examples are any indication—the population's winter die-off rate will stabilize at a sustainable level.

Once we see a greater than 50 percent winter survival rate, we will begin selling nucleus colonies in the spring. These five-frame hives will be produced from our overwintered stock. We will sell as many colonies as we can make from the colonies above thirty; that is, we want to keep at least thirty colonies to build up to a total of sixty hives (e.g., if thirty-six hives survive, we can sell nucs from six hives and keep the remainder to build up our own population to sixty by winter).

Expected Results and Pitfalls

In the first one to five years we expect to see significant die-off over the winter, especially in year two as mite levels rise. These attrition rates may range from 90–50 percent. By heavily splitting survivors, we will increase the genes of colonies with mite-tolerant traits. Starting in year three, we expect to see a gradual increase in winter survivorship. At the same time, mites will be present at a steady but low level in the colonies.

The biggest potential problem faced in this study will be getting through the tight genetic bottleneck of the first few years. If die-off does approach 90 percent, we will have to bring in colonies to help build the apiary back up to sixty hives. If only six hives survive the winter, for example, we will have to bring in fourteen nucs to bring us up to twenty total hives and then split aggressively to repopulate sixty hives. This is possible, though, as we will have drawn comb and resources from the dead-outs of the previous year. To preserve our genetic selection, though, all splits will be requeened from the original survivor hives and the queens and drones from the brought-in hives will be killed.

Another potential problem is noted by Dietemann and colleagues (2012: 127–28):

> Further progress in the selection of tolerant honey bee strains might be hampered by an inadequacy of selection methods, in which the role of intra-colonial genetic diversity for colony-level tolerance is under emphasised. Current research points to the importance of multiple mating of the queen resulting in a mixture of paternal genotypes.

By holding our breeding population in an isolated location and surrounding the breeding nucs with many hives containing our own genetic lines, we should be able to obviate this problem.

Dissemination of Results

Similar plans have been tested successfully in other locations, but the method has not gotten traction in the US. A big part of what we want to do with this project is build a community of beekeepers interested in developing their own strains of mite-tolerant bees. To that end, part of the funds will go toward outreach and building that community through presentations, publications, literature, videos, podcasts, colony sales, and an online forum.

Each year, we will seek out opportunities to share information about our breeding program and how beekeepers of every size can work towards breeding their own treatment-free bees. Many communities have beekeeping groups who are constantly looking for presenters (especially those from outside their immediate area). Most states have an apiary inspector, extension service, or other government-sponsored bee entities. Many universities have at least one person doing research on bees. We would approach beekeeping groups, state organizations, and universities in an expanding ring from our location in southern Wisconsin and use part of these funds for travel expenses to give presentations.

We will also produce and distribute written material at presentations, events, and online. The institute has started a series of technical manuals for different sustainability topics (the first one covers vermicompost, or worm composting). Our goal is to create a short but complete manual for a self-sustaining apiary aimed at small- and medium-scale beekeepers. This illustrated guide would walk through the rationale and genetics behind this method before describing step-by-step how to do it in one's own apiary. For larger-scale beekeepers, we hope to put together a proposal for an economically viable way to transition to tolerant bees (e.g., taking a percentage of bees out of direct production and breeding up their own tolerant stock to eventually replace their commercial hives). Funds will help us cover staff time and publication costs.

Additionally, we will write articles for publication in a variety of industry and public journals and magazines. General audience pieces will be sent to news outlets and magazines. More technical articles will be submitted to hobby and industry publications, such as *Bee Culture*, *Bee World*, the *American Bee Journal*, and *American Beekeeping Foundation Quarterly*. This will help spread the news about this process beyond the Midwest.

We will also produce and share videos and podcasts about this program. The institute has a running series of videos to which we will add a series of how-to episodes as well as some updates on how the program is going. Similarly, the institute's podcast series, the *Low Tech Podcast*, will cover updates and interviews related to the breeding program. We will also approach podcasts such as *Treatment Free Beekeeping*, *Beekeeping Today*, *PolliNation*, *Kiwimana Beekeeping Show*, *Bees and Such*, and the *Beekeeper's Corner*. This is time-intensive and funds will be used to subsidize staff hours.

After our population has stabilized, we will begin selling our extra hives. In the first few years, we'll have to split the fifteen to thirty colonies that survived the winter to repopulate our sixty hives. Once we have over 60–70 percent winter survival, we'll have too many bees. They will be sold as nucleus colonies to beekeepers in the region. If this creates a surplus in our budget we may begin a program of giving away mated queens for beekeepers to replace their current queen (this effectively replaces the old genetics with our new, tolerant ones). This will provide regional beekeepers with local, treatment-free bees at market rate (since we're not charging a premium for our bees), which will enrich our local genetic pool.

Finally, we will create and build an online community of people interested in and experimenting with this method of breeding mite-tolerant bees. Using a free online forum (such as Google or one hosted on the institute's website), we will have people available to discuss and answer questions of others carrying out this same selection process. Others can share their successes (to be replicated) and failures (to be avoided) with their fellow beekeepers. This will help us grow our reach beyond the Midwest as well. Funds will be used for staff time to create, manage, and contribute to this forum.

It is frustrating that a solution exists to solve one of the honeybees' biggest problems, but most beekeepers do not know it exists or are unwilling to adopt it. This program would demonstrate that a medium-size apiary can be viable with treatment-free bees. It will serve as an example to and resource for the beekeeping community. By providing information in many forms and reaching as wide an audience as possible, a group of interested beekeepers may begin to change the face of apiculture in North America. Funds from Project *Apis M.* would go specifically to purchasing hives and colonies to let us get to full scale the first year, instead of incrementally growing over the next five years. This is more effective because it creates a zone flooded with preferred genetics, instead of a smaller pool if we must start at a small scale. Funds will also support our outreach efforts, especially in the first year when travel and labor costs will be the greatest. In addition to the intrinsic benefit to beekeeping provided by this program, Project *Apis M.* will receive positive feedback for supporting this project, as we will happily acknowledge and display the Project *Apis M.* name and logo whenever it is appropriate. We'd be glad to work with your office in any way that can share this information and Project *Apis M.*'s central role in getting it off the ground.

Intended Outcome

By approximately 2025, we hope to have completed the majority of the goals set out by this proposal. After significant die-off of colonies in the first two or three winters, the population of our medium-sized apiary should stabilize and winter survival rates should begin to rise. At the same time, mite loads will still be present but at a low level managed by the surviving bees' genetic and behavioral resistance. Throughout this process, we will be sharing our data, results, analyses, and methods with the beekeeping community through a variety of outlets, allowing hobby, sideline, and even large-scale beekeepers to replicate our study.

We believe that this project's outcomes fulfill each of the goals of Project *Apis M.*'s funding objectives. As we lay out in this proposal, working towards treatment-free, mite-tolerant bees alone would provide a solution for the beekeeping industry and hobby in both the short and long term. Reducing disease vectors and increasing winter survivor rates directly contributes to this end. In addition to being a proof of concept, this project aims to freely provide our data and methods to the beekeeping community with targeted strategies for small-, medium-, and large-scale apiaries.

Although the project’s method may sound radical to those who treat prophetically and depend on their bees for their livelihood, it is based in successful projects from Sweden, Wales, and Africa. The first few years are admittedly the most precarious part, as the initial die-off may be severe, but this is simply a reflection of the weakness in our honeybee population that is masked by the availability of chemical treatments. Using natural and artificial selection on our bees, we are applying a well-known process from other animal husbandries.

We have built in what we believe to be scientifically robust processes. Our regular monitoring of hives will provide comparative data for both our own and other’s consumption and analysis. Our testing of hives for other diseases will also help reduce the likelihood of creating a large population to contaminate the local population. Most importantly, our method is driven by easy-to-follow criteria to dictate our splitting and other actions, which reduces the “human judgment” variable and makes our process replicable and testable by others.

The success of this breeding method in other locations supports the likelihood of success in this program over time. Furthermore, our organization has a robust track record of sharing our information with the public in online, audio, visual, and in-person venues. Finally, our budget is modest but will adequately cover the minimal costs of this study.

Economic Feasibility for New Products

This project will not generate new products *per se*, but instead may provide data and methods for beekeepers of all scales to breed mite-tolerant bees, leading to more cost-effective businesses and hobbies as well as healthier bees. If our study is successful in accepting the null hypothesis (that European honeybees can be bred to have mite tolerance), the breeding process will be written up and shared widely at no cost as the Low Technology Institute is a 501(c)(3) nonprofit research organization. Hobbyists will learn to split hives and raise their own local, tolerant stock, resulting in lower annual costs as they will no longer rely on purchasing nucs each year to restock dead-out hives. Side-line and commercial beekeepers can convert their stock through more targeted rearing of survivors and queen replacement, creating more resilient stock with fewer dead-outs. All beekeepers will save money and time by not having to purchase and apply miticide.

Project Timeline

Jan. 2020	Beehive placements finalized with Agrecol.
Apr.–May 2020	Johnson builds study hives.
May 2020	Johnson and Zelenski set up beehive stands.
May–Jun. 2020	Johnson and Zelenski place hives and install thirty purchased colonies.
Jun.–Jul. 2020	Johnson and Zelenski monitor hives biweekly and split those that are ready.
Jun.–Oct. 2020	Johnson, Zelenski, and/or assistants inspect hives monthly and record data.
Sep.–Oct. 2020	Johnson and Zelenski ready the hives for winter.
Oct. 2020–Apr. 2021	Johnson and Zelenski visit hives when conditions allow and record data. Also will write up 2020 report for PAm and create literature, presentations, and online resources. Requested PAm funding ends.
May–Oct. 2021	Repeat: inspections, splitting, mite counts, preparing for winter, and outreach; sell nucs when appropriate.
Oct. 2021–Apr. 2022	Write 2021 report; update literature, presentations, and resources updated. Continue outreach.

This cycle will repeat year on year. PAm will be updated with longer-term results.

Other Funding Sources

This is our first submission to Project *Apis M.* of this research.

This proposal has also been submitted to the Blooming Prairie Foundation for identical funds. We will continue to apply for funds from other organizations and agencies as funding opportunities become available (e.g., USDA).

If full or partial funds are received from any source, all other requested funders will be notified.

The funding requested here would fully fund our project, and therefore no matching or additional funds are needed if full funding is awarded.

Budget Detail

Annual Budget for Year(s):	**2020**	**2021 & 2022**	**2023 & After**
Woodenware	-$9,050	$0	-$1,500
Colonies	-$6,000	$0	$0
Labor	-$3,000	-$2,000	-$3,000
Outreach	-$2,000	-$1,000	-$1,000
Overhead	-$500	-$500	-$500
Project *Apis m.*	$17,050	$0	$0
Agrecol	$3,000	$3,000	$3,000
Low Technology Institute	$500	$500	$0
Nuc Sales	$0	$0	$4,000
Total	*$0*	*$0*	*$1,000*

Costs Covered by Project *Apis m.* Funds

Woodenware *(2020: 55 hives × $155 each + $500 hive stands = $9,050)*

This price is the complete "economy" hive from distributors (plus, but Johnson will build the hives with better materials for the same price (plus five hives from the pilot study) plus one hive strap. Six hives are placed on each of ten hive stands.

Bee Colonies *(2020: 30 colonies × $200 each = $6,000)*

We will start with thirty colonies from Zelenski's local stock in the spring of 2019 and split them into two colonies early in the summer; a typical practice that will bring us up to sixty hives quickly.

Outreach *(2020: $2,000)*

In the first year, literature, publications, presentations, videos, and podcasts must be created. Then, staff is needed to distribute and present this information: presentations, shows, and articles will be pitched to podcasts, radio programs, journals, magazines, and groups.

Other Costs and Income

Pollination Services *(Annually $3,000, pays labor and outreach)*

Agrecol, a local prairie seed company, pays for pollination services. This covers our labor.

Low Technology Institute *(Annually $500, pays overhead)*

Staff, website, and other institute costs of the project will be covered until a surplus is returned.

Colony Sales *(ca. 20 × $200 each = $4,000, pays woodenware and outreach)*

After 2023, surplus colonies will be sold as nucleus colonies ("nucs"; cost: $50 each). Study hives will need upkeep of ca. $500 per year. Surplus hives sold at market rate. Twenty hives is a conservative estimate of our surplus bees.

Surplus or Debt

Debt will be absorbed by reducing the payout to us for labor (i.e., we'll volunteer and/or work for less). Surpluses will be maintained in a dedicated bank account within the 501(c)(3) for later expansion.

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