The Saskatraz Project–A Review (2004-2009)

Most of the information in this review was reported on at invited presentations over the last two years at the Second World Symposium on Queen Breeding, in Nayarit, Mexico; Canadian Honey Council Symposium, in Niagara Falls, Ontario, The North American Beekeeping Conference in Orlando, Florida and in Brandon, Manitoba at the Manitoba Beekeepers Association meetings.

Report prepared by Albert J. Robertson; Principal Investigator, March 2010.

Abstract

The objective of the Saskatraz project is to breed gentle, productive honey bee colonies with tolerance to mites and brood diseases. Efforts are also being made to identify genetic diversity and correlate important phenotypes with molecular (microsatellites) markers.

These objectives were approached by assembling a large gene pool at an isolated apiary called Saskatraz. To access a source of honey bees adapted to the Saskatchewan environment and selected for many years for honey production, overwintering ability and good overall hive health, a request was made for Saskatchewan and Manitoba queen breeders to provide their best breeding lines to the program. Fourteen queen breeders provided 35 colonies. To provide breeding stock previously demonstrated to have mite tolerance, a few breeders provided reselected Russian and German breeding lines. All of the colonies at the Saskatraz apiary were normalized for varroa and tracheal mite infestation levels. No synthetic chemical miticides were applied and natural selection was used to identify the most productive and mite tolerant phenotypes. Initial selections were made over three and a half years. In the spring of 2007 varroa mite infestations and the stresses of associated pathogens killed all of the original Saskatraz colonies. Breeding lines selected in 2006 were back crossed at Saskatraz under high varroa mite pressure to generate breeder queens with increased varroa tolerance. The progeny of selected breeders are continually outcrossed and subjected to recurrent selection to preserve the selected gene pool, to maintain genetic diversity, and to enrich for economic traits. Re-selected colonies are returned to the Saskatraz apiary and the natural selection process is repeated in the search for genotypes with increased expression of mite tolerance and honey production without the use of chemical miticides. A model showing the logistics of the Saskatraz breeding program operation is presented in Figure 1.

In general, our approach has been to select for families with balanced traits, with increased honey production as our primary selection criteria. These families show varying degrees of increased honey production, good resistance to tracheal (*Acarapis woodi*) mites and chalk brood, and some tolerance to varroa (*Varroa destructor*) mites. None of the families show complete resistance to varroa mites and continued efforts are required to breed lines with improved varroa tolerance. Varroa infestation in *Apis Mellifera* is a serious world -wide problem, threatening the existence of the domesticated honey bee and is part of the cause of colony collapse disorder (CCD).

Since 2006, the Saskatraz breeding program has released 14 families (SAT -14, 17, 23, 28, 30, 34, 63, 65, 84, 86, 87, 88, 96, 98.) to queen breeders for multiplication. As of October 15, 2009, 4220 queen cells and 67 breeder queens were released to Canadian queen breeders

Introduction

The honey bee is indirectly responsible for one third of the worlds food supply through its pollination activity on more than 90 species of plants. (Greenleaf and Kremer. 2006).Since flowering plants and honey bees co-evolved together their survival depends on each other. However, the *Varroa destructor* mite and its effect on honey bee health threatens the honey bees existence. The exchange of honey bee colonies between Asia and Europe resulted in *Varroa Destructor* infecting *Apis Mellifera* in about 1960, and varroa mites were found in North American honey bees in 1987 (cf reviews Sanford, M. 2001). North American honey bees having no resistance died within 2 to 3 years after varroa infection. Chemical treatments with acaricides was initially effective at reducing varroa populations; however, the varroa mites soon developed resistance (Milani 1995, cf review Milani 2001.). In addition, chemical treatment of mites prevented selection pressure for the development of natural tolerance to parasitic mites and possibly makes honey bees even more susceptible to secondary infections (viruses, microsporidia, etc.) associated with mite infestations. Lack of genetic diversity in North American honey bee populations has also been a concern, since most commercial stock is produced by few queen breeders..

The Saskatraz project was initiated in 2004 with the intent of establishing a genetically diverse gene pool to breed for honey production, mite tolerance and resistance to brood diseases. Breeding for mite tolerance was approached by using natural selection, without using any synthetic chemical miticides. The first step was to gather together genetically diverse stock adapted to wintering in Saskatchewan, selected for honey production and brood diseases, such as chalk brood. Both tracheal (*Acarapis woodi)* and Varroa (*Varroa destructor*) mites arrived late into Saskatchewan, limiting exposure of local bees to these parasites. Most bee populations having limited exposure had no tolerance to varroa mites, but exposure to tracheal mites in some locations allowed some resistance to develop to the tracheal mite prior to initiation of the project. A request for breeding stock was responded to favourably by Saskatchewan queen breeders.

A collaborative initiative with the Ontario and Saskatchewan Beekeeper's Associations resulted in the joint funding of the importation of Russian stock from the USDA research facility in Baton Rouge, La., USA. This stock was demonstrated to show some tolerance to varroa mites (Rinderer, et al 1997), in previous studies and a few Saskatchewan queen breeders had reselected stock imported between 2001 and 2004. In addition, stock (semen) from a German program (Buchler, etal 2002;2008) involved in selecting for varroa tolerance by natural selection was imported in 2004 and 2005.A number of other programs selecting for survival of colonies under varroa mite infestation without miticides treatment have been described

(Rinderer etal. 2001; Leconte etal, 2007; Fries etal, 2007; Seeley etal, 2007).Other programs have focused on selecting for hygienic behaviour and suppression of mite reproduction, both traits which are correlated with varroa tolerance(Ibrahim and Spivak,2006;Harris.2007)

This report describes the results of selecting for both honey production and varroa tolerance with no synthetic chemical miticides, from a large, pre-selected, diverse gene pool over a period of three and a half years. In addition, close population mating by backcrossing selections at Saskatraz, with drone populations under high varroa mite infestations was performed. Methods are also described for enriching and maintaining the selected lines by outcrossing and recurrent selection. Microsatellite analyses is used to characterise genetic diversity and genotype specific breeding lines.

Methods and Materials

(i) Saskatraz apiary establishment and gene pool development

An apiary location operated by Meadow Ridge was chosen to establish the Saskatraz natural selection program. This apiary is located in an isolated area with native parklands and saline lakes to the south, east and west. The area is located in one of the major Saskatchewan migratory bird fly ways, with surrounding lakes and marshes. Willows, shrubs, poplars, native roses, both white and yellow blossom clover and native grass species are abundant. To the north of the apiary, typical parkland farm land predominates, producing mainly cereal grains and canola, with some alfalfa hay and pasture land. The apiary itself is well sheltered on all sides and surrounded by mature spruce, caragana and poplar, at one time being a family home stead. The site is not occupied and is seldom used except during harvest, because of grain bins located on the west side of the site. Isolation is necessary to protect the gene pool (closed population mating procedures) and to prevent infestation of other bee keepers with varroa mites, tracheal mites and associated pathogens.

Saskatraz was established in 2004 with 35 pre-selected colonies from fourteen different Saskatchewan and Manitoba queen breeders. Colonies were numbered in order of addition to the apiary. For example, Saskatraz -01 (SAT-01) stands for the first colony selection entered into Saskatraz. This gene pool included colonies naturally selected for over wintering ability in Saskatchewan, as well as selections for honey production, temperament, chalkbrood resistance and over all hive health. Since they were selected by different operations throughout Saskatchewan they would be expected to be genetically diverse. Thirteen producers donated 24 nuclues (nucs) bee hives and Meadow Ridge donated 11, as follows: Dwight Sollosy(1); Yves Garez(2); Steve Clifford (2); Trevor Rehaluk(2); Ron Bacon(1); Corey Bacon(1); Carl Meyers(1); Tim Wendell(4); Tony Lalonde(1); John Pedersen(6); Len Proctor(1); Wink Howland(1); Lester

Martens(1); Meadow Ridge(11). Reselected Russian stock (2000 to 2004) and breeding lines from the Manitoba Queen Breeders Association (MBQA) were also included in the breeding program. The MBQA is working with Dr. Rob Currie at the University of Manitoba. In 2005, 14 more selections were placed at Saskatraz with crosses made between Russian and German (Carnica) lines (semen) imported by Yves Garez, from Dr. Ralph Büchler, Kirchain, Germany The selections from the Russian (Rinderer et al., 1997) and German lines were introduced to add varroa and tracheal mite tolerance to the gene pool.

Since 2005, additional selections from new Canadian lines and reselections of Saskatraz stock have been added (Meadow Ridge, John Pedersen, John Polson, Robert Colbert, Jim Wood, Brent Mckee, Len Proctor, Tim Wendell, Yves Garez, and Robert Hamilton, Dr. Rob Currie, University of Manitoba (U of M.). Meadow Ridge maintains Saskatraz breeding stock by providing 50 yard sites for stock maintenance, outcrossing, backcrossing and closed population mating procedures.

All Saskatraz colonies showed trace to low levels of Varroa mite infestations, when measured by natural drop analyses, between August 7th and September 15, 2004. All colonies were treated with Apistan (2 strips per colony) for 32 days (September 15 to October 15) to normalize varroa populations. Two independent samples of 100 bees per colony failed to detect tracheal mite infestations. On October 15, 2004 all colonies were infected by adding 200 worker bees from a colony provided by John Gruszka showing 58 to 60% tracheal mite infestation

Meadow Ridge maintained four apiaries with “near pure” Russian lines established by back crossing selections released from Baton Rouge, USDA between 2000 and 2005. Initial stock importation was funded and implemented as a joint effort by the Saskatchewan and Ontario Beekeepers Associations. These Meadow Ridge apiaries were made available for re-selection of Russian stock for evaluation at Saskatraz, and used for both out crossing and back crossing procedures between 2004 and 2007.

In 2005, more honey bee semen was imported from Dr. Büchler’s program in Kirchhain, Germany. This program involves selection for varroa tolerance, honey production, grooming and hygienic behavior, and has been in progress for about 15 years. Susan Cobey assisted us in making 35 new crosses with this semen, (G-08 and G-72) by instrumental insemination of virgin queens from the following selected lines (yellow-green-05, yellow-blue-05, UM (University of Manitoba)-163, 234, 147, SAT 28, 30 and BTP-30). Yellow-green-05 and yellow-blue-05 virgin queens were derived from Russian breeder queens obtained from Charlie Harper (Queen breeder for USDA, Baton Rouge, Louisiana) in 2006.UM lines were obtained from Dr. Rob Currie at the University of Manitoba, SAT-28 and 30 virgin queens were obtained from SAT-28 and SAT-30 selections made at Saskatraz. BTP-30 is a breeder queen obtained by crossing a Buckfast

queen with Russian P-30 drones. Some daughters of this cross were inseminated with G-08 and G-72 semen.

(ii) Measuring honey production and mite infestations

Honey production was measured from July until September each year (2005-present) by weighing individual honey supers from all Saskatraz colonies every time surplus honey was removed. The error associated with the variation in empty super weights was calculated to be 2.3 (mean), 1.7 (standard error), n=20.

Tracheal mite infestations were monitored on a monthly basis between May and October of each year. All colonies were sampled by collecting a minimum of 100 adult bees in glass jars containing 70% methanol. Tracheal mite analyses were carried out at the provincial apiculture laboratory in Prince Albert, Saskatchewan, under the direction of John Gruszka. Tracheal mites were detected following procedures similar to those described by Peng and Nasr 1985. Varroa mites were also counted after alcohol washes by straining out the bees and counting the remaining varroa.

Varroa mite populations were also assayed on a weekly basis, by natural drop, following procedures described by Martin, 1998. Commercially available Apinovar boards were placed under all Saskatraz colonies to monitor mite populations. The screened bottom boards allow dead or groomed mites to fall on a coroplast sheet which can be removed like a drawer from the bottom board for mite counting and analyses. Mites were counted and cumulative drop was recorded for each colony. Mature and immature (white mites) were recorded as well as total mites. In some cases, the percent mite infestations in worker and drone brood were also assessed. To identify varroa sensitive hygienic (VSH) phenotypes the number of mites per brood cell was recorded. Mite damage was also microscopically assessed using a phase contrast microscope, and mites were selectively sampled for molecular analyses.

(iii) Hygienic and grooming behavior assays

Assays for hygienic behavior were carried out as described by Spivak and Boecking, 2001, and for Varroa Sensitive Hygiene following procedures outlined by Ibrahim and Spivak, 2006. In 2005 hygienic tests using liquid nitrogen to kill selected brood areas to test Saskatraz colonies were carried out and sub sequentially modified in 2006. Brood was killed (whole frame) by repeated freezing and thawing at-20C and +4C, respectively over a 2 week time frame. Colonies were tested for uncapping and removal of dead pupae by placing test frames in third story supers with excluders placed over the second super. This method is demonstrated in Robertson(2007). Dr. A. Ibrahim performed hygienic assays on Saskatraz breeding lines and out crosses in 2007, defining VSH phenotypes as colonies that uncapped and removed 95% or

better of freeze killed pupae in 24 hours. VSH phenotypes were also identified by opening one hundred or more sealed brood cells and scoring for the number of varroa mites per cell as described by Harbo and Harris,2005, on selected and non-selected Saskatraz breeding lines.

Grooming assays were performed on 4 daughters from each of 6 Saskatraz breeding lines in the fall of 2008. Although a number of grooming assay procedures exist for laboratory work (Aumeier, 2001; Mondragon etal 2005); we are developing grooming assays that work at the colony level with a minimum amount of invasiveness or manipulation. All selected colonies were normalized for varroa mite populations by Apistan treatment in the fall (late-September to early November). Varroa infested (varroa nursery) colonies were used as a source of inoculums for these studies. Each colony was inoculated with a sufficient number of adult bees carrying varroa mites to introduce 300 adult mites to the test colonies, which were indoor wintered. This simulates in part, natural methods of Varroa mite infection. Mite drop was assessed every 24 hours for 21 days using apinovar boards. All mites dropping on boards were microscopically assessed for damage using a stereo microscope. In the spring (April) all surviving colonies were assessed for varroa mite infestations to determine varroa population growth rates.

(iv) Molecular Marker Analyses

The technical aspects of DNA isolation and analyses, Polymerase Chain Reaction (PCR)and Reverse Transcriptase, (RT-PCR), marker analyses of this project were carried out on a contract basis by GenServe Laboratories., Saskatchewan Research Council, Saskatoon, Saskatchewan, by Bruce Mann in association, with Dr. Yves Plante. General methods are as described In DNA MARKERS: Protocols, Applications and Overviews. 1997. A brief description of materials and methods used will be described here. A Qiagen DNeasy tissue kit was used to extract DNA from 50mg of drone pupae or larvae. The DNA markers used (108 microsatellites) in the initial screening were selected from Solignac et al 2003 and Genbank. DNA was quantified by flourimetry and assessed for quality by agarose gel electrophoresis. Initial PCR reactions were performed using a 55C annealing temperature and 1.5m M MgCl2, DNA products were separated on polyacrylimide sequencing gels using the LICOR system and fragments scored using GeneImagIR (Licor). DNA was extracted from drone pupae collected from Canadian lines at Meadow Ridge and from Russian lines sent to us from the USDA, Baton Rouge, La. USA. Russian lines reconstructed by backcrossing procedures at Meadow Ridge using embryo's obtained from Baton Rouge, were also sampled. Other samples included German lines (semen), Russian x Canadian hybrids, alcohol preserved tissue samples of Scutellata and Scutellata hybrids from the Yucatan area of Mexico (Felipe Brizuela), and Saskatraz breeding lines. Microsatellite analyses were performed using methods similar to those of De La Rue et al 2001,

Franck etal 2001 and Estoup etal 1995. Dendograms were constructed using a TREECON software package (Van de Peer and Wachter, 1990).

RT-PCR procedures were carried out at GenServe Labs, SRC by Bruce Mann and at the Veterinary Infectious Disease Organization (VIDO), University of Saskatchewan, (U of S) Saskatoon, Saskatchewan by Wayne Connor according to published procedures (Chen etal. 2005).RT-PCR kits were purchased from Invitrogen. Sequencing of amplified bands was performed to confirm identity.

Results and Discussion

(i) Selection of SASKATRAZ breeding lines for Honey Production, Tracheal Mite Resistance and Varroa Tolerance.

The Saskatraz apiary was established during the summer of 2004, and by October 15 all 35 colonies were normalized for both tracheal and varroa mite populations as described in Materials and Methods. No further chemical miticide treatments were used and measurements on mite infestation levels and honey production were initiated in May, 2005. The genetic diversity and gene flow in and out of Saskatraz is shown in Figure 1. The operation and logistics behind the breeding program are summarized showing the time frames involved. This model was developed over the past three years based on the progression of the program. Blocks A, B, C, D and E represent gene flow from different sources in to Saskatraz. All of these colonies were preselected (as described in materials and methods) prior to placing into the Saskatraz natural selection site for evaluation. Colonies were managed by Meadow Ridge using standard procedures for outdoor wintered colonies in Saskatchewan. Fall feeding consisted of up to 30kg of 50% sucrose with Fumidil B and spring feeding up to 15kg of 50% corn syrup. No pollen or pollen substitute were used. Colonies were wrapped in insulated 4 packs on pallets prior to October 31, and opened in May for inspection and preliminary selections. Colonies were scored for wintering ability, cluster size and location, brood pattern, brood diseases (chalk brood), temperament, burr comb, queen characters (size, age, color, egg placement, etc.). In 2006 the first six breeding lines were released to commercial queen breeders and beekeepers, followed by ten additional lines between 2007 and 2009. Block F shows the gene flow out of Saskatraz, representing 14 breeding lines certified for distribution as of 2009. Queen breeders are encouraged to return lines subjected to out crossing and reselection (RC) to maintain genetic diversity and enrich for economic traits. These lines as well as those selected by Meadow Ridge outcrosses are reselected for evaluation at the Saskatraz natural selection yard site. Block G represents Saskatraz satellite yards set up by Meadow Ridge for close population mating and

for maintenance of daughters (stock maintenance) of selected lines. The Saskatraz natural selection apiary is also used for close population mating procedures, to select for drone populations surviving high varroa infestations. Drones from colonies suppressing varroa population growth would be expected to enrich for varroa resistance in colonies headed by queens mated with the most fit drones from the Saskatraz drone population

Mite populations and honey production were monitored as described in Materials and Methods. Honey production data is summarized for 2005 and 2006 for non-selected (SAT-04, 24) and selected (SAT-14, 17, 23, 28, 30, 31, 34) colonies in Figure 2. The best selections for honey production were SAT-14, 30 and 31. SAT-31 superseded at Saskatraz in 2005, showed the highest honey production in 2006, but was lost prior to multiplication of daughters. The increased honey production and vigor of this colony after supersedure at Saskatraz is of interest. Controlled studies (Matilla and Seeley, 2007) on genetic diversity have shown "enhanced productivity and fitness" in colonies where the queens have mated with genetically diverse drones, such as the drone population at Saskatraz. We have reselected out crosses of SAT-30, and 14 daughters in 2008 and 2009.Both are showing increased honey production, and are currently undergoing revaluation at Saskatraz.

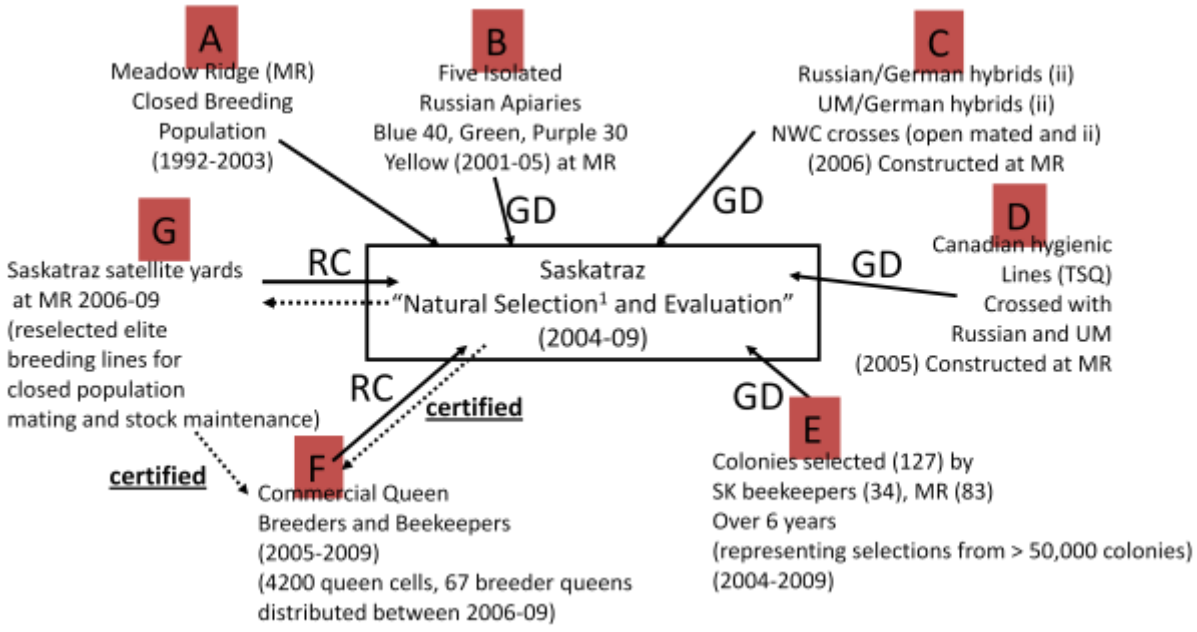


Figure 1: Letters A to G represent isolated apiaries and the year of establishment at Meadow Ridge. Solid arrows indicate genetically diverse gene (GD) flow into Saskatraz, dashed arrows gene flow out of Saskatraz. (ii) denotes instrumental insemination. RC denotes recurrent selection. 1 Denotes no chemical miticides.

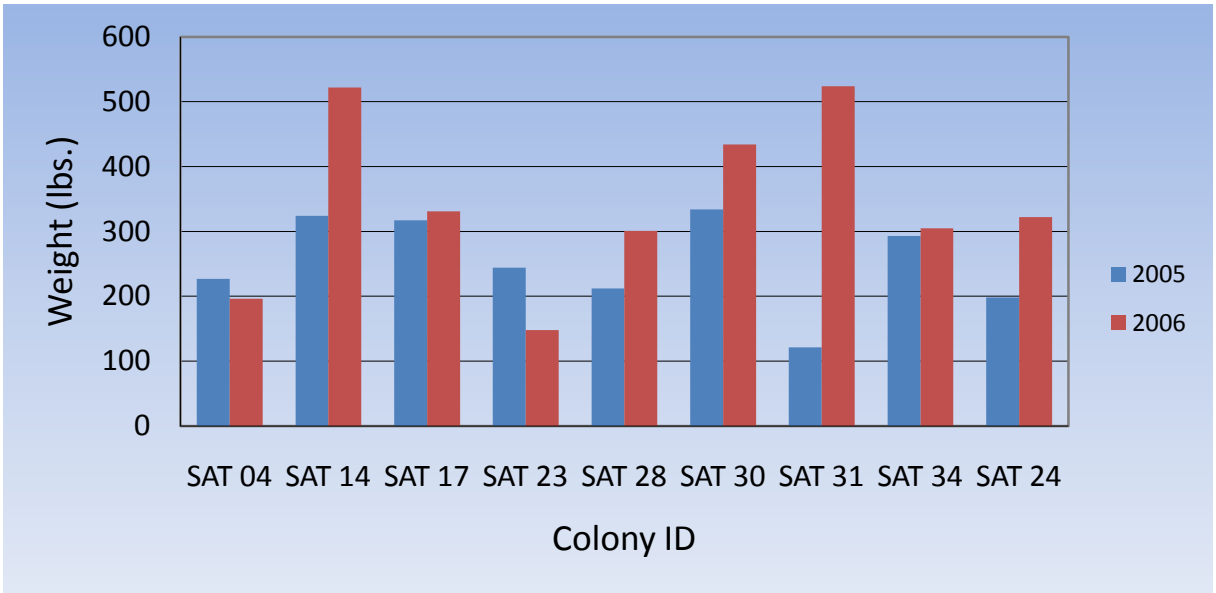


Figure 2.Saskatraz honey production measured during the summer of 2005 and 2006.

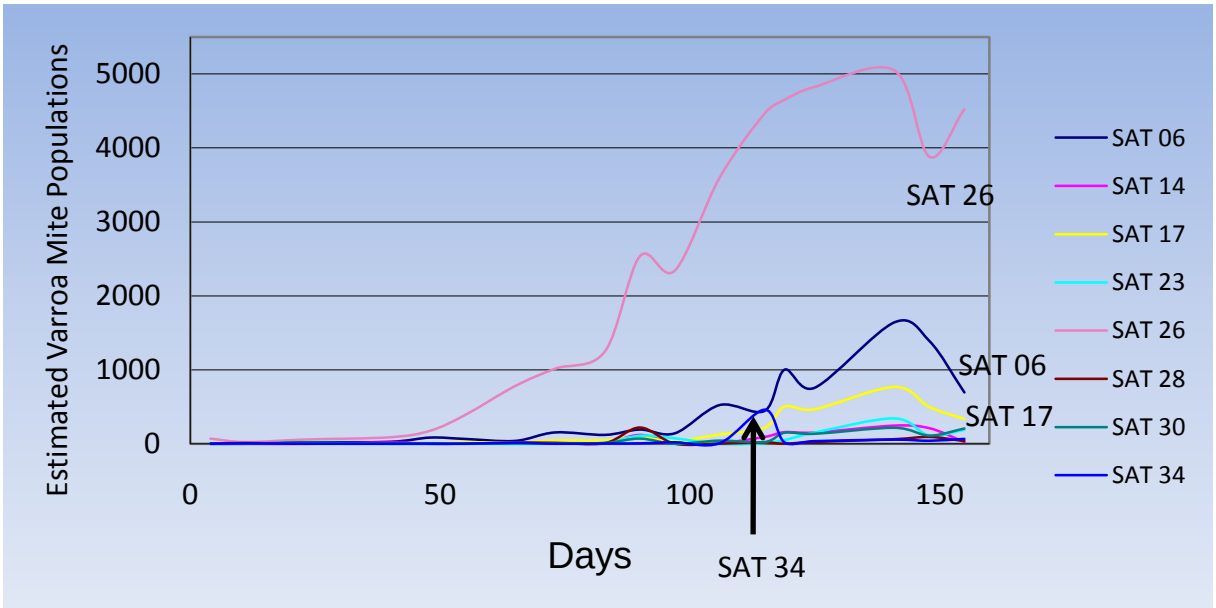


Figure 3.Varroa mite population growth for selected and non-selected lines were estimated by the natural drop method, and counted on a weekly basis between May 7 and Oct.15, 2005

Figure 3 shows mite population growth data measured by natural drop in 2005.Data from eight test colonies are shown, identifying high (SAT-06, 26), intermediate (SAT-17) and low (SAT-14, 23, 28, 30, 34) varroa population growth. Figure 4 shows the same analyses done during the summer of 2006, after 21 to 24 months with no chemical miticide treatment. Although spring mite levels were low and comparable to fall 2005 levels an accelerated rate of varroa population growth occurred in July and August of 2006, in both selected and non-selected

colonies. Non-selected colonies (SAT-24 and 04) and SAT-23 failed in early fall of 2006. Selected colonies (SAT-28, 31 and 34) showed the best suppression of varroa mite population growth in 2005, maintaining the same status in 2006 (figure 4).

The values shown in Figure 5 are cumulative counts obtained by natural drop, at 7 to 10 day intervals, comparing the results obtained for 9 Saskatraz lines during the summers of 2005 and 2006. Figure 6 shows the average mite drop per day for selected and non-selected lines between May and October, 2006, showing the rapid increase in mite populations in sensitive colonies and suppression by tolerant lines. Mite populations tended to follow the increase in adult bee populations, a reflection of increased brood production required for varroa reproduction. The average mite drop per day per month dropped off with reduced brood production and adult bee populations. Sensitive colonies (SAT-04, 24) were dead by September, and selected colonies were showing reduced drop rates in October.

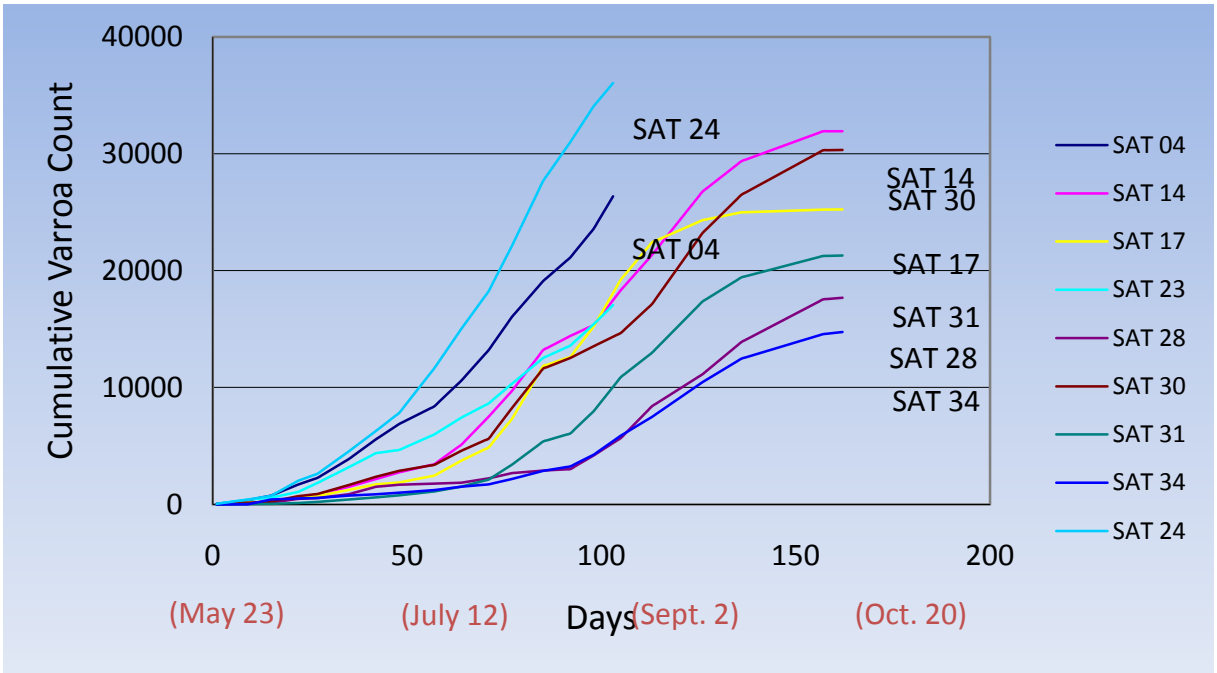


Figure 4. Cumulative varroa mite population growth measured as in figure 3, by natural drop in 2006

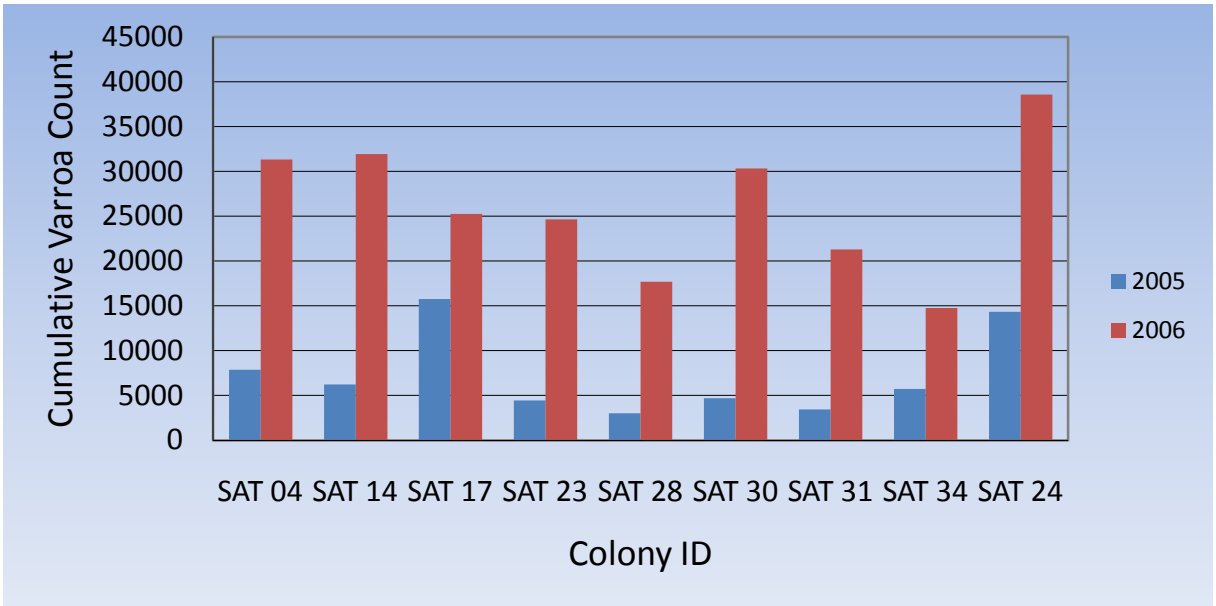


Figure 5. Comparison of total varroa mite drop in 2005 and 2006 measured by natural drop.

Adult bee populations were also monitored for tracheal mite infestations and varroa mites by alcohol washes on a monthly basis. Tracheal mite infestations and varroa mite levels were determined by the provincial apiculture laboratory in Prince Albert under the direction of John Gruszka. Figure 7 shows Saskatraz hive locations and tracheal mite levels as determined in May (Blue) and October (Red) of 2005. Hive locations bordered by red are breeder selections (SAT-14, 17, 23, 28, 30, 34). All colonies were inoculated with 200 to 300 bees carrying 60 % tracheal mite infestations in October, 2004.In the spring only 4 colonies tested positive for tracheal mites, the highest being 3% (SAT-33). In the fall one year after infestation 11 tested positive; however, 2 that tested positive in the spring were negative in the fall. The colony showing the highest infestation level in October (SAT-8=14%) came from a Saskatchewan breeder that has never tested positive for tracheal mites, and therefore no selection pressure for resistance.

Figure 8 a to e follows both tracheal and varroa mite infestations for adult bees between May and September 2006, for selected (SAT-14, 17, 23, 28, 30, 31 and 34) and non-selected (SAT-04, 24) colonies. Tracheal mite populations remained relatively low throughout the summer and fall, peaking in non-selected colonies SAT-04 and 24 and SAT-23 in July. No tracheal mite infestations were found in SAT-34, and other selected colonies maintained very low levels. SAT-17 had a Buckfast background and also showed good tracheal mite resistance. All of the selected Saskatraz breeders except SAT-23 showed good to excellent tracheal mite resistance. Danka and Villa, 2003, showed that honey bees resistant to tracheal mites were more responsive in autogrooming behavior when challenged with tracheal mites than sensitive bees. In this study, tracheal mite resistance and suppression of varroa mite population growth were correlated in SAT-28 and 34. Varroa mite populations on adult bees exploded in August, Figure

8d) resulting from large flushes of varroa emerging with hatching bees. Colonies reduced in brood rearing in August resulting in increased adult varroa mite infestation of the adult bee population. The alcohol wash results presented in Figures 8 support those shown for previous natural drop analyses. Colonies showing the lowest levels of adult varroa infestations, best suppressed the varroa population growth rate.

SAT-28 superseded in 2005, and in 2006. A number of SAT-28 daughters are being used as breeders by Saskatchewan bee breeders and we have reselected out crosses of this breeding line in 2007, 2008 and 2009, which show excellent over wintering ability and honey production. On May 23, 2006 John Pedersen and I, while evaluating Saskatraz colonies, noticed this colony showed aggressive behavior towards varroa mites. More than one worker bee was observed to simultaneously attack and bite an adult mite. SAT-34 and a number of her daughters have shown good to excellent suppression of varroa mite population growth; however, the temperament of the SAT-34 line was aggressive and required selection of less aggressive progeny.

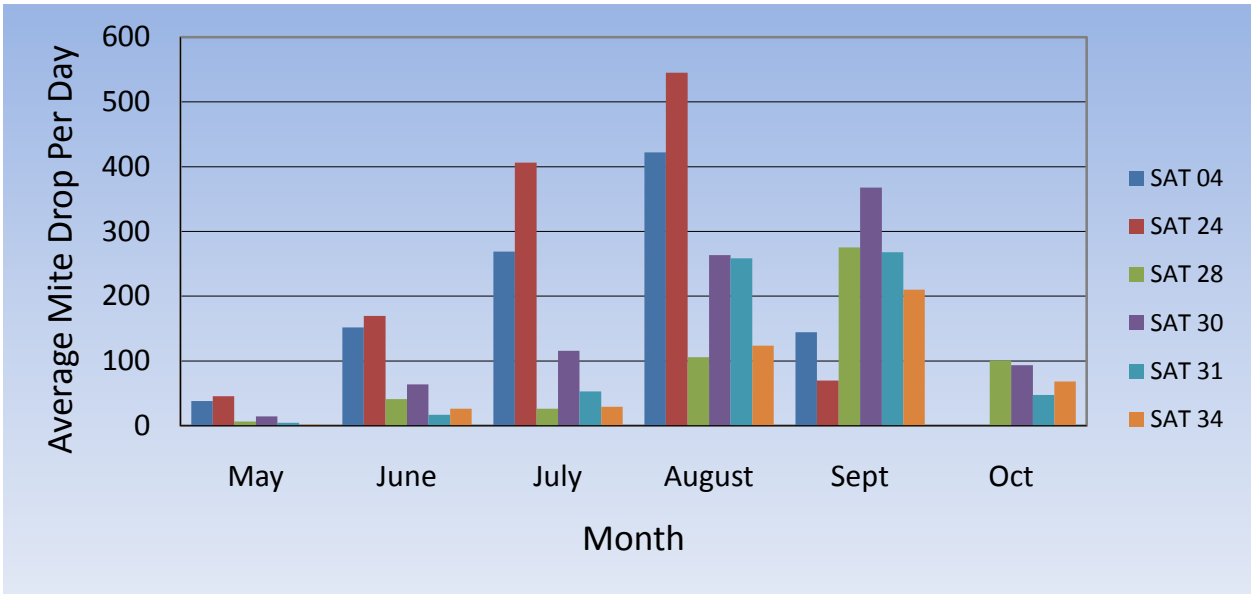


Figure 6: This bar graph shows the average mite drop per day from May until October 2006 for selected SAT-28, 30, 31, 34 and non-selected colonies.

Saskatraz Tracheal mite levels and hive locations

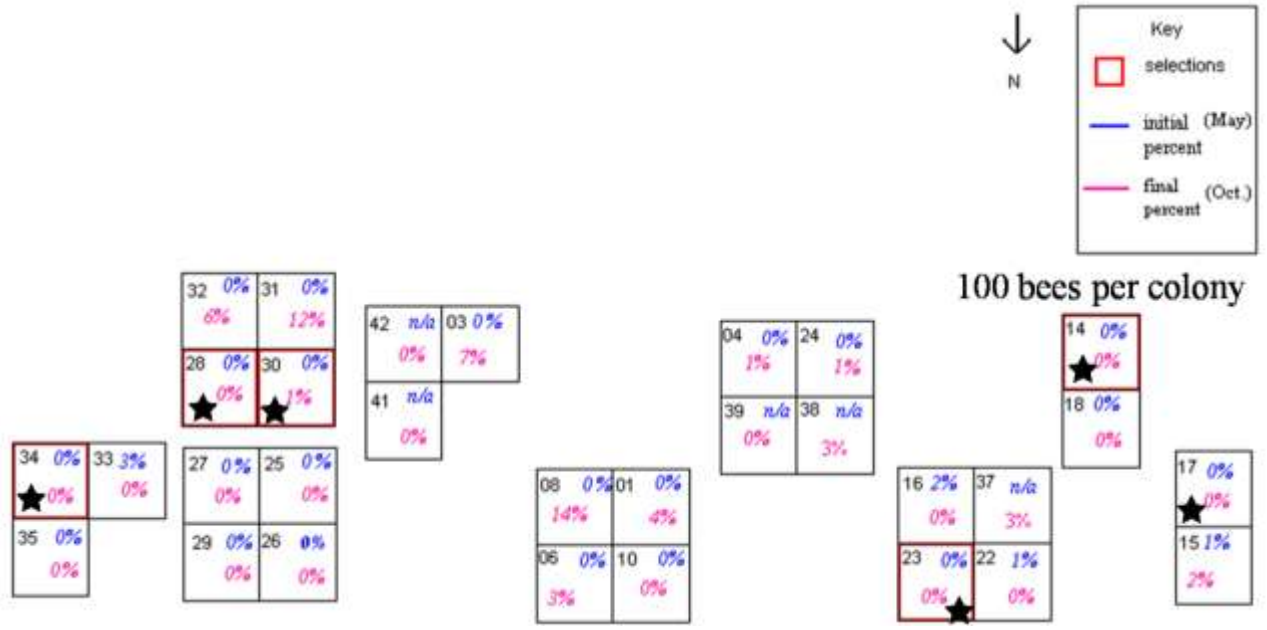


Figure 7: Per cent tracheal mite infestations were determined on a monthly basis by sampling 100 bees per colony from May to October. May (blue), October (red) values for each colony at each location are indicated in the upper right hand corners of each hive location. Red squares and stars denote selected colonies.

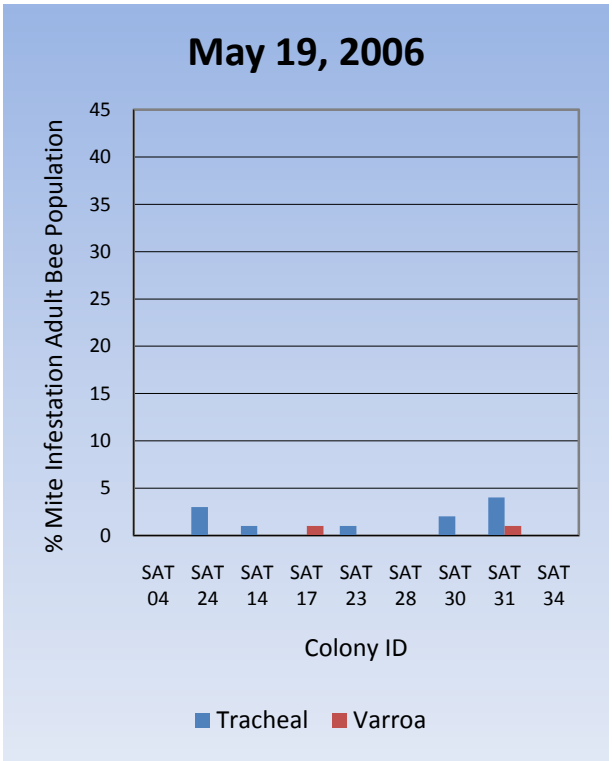


Figure 8(a)

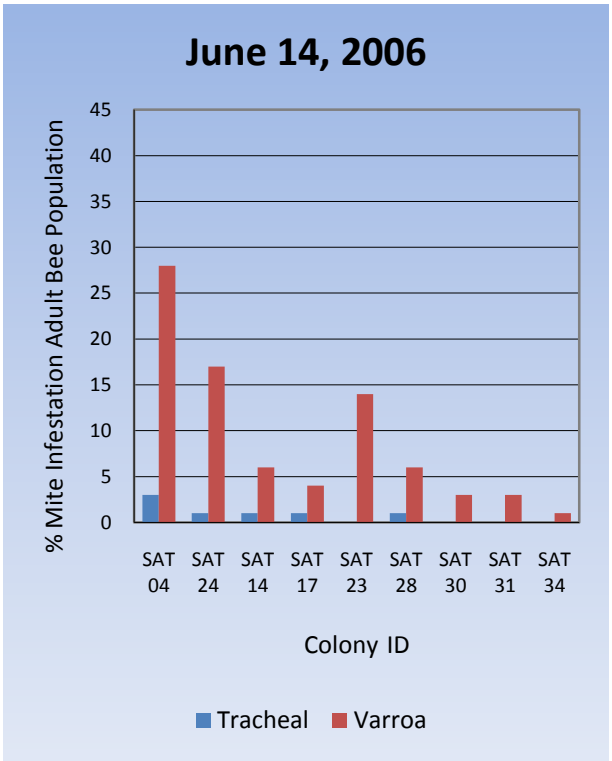
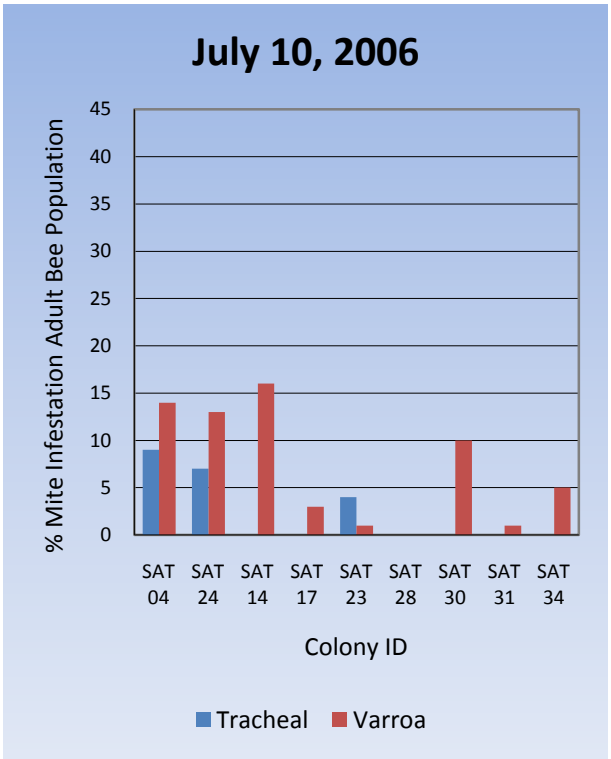


Figure 8(b)



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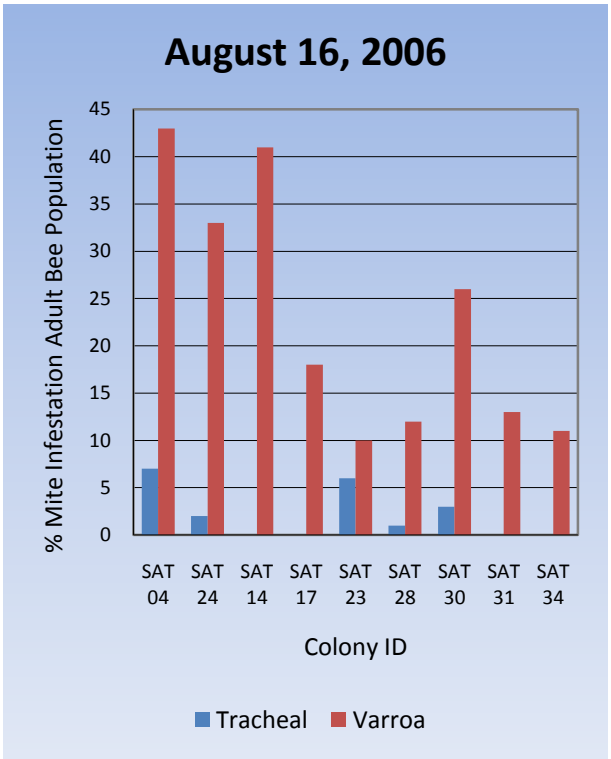

Figure 8(d)

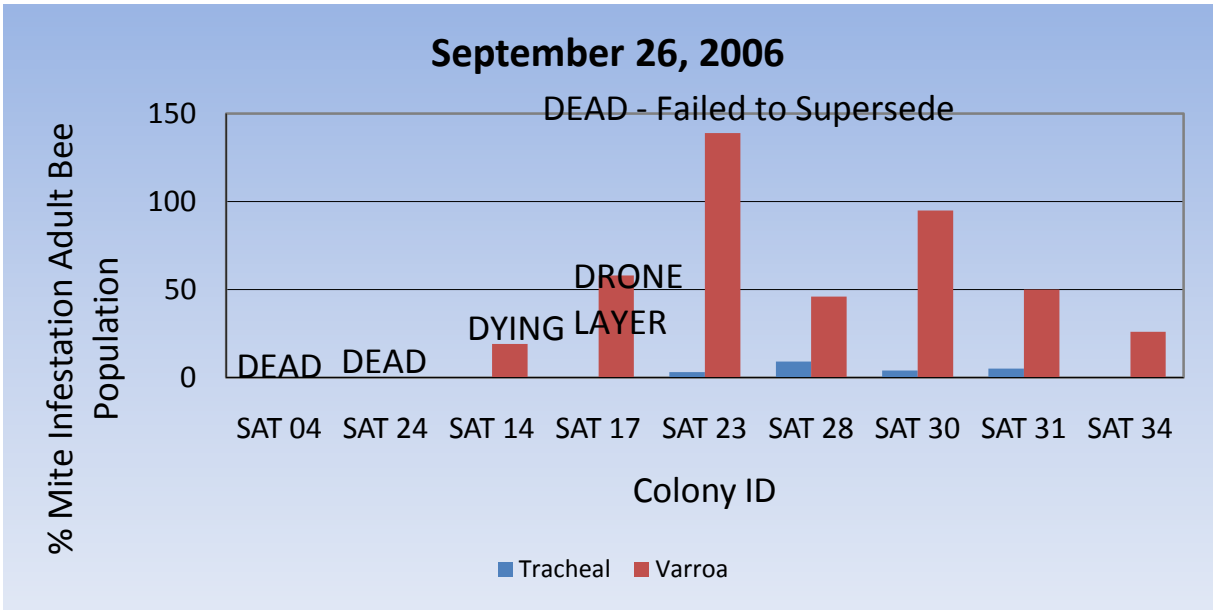

Figure 8e

Figure 8(a) to (e) show the results of alcohol wash assays on 1 to 2 hundred bees sampled from selected and non-selected colonies between May and September 2006. Both percent tracheal (blue bars) and varroa (red bars) infestations for adult honey bees are shown.

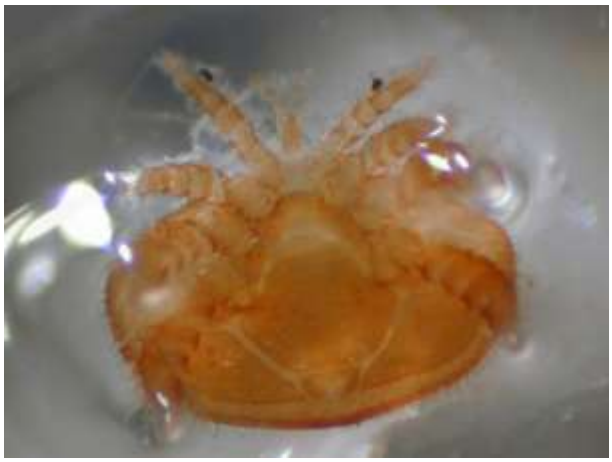

Figure 9(a)

Figure 9(b)

Figure 9(a) and (b) are microscopic ventral images of varroa mites collected by natural drop on Saskatraz test colonies. Figure 9(a) is an example of an intact mite showing mouth parts and eight legs, in (b) the varroa mite has two severed legs on the right side and is missing mouth parts. The damage results from honey bees biting varroa mites during autogrooming activities or as aggressive behavior towards the mites.

When assessing varroa mite population growth by natural drop in 2006, 100 mites from each sticky board were sampled and microscopically analyzed every 6 to 7 days for 65 days (late July on) for mites damaged(Figure 9) due to grooming behavior (Figure 10), and for the number of light immature mites present (Figure 11). Figure 10 shows that SAT-28, 30 and 34 damaged more mites than SAT-24 and 06, but the number of mites damaged by SAT-14 and 17 were similar to non-selected colonies. Figures 11a and b show that colonies with high numbers of immature mite drop (SAT-06, 24) showed greater increases in varroa population growth and fewer damaged mites relative to the number of immature mites and total mites in the hive. SAT-34 and 28 (Figure 11c and d), respectively, showed higher numbers of damaged mites and lower numbers of immature mite drop throughout the summer, with damaged mites equaling or exceeding immature mite drop in some cases. SAT-28 and 34 also showed more variability in the number of mites damaged compared to immature mite drop, which may reflect more grooming activity or hygienic behavior depending on environmental conditions throughout the summer. SAT-31 (Figure 11f) showed the next best grooming activity, but similar levels of mite population growth to SAT-30 (Figure 11e). SAT-14 and 17 showed phenotypes for grooming and immature mite drop intermediate to selected and non-selected colonies (Figure 11g and h),respectively.

Hygienic testing of selected Saskatraz colonies (SAT-28, 30, 31, 34) and non-selected lines SAT-04 and 24 were performed in 2006. These results were described in detail previously (Robertson, A. 2007). Briefly, SAT-34 was most hygienic followed by SAT-28. These colonies showed intermediate honey production, but excellent suppression of varroa mite population

growth, where as SAT-30 showed intermediate hygienic behavior and excellent honey production. The most hygienic hives (SAT-28, 34) showed the highest percentage of chewed mites due to grooming behavior and the lowest overall percentage of light mites (cf Figure 11 c and d).

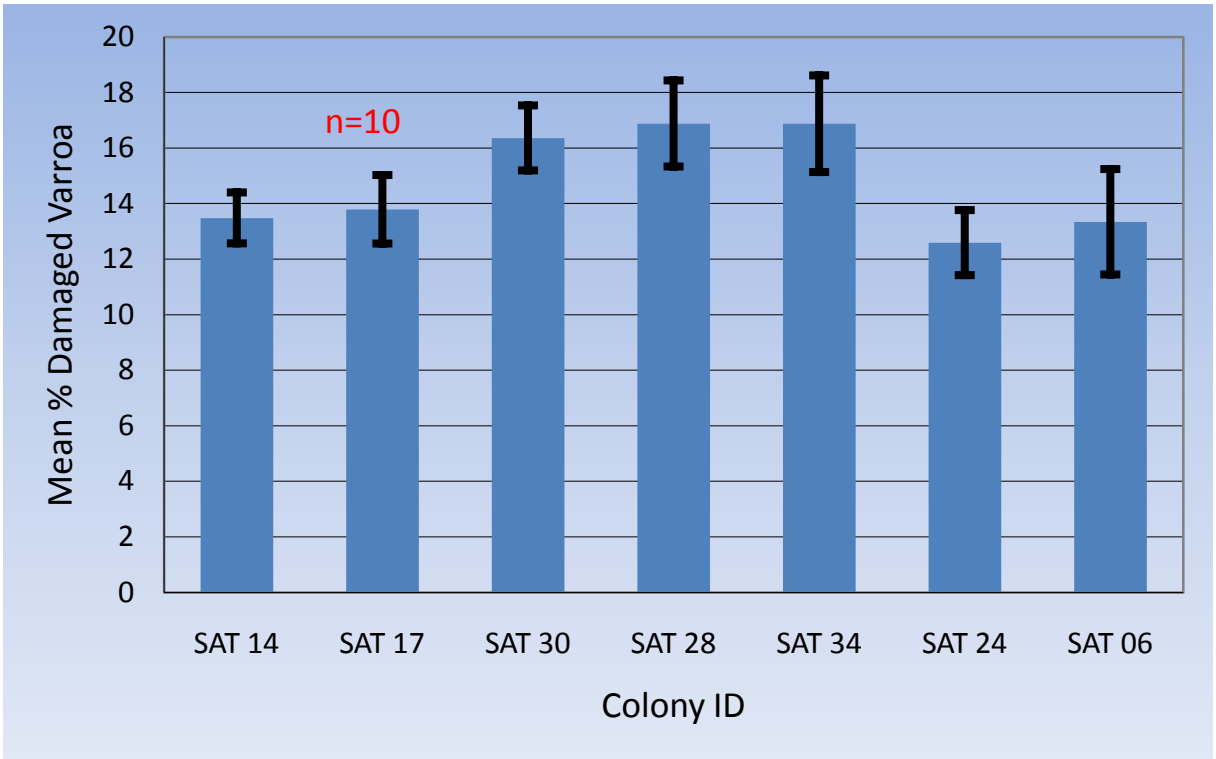

Figure 10: Samples of varroa mites from Saskatraz test colonies were collected every six to seven days and scored for damaged legs and mouth parts. Damage to the dorsal shield was not scored as due to grooming activity. The values plotted are mean plus or minus SEM(Standard Error of the Mean), n=10.

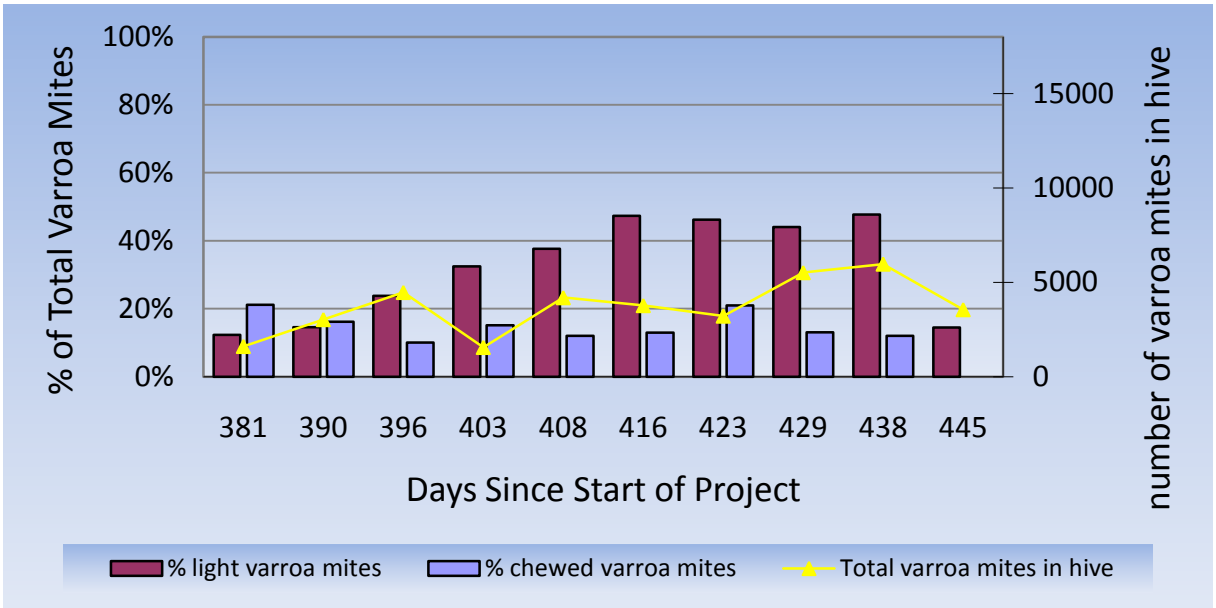

Figure 11(a). SAT-06

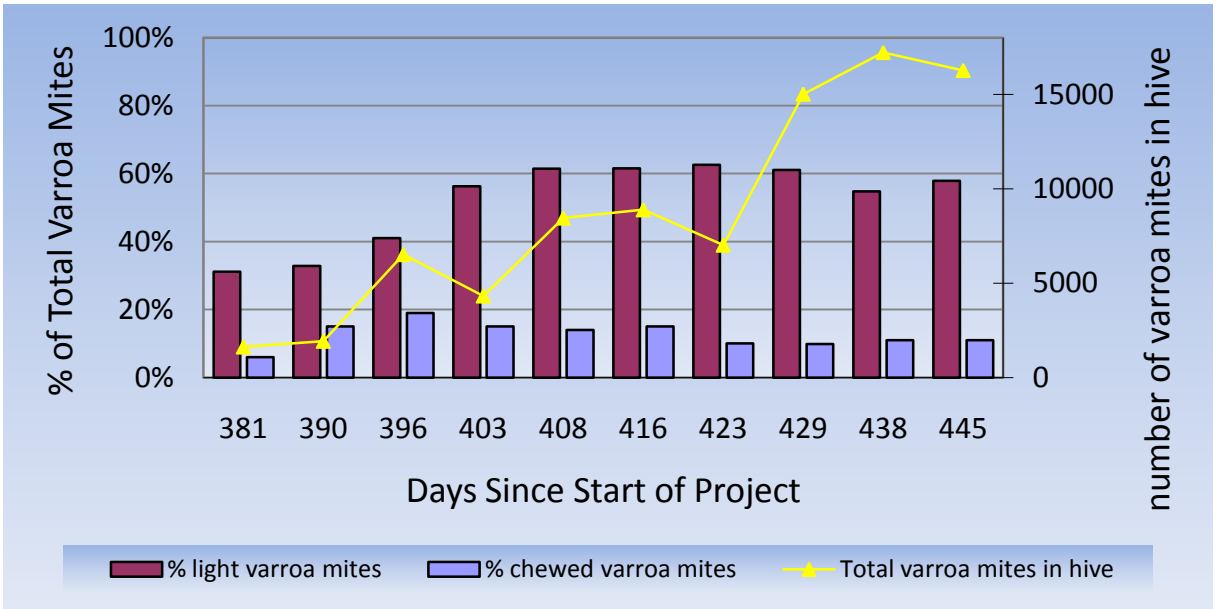

Figure 11(b) SAT 24

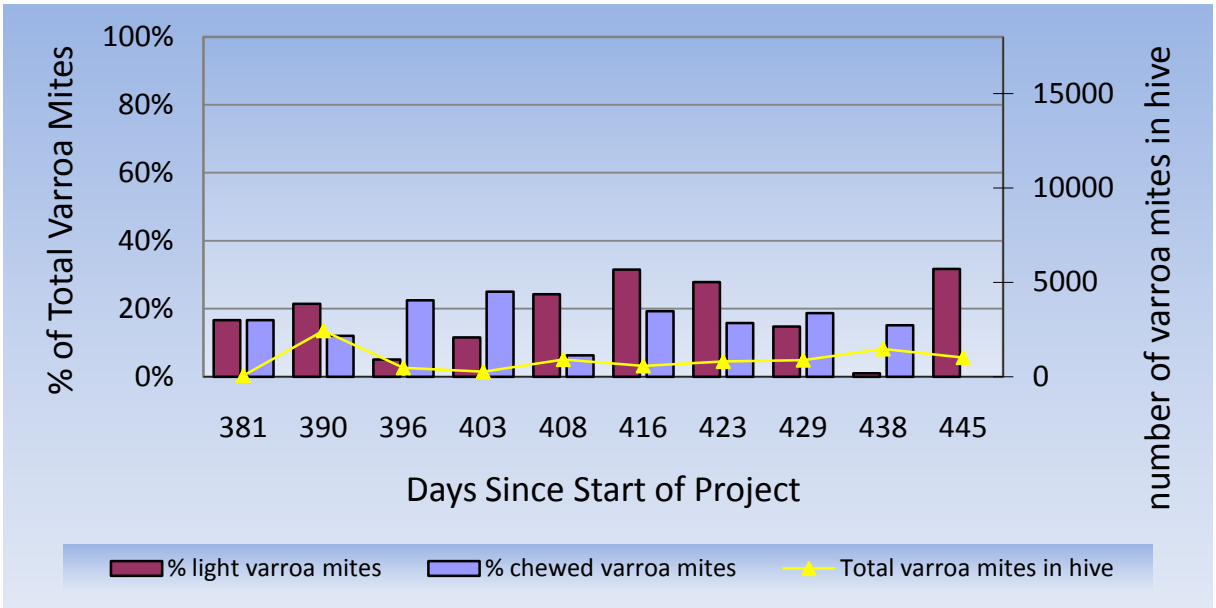

Figure 11(c) SAT 34

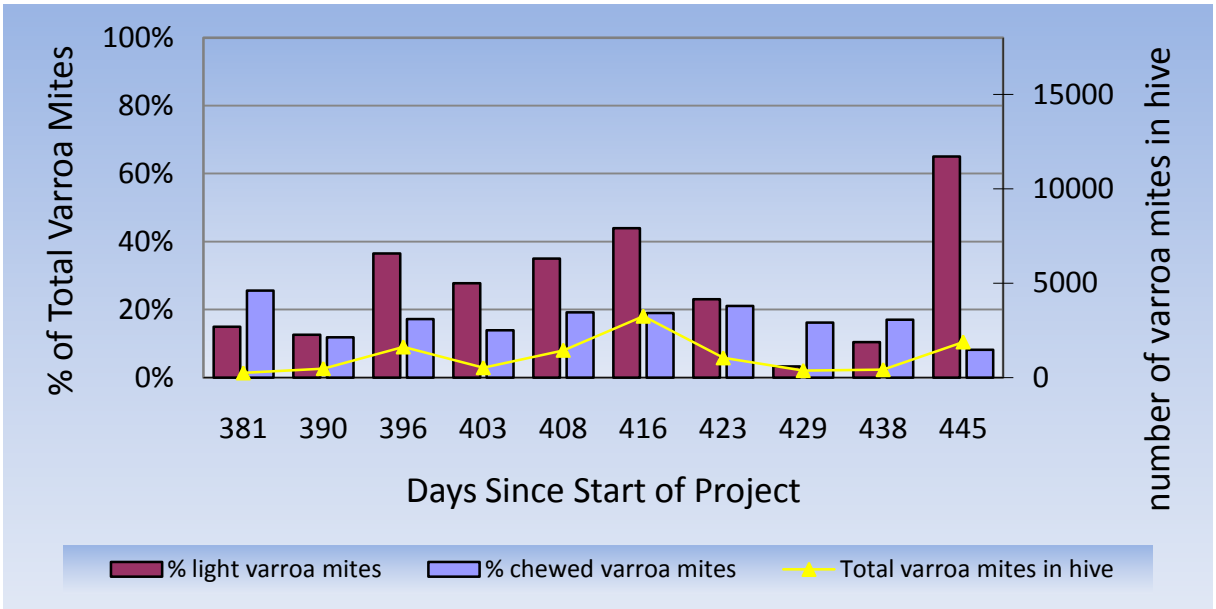

Figure 11(d) SAT 28

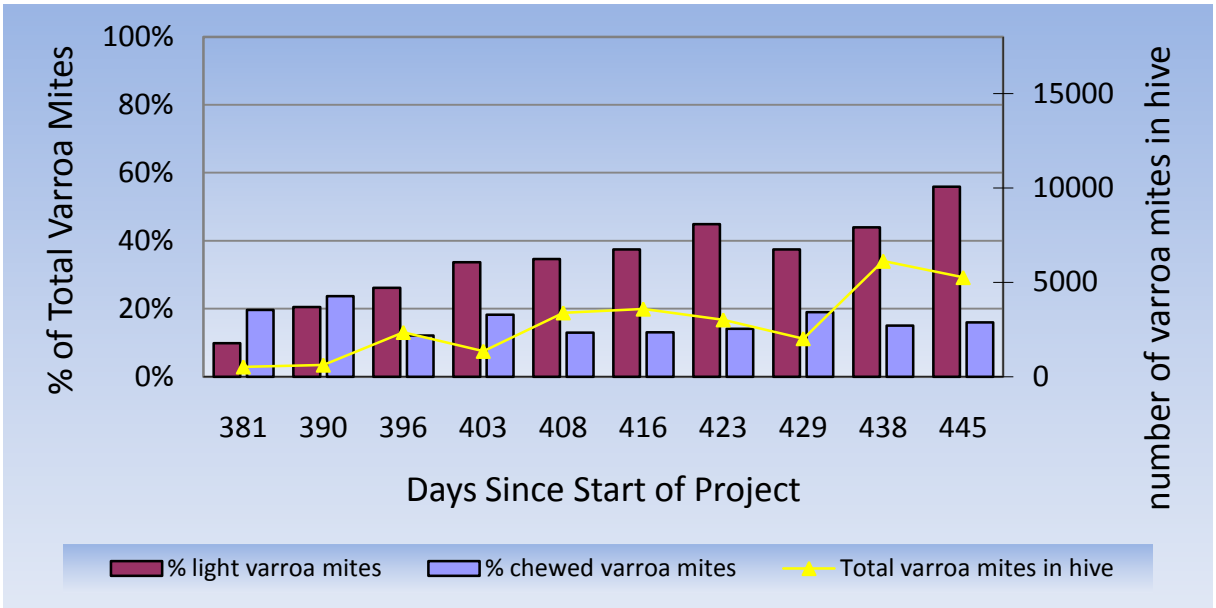

Figure 11(e) SAT 30

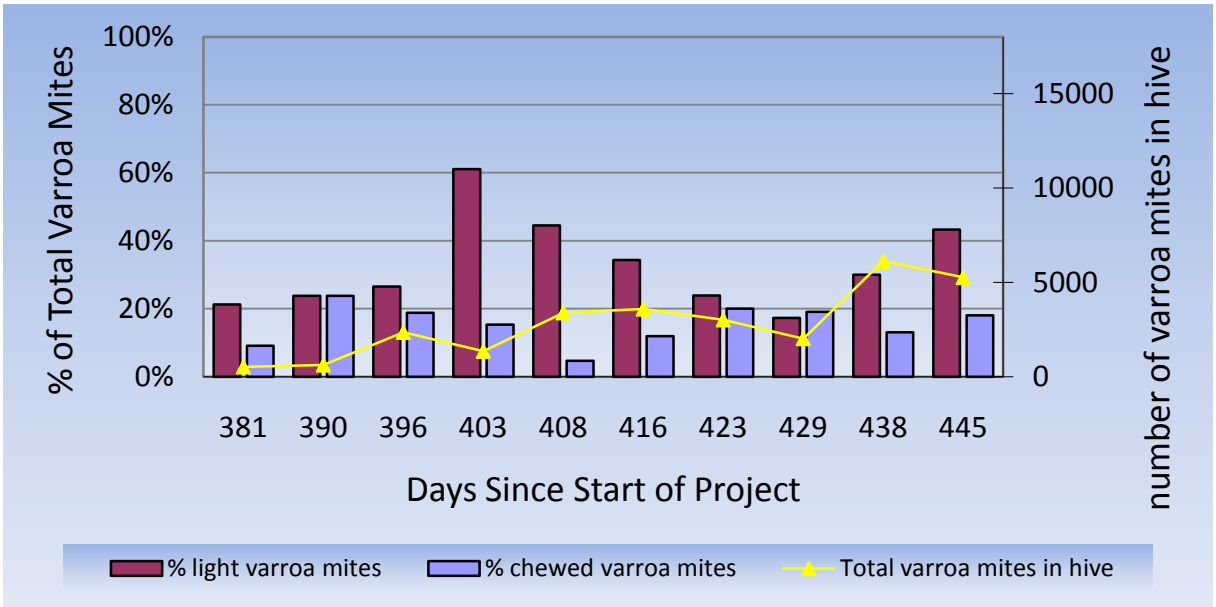

Figure 11(f) SAT 31

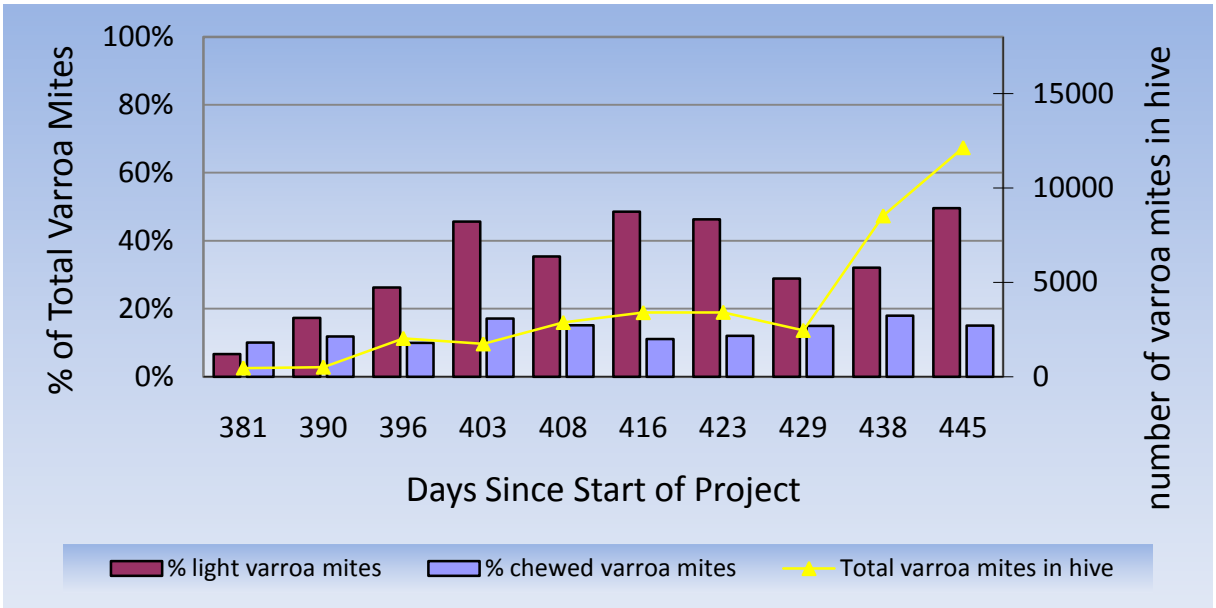

Figure 11(g) SAT 14

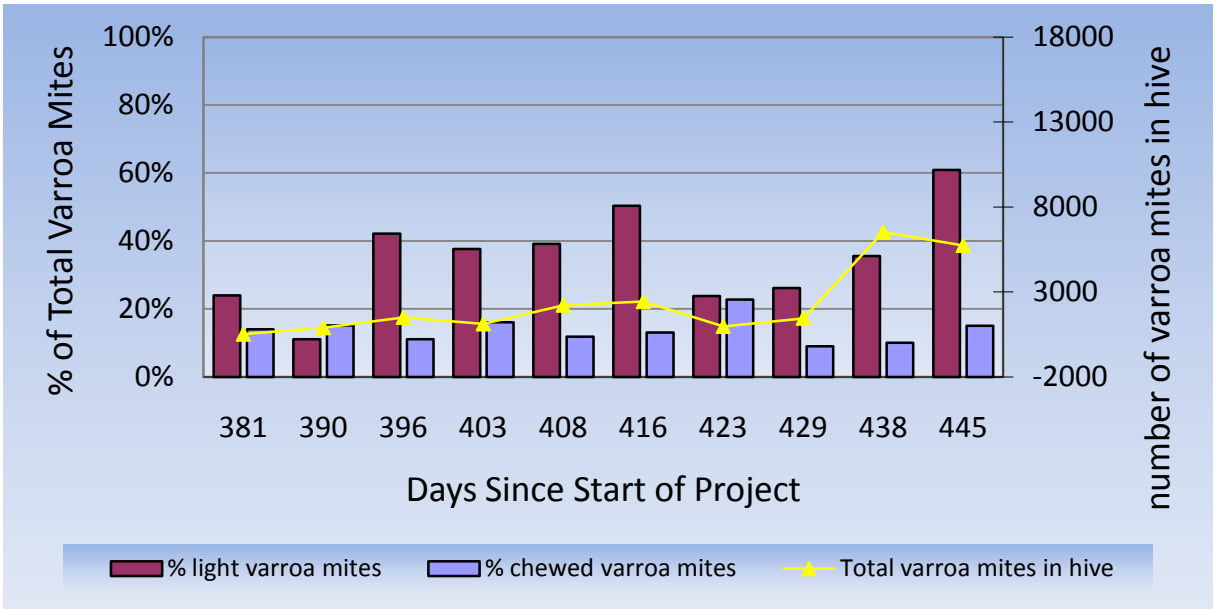

Figure 11(h) SAT 17

Figure 11 a and b show the number of varroa mites in the hive for non-selected colonies (Figure 11a and b, SAT-06 and 24) and a colony selected for mite tolerance (Figure 11c, SAT-34). Varroa mites were collected by natural drop on sticky boards on a weekly basis for data presented in figures 11a to h, inclusive)

An experiment was performed on Saskatraz test colonies to determine if some of the varroa tolerant lines showed phenotypes similar to *Apis cerana* (Roth, 1999). *Apis cerana* limits varroa population growth by removing varroa infested worker brood, but allows drone brood to become more infected. Grooming, and hygienic traits (detection, uncapping and removal of varroa infested brood) may help *cerana* to survive varroa infestations and co-exist with the parasite. *Apis cerana* also "entombs" varroa infected drone brood.

Preliminary experiments were performed by random sampling (n=4) and counting the number of varroa in worker and drone brood (100 brood cells per sample) over the summer. Saskatraz 34 (Figure 12) shows a high drone brood infestation in August, but suppresses the number of varroa present in worker brood. Some studies with the varroa sensitive hygiene trait (VSH),which refers to bees that detect and remove reproductive varroa from sealed worker brood with high efficiency (Boecking, 2000) are consistent with this observation. In studies of the effects of drone and worker varroa infested brood on the VSH phenotype, Harris (2007) showed that VSH bees removed more worker pupae infected with varroa than drone infected brood.

Higher levels of brood infestation were found in both worker and drone brood in a non-selected colony (SAT-04) in June and July (figure 12). In August there was no drone brood detected in SAT-04, and the worker brood showed over 60% infestation levels. Worker brood infestation levels were less than 9 per cent +/- 4.1 SEM in SAT-34. Table 1 shows the data for 14 Saskatraz test colonies determined throughout the summer. SAT-28 shows a phenotype similar to SAT-34, maintaining low levels of varroa infestation in the worker brood. Both of these colonies showed good suppression of varroa population growth, grooming behavior and hygienic behavior. SAT-14, 17 and 30 maintained low levels of worker brood infestation until late July and August. Soon after drone brood infestation levels increased, drone brood production in the colonies decreased and worker brood varroa levels increased to higher levels (Table 1). *Apis ceranae* entombs infested drone brood, *Apis mellifera* does not, and drones that successfully emerge carry high numbers of phoretic mites serving as a source of infection to neighboring colonies, and worker brood within the colony. Table 1 shows that by mid August varroa mite susceptible colonies (SAT-04, 14, 17, 23, 24, 25, 30) were critically infected.

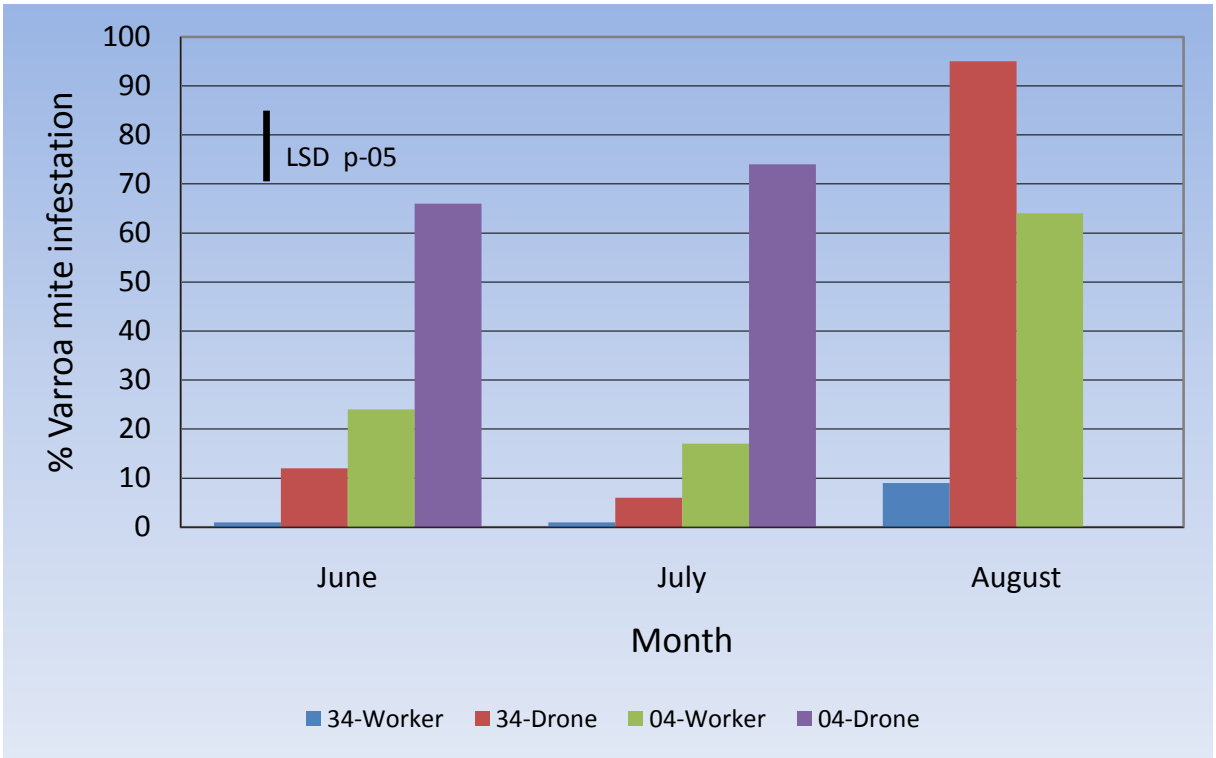

Figure 12: Drone and worker brood was randomly sampled between June and August 2006 to determine if differences exist between colonies in percent varroa mite infestations of worker and drone brood. SAT-34 showed low levels of infestation in worker brood, but high levels in drone brood by August. A non-selected colony, SAT-04, showed a general increase in worker brood infestation by August. No drone brood was present in SAT-04 in August. LSD p-05 (least significant difference)

The total number of colonies at the Saskatraz yard site from which honey was harvested in 2006 was 49. Colony numbers are constantly changing because of queen failures, failed supersedures and wintering losses. All colonies entered in 2004 have numbers 35 and lower, by August 30, 2006 SAT-72 was the highest recorded entry from which honey was harvested. Colonies requeened or nucs moved into the yardsite were given consecutively higher numbers.

Table 1: Comparison of % Brood Varroa Mite Infestation in Saskatraz Test Colonies May-to August 2006.

Saskatraz Colony ID	May 29		June 14		July 19		Aug. 16	
Brood type	worker	Drone	worker	drone	worker	drone	worker	drone
01	Δ	Δ	Δ	Δ	17+/-3	61+/-4.7	Δ	Δ
04	Δ	Δ	24+/-4.9	66+/-4.2	17+/-3	74+/-9	55+/-3.7	ND
06	Δ	Δ	Δ	Δ	12+/-2.3	33+/-3.4	Δ	Δ
14	3 +/-1.0	13+/-1.4	0	24+/-2.8	3+/-1.9	37+/-5	29+/-13	36
16	Δ	Δ	22+/-5.8	32+/-4.9	13+/-1	39+/-5	Δ	Δ
17	3+/-1.0	6+/-2.0	2+/-0.71	37+/-3.0	9+/-1	21+/-1.9	48+/-2.8	80+/-8.0
23	0	ND	5+/-1.9	56+/-8.1	Δ	Δ	37+/-2.8	77+/-9.8
24	Δ	Δ	16+/-2.8	53+/-9.4	Δ	Δ	34+/-3.8	ND
25	Δ	Δ	14+/-1.2	54+/-4.8	Δ	Δ	82+/-2.6	96
26	Δ	Δ	23+/-9	79+/-9.5	Δ	Δ	Δ	Δ
28	0	23+/-1.9	5+/-2.5	30+/-2.6	Δ	Δ	9+/-5.3	ND
30	2.7+/-1.2	17+/-2.4	6+/-2.6	41+/-4	7+/-3	65+/-2.5	84+/-7.1	ND
31	Δ	Δ	Δ	Δ	Δ	Δ	51+/-20	ND
34	Δ	Δ	1+/-1.0	12+/-2.8	1+/-1	6+/-3.5	9+/-4.1	95

Sample size = 100

Values reported are mean =+/- SEM; n=4

ND = no drone brood present

Δ = No Data

Close population mating of virgin queens from 2005 and 2006 selections (SAT-14, 17, 23, 28, 30, 34) were made by backcrossing at Saskatraz after June 19, 2006. The objective of these crosses was to mate the best queen selections with drones from colonies best tolerating high varroa infestations, thereby enriching for varroa tolerant phenotypes in the original selections. Varroa preferentially infest drone brood putting a high selection pressure on drones from varroa sensitive colonies. Table 1 shows the most varroa tolerant colonies had the lowest drone infestation levels, and would be expected to produce the largest number of drones fit for mating with virgin queens from selected colonies. This should be an effective method for combining both drones and queens with the best varroa tolerance. However, this proved to be difficult, with poor mating success and frequent supersedures. Out of 60 mating nucs only 24 queens successfully mated and established. Extensive analyses of progeny from these close population mated queens is still in progress. This natural selection method produced not only colonies with improved mite tolerance, but with superior honey production.SAT-61,63,65,87 and 98 show excellent honey production and good varroa mite suppression (Figure 13 and 14). SAT-65, 84 and 93 showed excellent varroa suppression, with SAT-84 having a VSH (Varroa Sensitive Hygiene) phenotype, but less than average honey production. SAT-65 produced excellent honey yields and good varroa tolerance.

On May 18, 2007 inspection of the Saskatraz yard site revealed that all of the colonies but seven died between November 2006 and May 2007. The surviving colonies consisted of a selection SAT-65, provided as a queen by a collaborating queen breeder and established at Saskatraz on July 10, 2006. SAT-65 swarmed during the summer, and a daughter mated at Saskatraz re-established the colony. The remaining survivors were nucleus's made up at Saskatraz in 2006. Extensive post mortem analyses are still in progress. We recently partnered with VIDO to perform virus and microsporidia analyses on colonies dying or collapsing because of varroa infestations. The results of these studies will be reported when complete. We visually assessed the dead colonies when the yard was first opened and found hives full of pollen and honey with no evidence of dead bees. Last inspection of the yard on November 10, 2006 indicated most colonies; particularly the selected breeders had good wintering populations. We found no evidence of dysentery, starvation, nosema or queen failures. Only a few colonies had significant numbers of dead bees, these colonies likely resulted from failed queens or failed supersedures.

On June 7, 2007 all remaining colonies were treated with Apistan to normalize varroa mite populations. Saskatraz stock outcrossed to Meadow Ridge apiaries in 2006, to maintain the Saskatraz gene pool, were reselected in 2007 for further testing at the Saskatraz natural selection yard site. Back crosses to Russian stock and closed population mated Saskatraz daughters, as well as new selections were added in 2007. The Saskatraz apiary produced an average of 200lbs per colony in 2007.

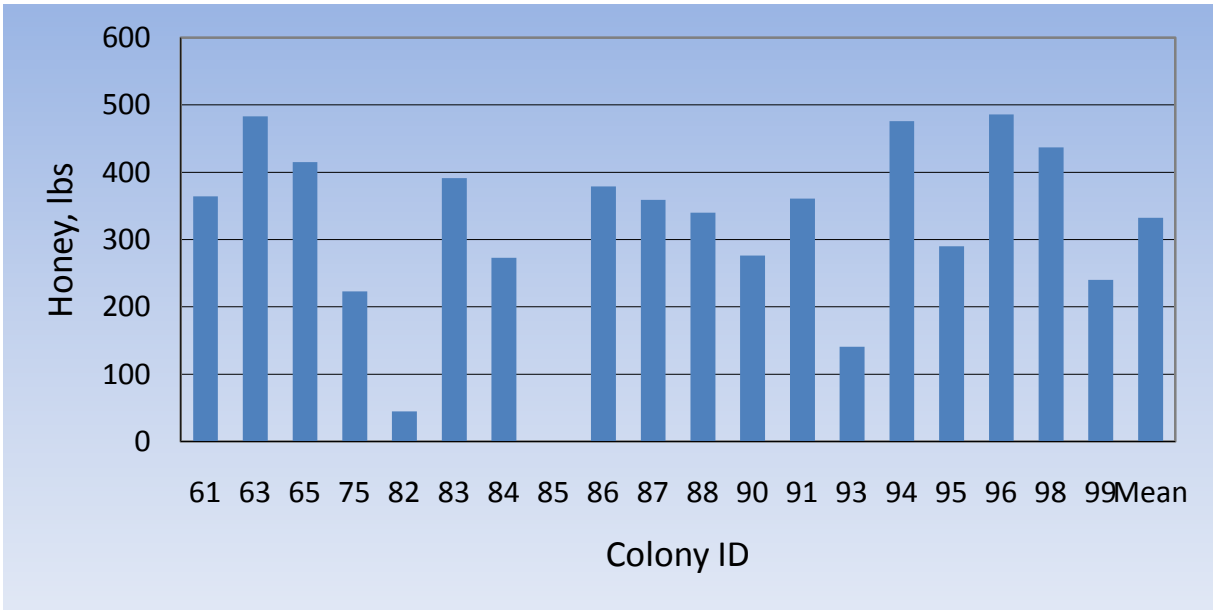

Figure 13: Total honey production per colony in 2008. SAT-63, 65, 94, 96 and 98 all produced over 400 lbs.

During the fall of 2008 varroa mite populations were closely monitored at the Saskatraz yard site by analyzing adult honey bee populations (alcohol washes), natural drop (Figure 14) and brood infestation levels. SAT-65, 84, 88, 96, and 98 produced economical honey yields (Figure 13) and showed the best suppression of varroa mite population growth (Figure 14). Some colonies showed no detectable varroa, but no significant honey production (SAT-82). The percent varroa infestation in Saskatraz worker brood was assayed in detail for SAT-63, 65, 84, 86, 87, 88, 90, 91, 94 and 96 (Figure 15).

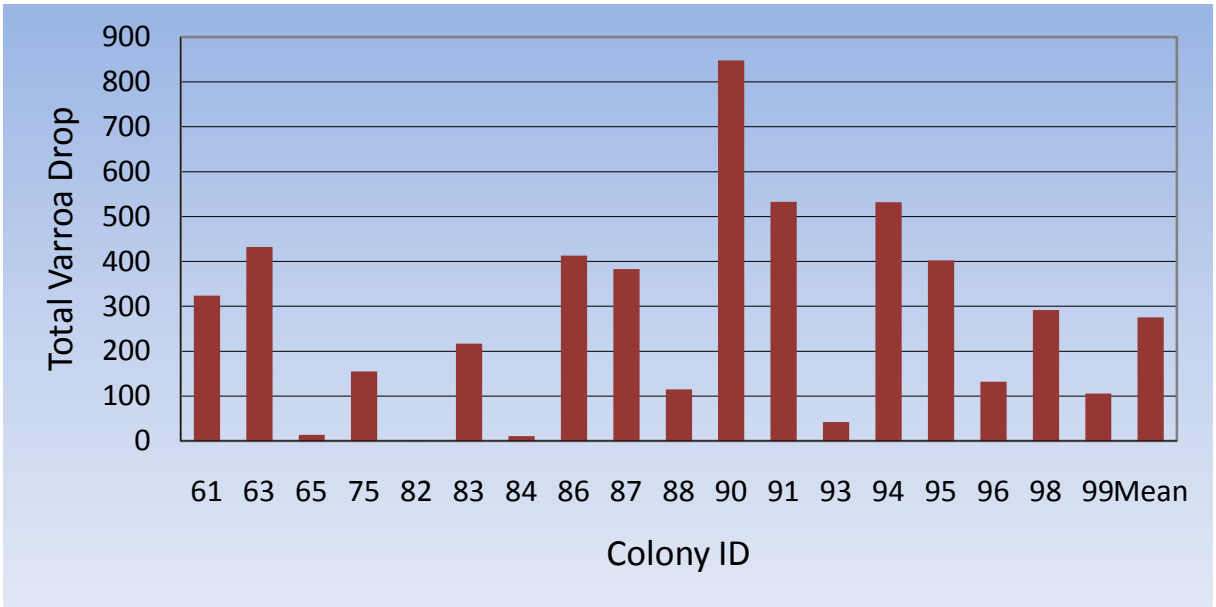

Figure 14: Total varroa drop per week for Saskatraz test colonies in 2008. SAT -65, 82, 84 and 93 showed the lowest drop per week from May 28 to Sept 30.

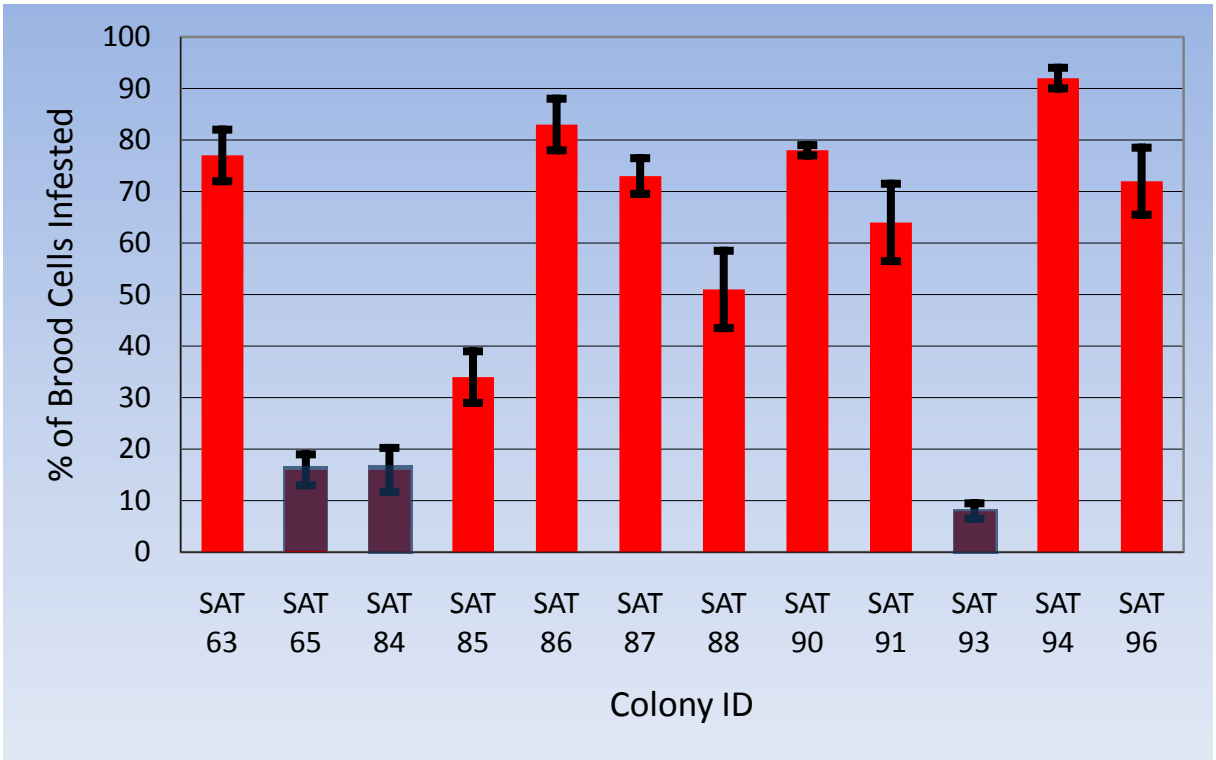

Figure 15: One hundred brood cells were randomly sampled for varroa infestation from Saskatraz test colonies on September 16, 2008. Error bars are SEM, n=5. Red bars show colonies showing visual signs of virus; Deformed Wing Virus, (DFW) or testing positive for Israeli Acute Paralytic Virus (IAPV) by RT-PCR (Figure 18). No virus was detected in SAT-65, 84 or 93.

SAT-65, 84 and 93 showed the lowest percent varroa infestation in worker brood. Varroa reproduction per brood cell was also analyzed in order to select for colonies which suppress varroa mite reproduction (SMR), now called VSH or Varroa Sensitive Hygiene (Figure 16). Eleven colonies (100 cells per colony) were randomly sampled (SAT- 63, 65, 84, 86, 87, 88, 90, 91, 94, 96) and assayed for the number of varroa per cell. SAT-65 and 84 showed the lowest levels of varroa per cell. SAT-84 showed the lowest level of infestation with a mean number of 2 varroa per cell. This phenotype fits the definition of a VSH phenotype. This phenotype removes the most reproductive varroa mites from worker brood cells, leaving only brood cells with low numbers of varroa mites. This results in the overall suppression of varroa mite population growth. It is thought that the mites with low reproductive rates may undergo delayed oviposition, but it is not known whether this is due to the pupae parasitized or the varroa mite (Jeff Harris, 2010. Orlando meetings).

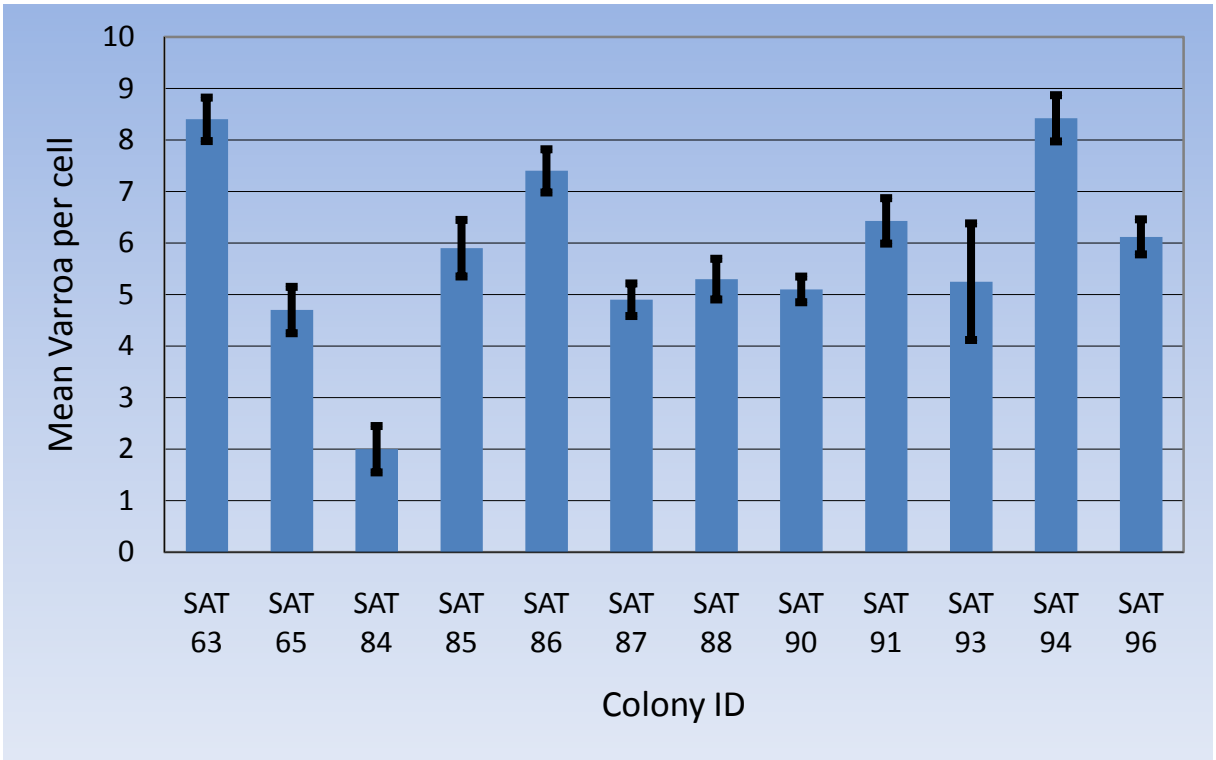

Figure 16: Brood comb from all Saskatraz colonies was randomly sampled for varroa infestation and the number of varroa per cell determined by stereo microscope analyses. One hundred cells were analyzed per colony. Mean values are plotted, error bars are SEM.

Pre-emergent pupae infected and not infected with varroa were sampled for both morphological (phase contrast microscopy) and molecular analyses (RT-PCR for viruses). Pre-emergent pupae that were heavily infected with varroa (8 mites/cell) showed morphological (Figure 17) anomalies (deformed wings, extruded proboscis, deformed and atrophied abdomens). Ten Saskatraz selections with levels of varroa infestation between 15 and 90% were screened for the expression of IAPV (Israeli Acute Paralytic Virus) and Deformed Wing Virus (DFW) by RT-PCR at GenServe Labs, SRC (Figure 18). Some critical observations were made. Colonies showing visual symptoms (DFW) of virus infections (SAT-63, 90, 91, 94, 96) had high levels of varroa infestation (65 to 95%) and tested positive by RT-PCR for IAPV and DFW virus infections. However, 2 colonies (SAT-65, and 84), showed no virus symptoms or expression of viruses by RT-PCR. Figure 18.

Figure 17: Picture of a pre-emergent pupae (SAT-94) removed from a brood cell infested with 8 varroa mites.

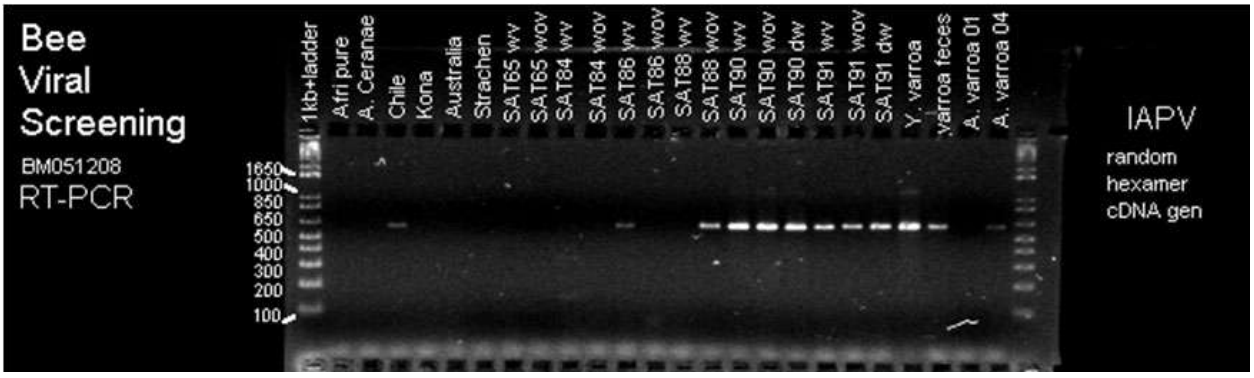

Figure 18: Screening of pre-emergent pupae from varroa tolerant (SAT-65, 84) and sensitive (SAT -90, 91) breeding lines for IAPV using RT-PCR (Bruce Mann, SRC).

Figure 18 also shows IAPV screening of adult bees preserved in alcohol, from samples of Africanized bees from the Yucatan area of Mexico, *Apis ceranae* (unknown source) and attendants from queens shipped from southern Chile, Hawaii (Kona), Australia and California (Strachan). A trace of IAPV was found in an attendant from Chile, while all others showed no detectable viruses. SAT-86 pupae parasitized with varroa tested positive, where as pupae not infected showed no detectable virus. Both pupae parasitized with varroa and not parasitized showed IAPV infection in SAT -90 and 91. Y. varroa is a sample of varroa mites from a producer who experienced some high colony varroa levels in 2007. Varroa feces collected from SAT-90 brood comb also showed a strong signal for IAPV infection. This finding indicates that the potential exists for brood comb to be carrying viruses for infecting subsequent brood production. Experiments are in progress to determine the infectivity of virus particles in varroa feces. Varroa samples collected from Ontario (Alison Skinner) in 2001 and 2004 showed the presence of IAPV virus in 2004, but not 2001.

In the fall of 2008 the varroa levels increased to critical levels (both in brood and on adult bees) in most colonies, and an attempt to rescue these colonies was made by treating with organic acids (Figure 19). Formic acid treatment did not increase the varroa drop rate except in SAT-96 between September 30 and October 17. A slight increase was noted in SAT-65, 61 and 86 between October 17 and 25. After oxalic vapor treatment on October 25 varroa drop rates increased most dramatically in SAT-61. There was an overall increase in the mean drop rate for all colonies showing the effectiveness of oxalic treatment.

In the spring of 2009 only three colonies were found to survive the winter SAT-85, 88 and 96. These colonies were established at Saskatraz in 2007, and showed lower levels of mite drop in the fall of 2008, but significant virus infections. Scanning electron microscopy showed considerable damage to surface hair on the bees from colonies treated with oxalic vapor compared to untreated colonies (data not shown). Formic and oxalic treatments may have imposed considerable stress on the bees in the fall contributing to their death. SAT- 65, and 84 both died even though varroa mite (Figure 19) and virus levels (Figure 18) were low. The fall treatment protocol was not successful and may have resulted in high mortality rates. The Saskatraz yard site was restocked during July of 2009, with reselected colonies managed under standard commercial procedures. All colonies received three formic acid treatments (mite wipe pads) and Apistan during the spring of 2009. SAT- 85, 88 and 96 did not receive any treatments except with organic acids as described in Figure 19, in the fall of 2008.

SAT-110, 113, 121, 125, 126 all produced over 370 lbs in 2009 (Figure 20). SAT-125 was a daughter of SAT 30, out crossed in 2006 and reselected at Meadow Ridge in the spring of 2009. This colony was selected for high honey production in 2006.

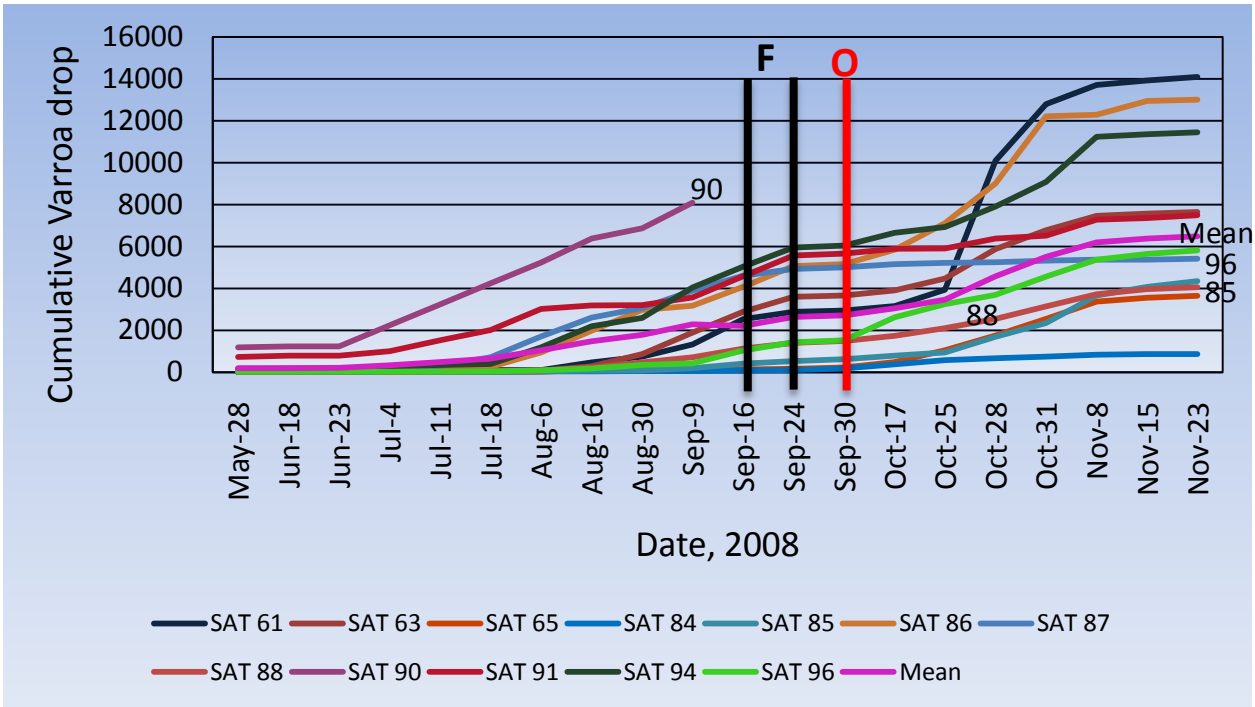

Figure 19: Cumulative varroa drop at Saskatraz apiary between May 28 and November 23, 2008. Vertical black bars show times (September 30 and October 17) of treatment with formic acid (mite wipe pads-60% formic acid), followed by oxalic vapor (O) on October 25, 2009. Oxalic vapor treatment was performed by Calvin Parsons.

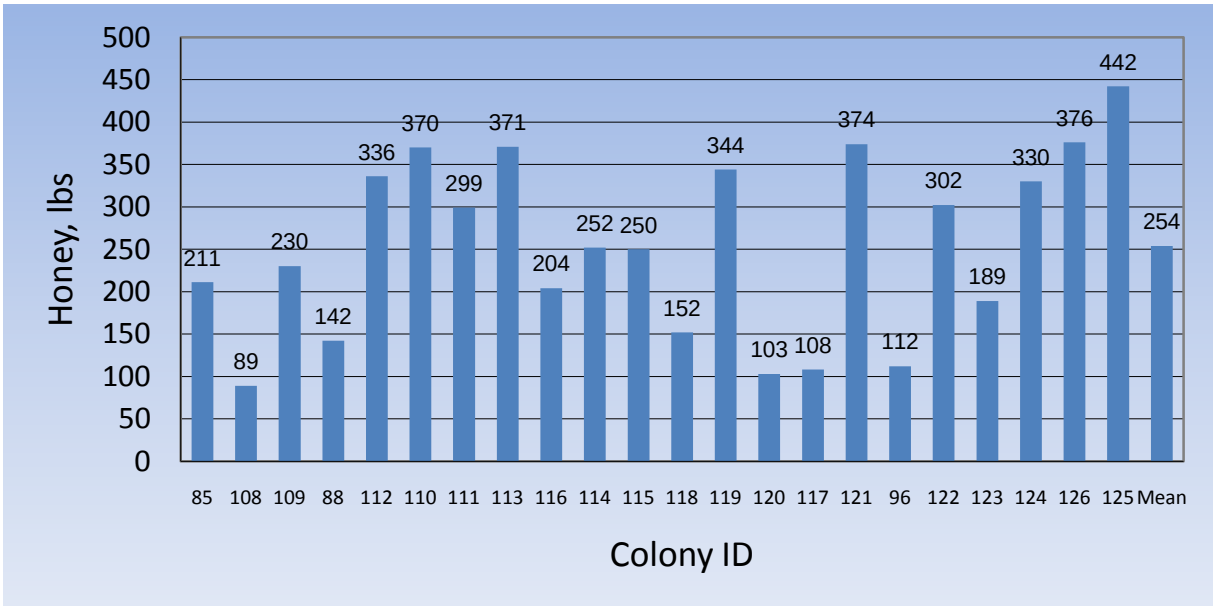

Figure 20: Total honey production per colony in 2009. SAT-125 and 126 produced the most honey per colony in 2009. The survivors SAT- 85, 88 and 96 produced 211, 142 and 112 lbs respectively

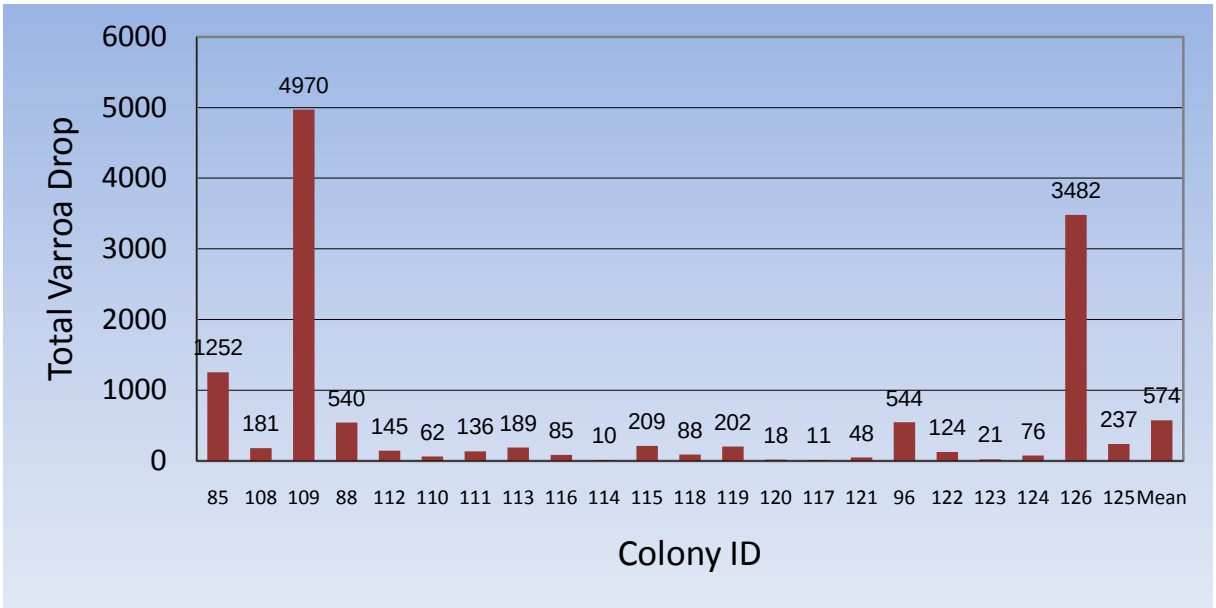

Figure 21: Survivor colonies SAT- 85, 88 and 96 showed total varroa mite counts of 1252, 540 and 544 respectively. Two colonies provided by a collaborating queen breeder for testing SAT-109 and 126 showed varroa mite levels increase to 4970 and 3482 respectively.

The surviving colonies SAT 85, 88 and 96 produced 211,142 and 112 lbs of honey respectively, all below the yard average (254lbs). When first inspected in the May 2009 these colonies were very weak averaging about 3 partial frames of bees and small patches of brood. Varroa mites were visible on the adult bees and deformed wings were noted. These colonies all showed some virus infections (DWV, deformed wing virus) in the fall of 2008 and significant levels of varroa mites in the brood (Figure 15).They were not treated for varroa mites, worked down to a single box, fed and expected to die. On July 3, 2009 the colonies had recovered, being full of brood and bees with bees hanging out the entrances. On inspection no visible mites were observed. The data from the spring of 2009 is difficult to interpret because of the organic acid treatments made in the fall of 2008, which may have imposed other complicating stresses on the Saskatraz colonies. A detailed post mortem analyses was performed on the dead colonies in the spring of 2009, (unpublished data) indicating severe loss of the hairs on worker bee external surfaces. Visual damage to the bees antennae was also observed in scanning electron microscope studies. These damages could result in loss of thermal protection, water balance and sensory mechanisms The Saskatraz survivors showed excellent tolerance to all these stresses and were able to make a strong recovery.

Although low levels of varroa mites were present in all colonies reselected for testing at Saskatraz, and varroa populations were normalized by spring treatments with Apistan and formic acid, two colonies SAT-109 and 126 showed high varroa population growth rates. The queens heading these colonies were provided by a collaborating queen breeder in 2007 and

reselected for Saskatraz in 2009. The colonies showed excellent honey production, but were sensitive to varroa. This line was traced back to a Russian release (yellow) made in 2004, which should have had some tolerance to varroa. The colonies from which the test lines were selected were managed with chemical miticide treatments up until June 2009. Most of the other colonies were daughters of Saskatraz breeders out crossed between 2006 and 2008 at Meadow Ridge and subjected to recurrent selection prior to re-introduction into Saskatraz. These colonies were also managed under commercial conditions, but with different miticides treatments. This data suggests that certain chemical miticide treatments and removing varroa infestations (selection pressure) results in decreased varroa tolerance over time. This requires further investigation and return of varroa tolerant selections released since 2006 for retesting at the Saskatraz natural selection site.

COMPARISON OF SASKATRAZ FAMILY HONEY YIELDS TO PROVINCIAL AND MEADOW RIDGE YIELDS

Figure 22 compares the mean honey production in Saskatchewan over the last 20 years to that of Saskatraz over the last 5 years. Although location effects are not accounted for the Saskatraz apiary out yielded the provincial averages every year, since 2005 without chemical miticide treatments. During years where varroa mite levels reached critical levels in the fall of the year (2006 and 2008), honey production continued to exceeded provincial averages.

Figure 23 shows the results of comparing Saskatraz honey production as a percent of provincial and Meadow Ridge values. Comparing to Meadow Ridge values helps assess management effects, and climatic effects within a 100 mile radius. The trend has been for Saskatraz production to move closer to Meadow Ridge values, even though Saskatraz colonies are not treated with chemical miticides. This effect can be explained by considering the effects of recurrent selection for honey production. In 2005 honey production was only 65 percent of Meadow Ridge; however, outcrossing colonies selected for honey production and reselecting for wintering ability and honey production should serve to further enrich for genes involved in these multi-genetic traits. This process not only maintains the majority of the gene pool originally selected from Saskatraz, but maintains genetic diversity, which has significant effects on hive performance (Matilla and Seeley, 2007). The mean honey production is now similar for Saskatraz and Meadow Ridge, and is showing mean Saskatraz honey production as 30% more than the provincial average.

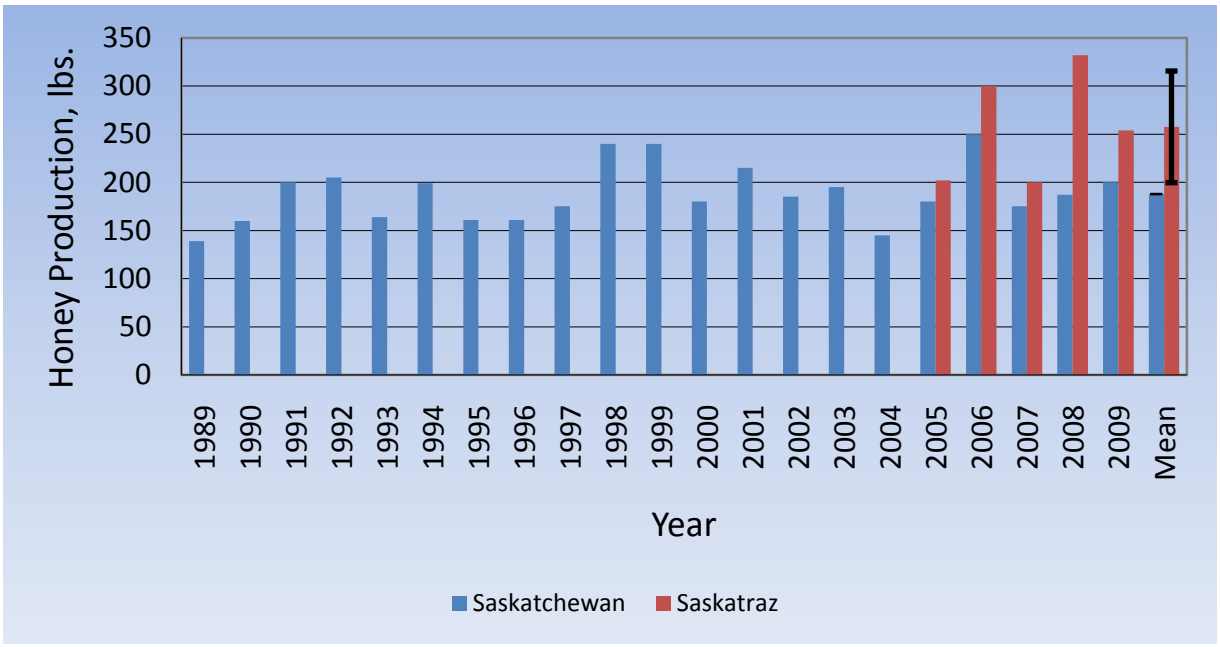

Figure 22: Honey production in Saskatchewan between 1989 and 2009 (source Saskatchewan Agriculture) reported as average production per year from approximately 63,000 colonies. The 20 year mean plus or minus SEM is shown. The average honey production per year at Saskatraz represents values calculated from colony numbers which varied between 25 and 35. The mean represents honey production between 2005 and 2009 plus or minus SEM.

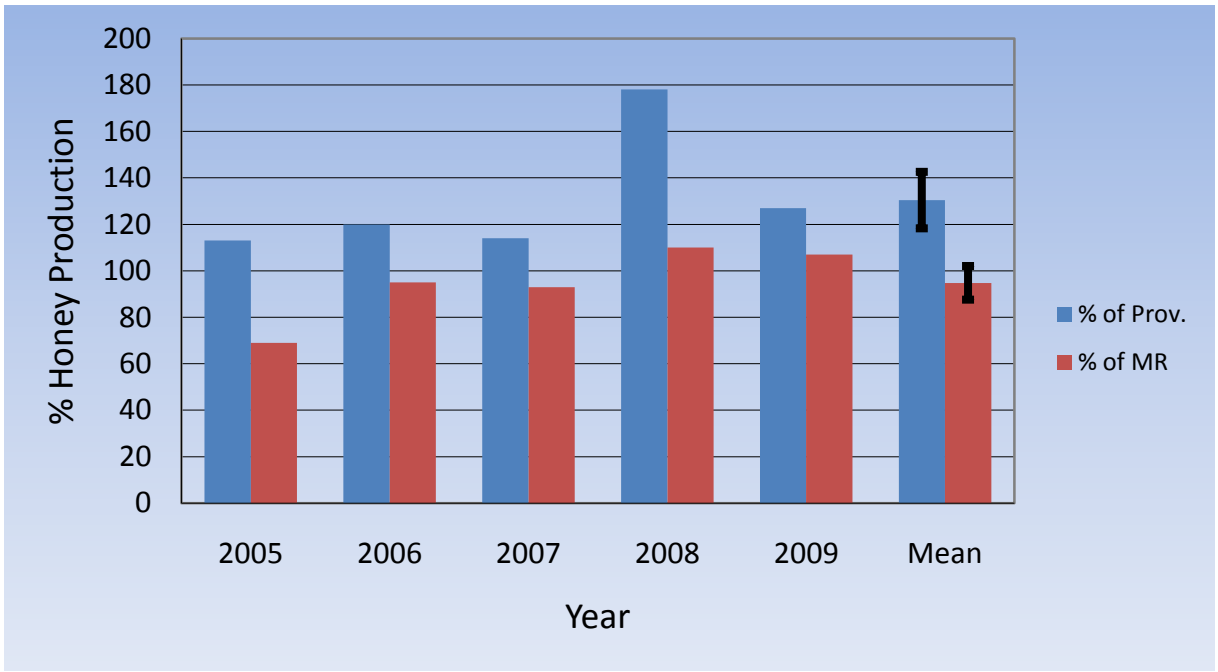

Figure 23: Shows the average honey production at Saskatraz per year as a percent of that for provincial and Meadow Ridge averages.

Figure 24 shows the results of honey production data collected from selected Saskatraz colonies and reselected daughters from out crosses of these original breeders. The honey production of selected Saskatraz families is compared as a percentage of provincial and Meadow Ridge averages. The Saskatraz colonies and their daughters selected for honey production (SAT-14, 17 and 30, 94, 86 and 125) consistently produced 40 to 60% more than the provincial average and 20 to 40% more than Meadow Ridge. Saskatraz colonies originally selected for both varroa tolerance and honey production and reselected daughters from them (SAT-28, 34, 85, 87, 93, 96, 112) consistently produced from 30 to 40% more honey than provincial averages, but did not show any increased production when compared to Meadow Ridge values. This implies that increased varroa tolerance comes at a cost of reduced honey production. A 30% increase in honey production with a current average value of approximately $30 million per year in Saskatchewan, would translate into a considerable economic impact (9 million dollars per year).

The initial concept of establishing a large diverse gene pool at Saskatraz, selecting the best colonies for honey production and varroa tolerance, using natural selection, out crossing and reselecting progeny to maintain and improve the selected gene pool and to maintain genetic diversity has proven successful. Improved honey production, wintering ability and varroa tolerance improves economic benefits for bee keepers. Healthy colonies produce economic honey crops and provide improved pollination efficiency. However, this approach to bee breeding requires a large colony base and millions of dollars of infrastructure and high labor costs. The project was designed initially using selections from 14 queen breeders who had been overwintering honey bees in Saskatchewan for many years. These colonies were selected for overwintering ability, honey production and resistance to brood diseases (chalkbrood). None of these colonies showed significant tolerance or resistance to varroa mites, but some showed good tracheal mite resistance. The best varroa mite tolerance was introduced through the introduction of Russian stock in a joint venture with the Ontario Beekeepers Association, and by obtaining German stock (drone semen) from Dr. Ralph Buchler, Kirchhain, Germany. Semen importation was made possible by the efforts of Yves Garez, a Saskatchewan queen breeder. The selection process could be improved significantly by identifying biomarkers to assist selection of important traits.

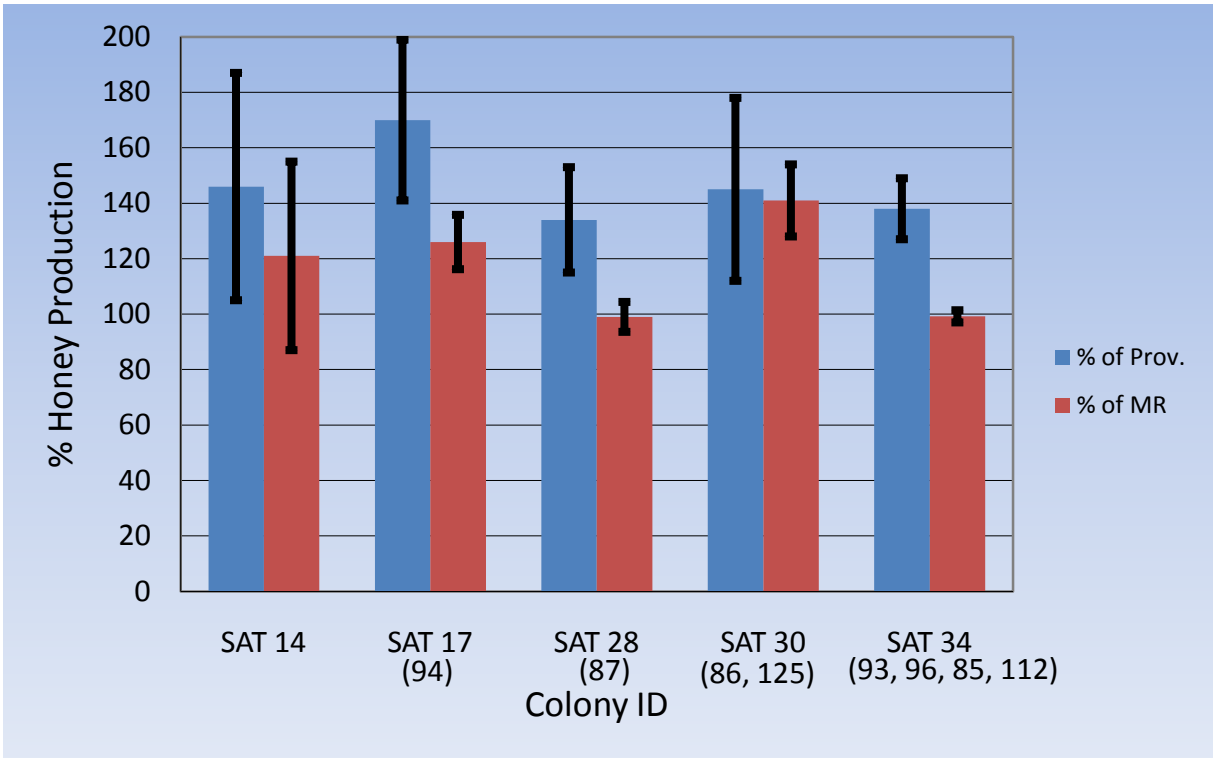

Figure 24: Saskatraz families selected for honey production (SAT-14, 17 and 30) and varroa tolerance (SAT-28 and 34) compared as a percent of provincial and Meadow Ridge production.

IDENTIFICATION OF MOLECULAR MARKERS FOR MONITORING GENETIC DIVERSITY AND CHARACTERIZING IMPORTANT PHENOTYPES.

Microsatellite marker analyses has been carried out in collaboration with Dr. Yves Plante and Bruce Mann, GenServe Laboratories, Saskatchewan Research council on a contract basis since 2003. Only data relevant to the current report is described here. Detailed methods and results showing identification of 20 informative microsatellite markers for genotyping different honey bee populations will be published elsewhere

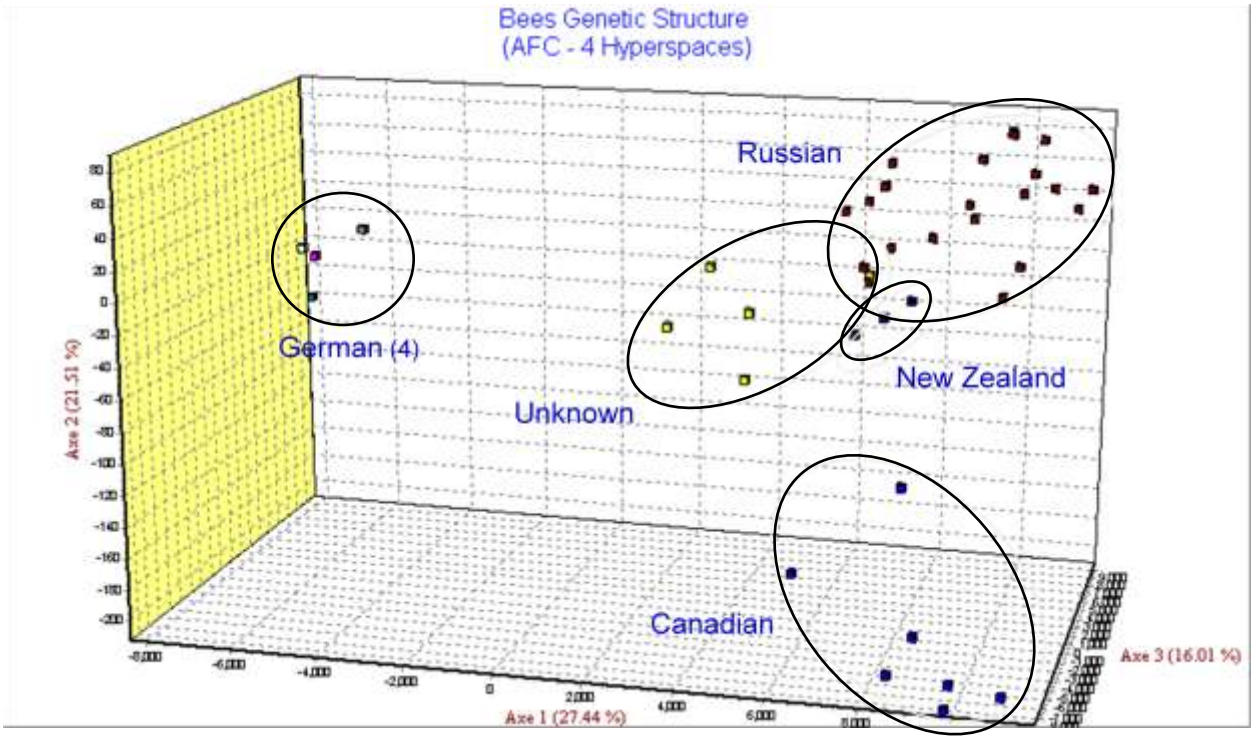

Figure 25: A three dimensional plot showing the grouping of 5 different honey bee populations using 20 informative markers.

Figure 25 shows the results of screening 5 different sets of drones collected from sources of stock selected for introduction into the Saskatraz natural selection yard site. The statistical programs used for generating these plots were provided by Dr. Yves Plante, some of which can be accessed at http://bioinformatics:psb.ugent.be/psb/Userman/treecon. The plot clearly shows the genetic diversity between the different populations. The unknown samples represent hybrids generated by Russian Canadian crosses, and the New Zealand bee samples clustered between the Russian and Canadian samples.

The 20 informative markers were also used to genotype Saskatraz breeding lines Figure 26. Drones collected from each of 14 Saskatraz breeding lines. SAT-28, 34, 65, 84 and 96 all showed some degree of varroa tolerance, but no grouping similarities. Saskatraz selections sensitive to varroa population growth (SAT-04, 24, and 90) grouped closer together, but close to some lines showing varroa tolerance, such as SAT 84. This study indicates more markers are required to increase the resolution required to discriminate between different phenotypes, or higher resolution methods are required. However, the 20 informative markers can be useful to identify individual Saskatraz breeding lines. Figure 27 shows the map and linkage group location of each of the 20 informative markers. The map (Figure 27) was constructed using information at the www.ncbi.nim.nih.gov website and microsatellite sequences.

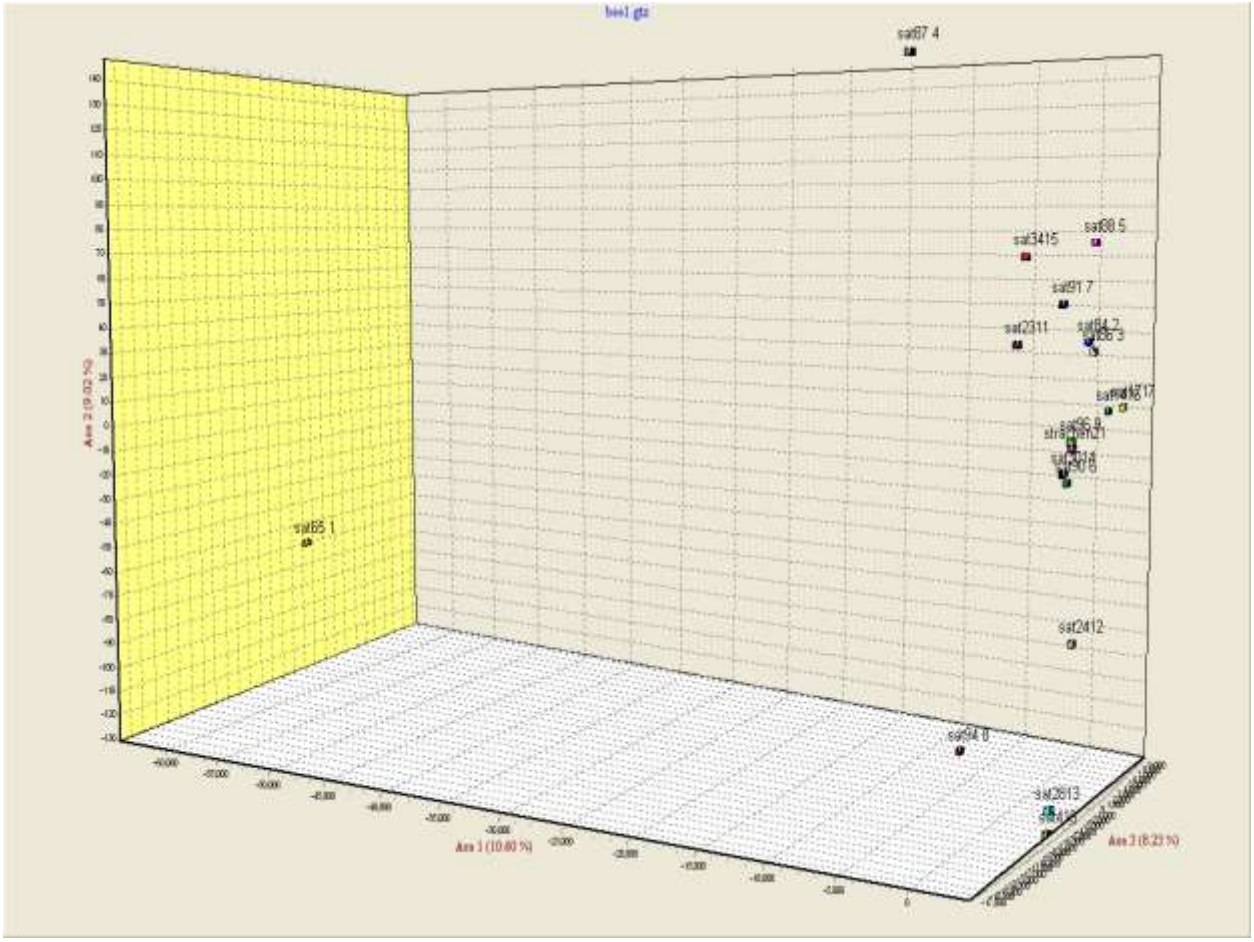

Figure 26: Genetic relatedness (genotyping) between Saskatraz breeding lines.

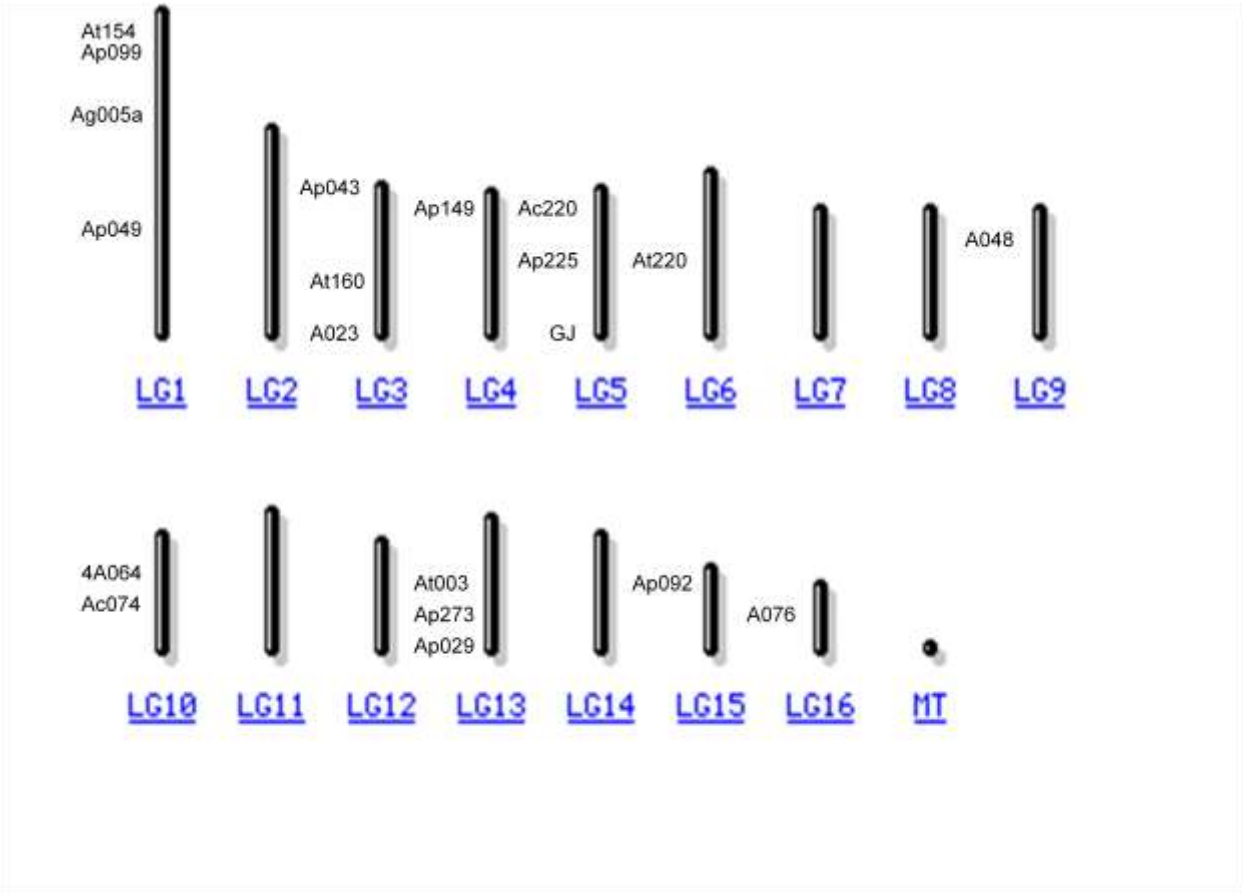

Figure 27: The 20 informative microsatellite markers used to identify different honey bee populations were mapped using sequencing information from the honey bee genome project.

Current research activities involve progeny analyses of out crossed and re- selected Saskatraz breeding lines for grooming behavior, morphological characteristics, hygienic behavior, VSH phenotypes, molecular marker analyses (microsatellites, microarrays, kinome arrays) and testing Saskatraz breeding lines for susceptibility to virus infections. Mohammad Mostajeran, a research associate on the project is working on morphometrics, grooming behavior and VSH phenotypes and we are collaborating with VIDO (Dr.Philip Griebel and Wayne Connor) and the University of Saskatchewan, Food and Bioproducts (Dr. Xiao Qui and students) on virus immunity and microarrays, respectively. Extensive data has been collected in 2008 and 2009 on progeny analyses and will be described in future newsletters.

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Research Personnel and contributions-The Saskatraz Research Team.

The continued professional assistance of Meadow Ridge Employees since the inception of the project in 2004 (Tom Robertson, Neil Morrison, Jennifer Robertson and Cecilia Robertson) for helping with Saskatraz selections, maintenance, honey harvesting, yield analyses and reporting as well as multiplying breeding stock for distribution to queen breeders and commercial

beekeepers has made this project possible. We were fortunate to have John Pedersen help with breeding stock multiplication and distribution in 2006. The Principal Investigator, Dr. Albert J. Robertson volunteered most of his time in kind for experimental design, colony selection, data analysis, data presentation, writing grant applications and publications, as well as grafting, propagation, and distribution of breeding stock for members of the Saskatchewan Beekeepers Association. We are grateful to John Gruszka not only for his help with mite analyses at the Provincial Apiculture Labs, but for his dedicated and loyal support of the project. We thank Dr. Abdullah Ibrahim for assisting with the breeding program April to December 2007. Dr. Abdullah Ibrahim helped perform initial recurrent selection analyses on Saskatraz breeding families for varroa suppression and assayed colonies for hygienic behavior. He also assisted with stock distribution, honey production analyses, and establishing closed population apiaries for stock maintenance and mating. Dr. Felipe Brizuela assisted with stock distribution, recurrent selection, Saskatraz varroa analyses and closed population breeding procedures from June 2008 to October 2008, and April 2009 to November 2009. Felipe also assisted with a feasibility study in Chile for multiplying Saskatraz stock January-March 2009. Mohammad Mostajeran has worked on the Saskatraz project since July 2008. His work has focused on varroa analyses at the Saskatraz natural selection yardsite. He has also worked on recurrent selection procedures for varroa population growth on outcrossed Saskatraz stock, new selections and establishing and improving closed population breeding methods. His work has also involved morphometrics and progeny analyses of Saskatraz daughters during indoor wintering for grooming behavior, hygienic behavior and pathogen analyses. We are pleased to have the expert technical assistance of Bruce Mann, GenServe Labs, SRC and Wayne Connor, VIDO, U of S. The expert help of Rob Peace with the preparation of power point presentations, manuscripts and microscopic analyses is gratefully acknowledged. We are also grateful to Wink Howland for taking care of financial statements and payroll, and all of the Saskatchewan and Manitoba beekeepers that donated breeding stock to the program.

(iv) Equipment and Materials

All equipment for operation and maintaining hives was supplied in kind by Meadow Ridge Enterprises Ltd. Meadow Ridge also supplied land, 50 apiaries, buildings and laboratory facilities in kind (microscope, insemination equipment, cameras, photo's, computer, incubators) for mite analyses and specialized breeding. Some costs were incurred to upgrade insemination equipment and microscope capabilities, as well as add a laptop computer. Vehicles were used at standard mileage costs. Direct costs were incurred to purchase sticky boards for varroa mite analyses, brood and bees for nucs and cell builders for SBA cell and breeder queen sales, and specialized apinovar bottom boards to efficiently sample varroa populations. The provincial

apiculture lab, Prince Albert, Sk, supplied alcohol and sample bottles for sampling mites and provided tracheal mite analyses.

Appendices:

APPENDIX A

The following chart summarizes the pedigrees of the Saskatraz lines released since 2006.

Pedigrees of Saskatraz Breeders

Key: First two letters = queen breeder initials

BL = Blue	**BF** = Buckfast	**SC** = Supercede	**up** = unknown progeny
Y = Yellow	**C** = Carniolan	**S** = Year of Selection	**bc =** backcross
YB = Yellow/Blue	**T** = Tracheal	**d** = Daughter	**cp =** Closed Population
R = Russian	**RP =** Russian Purple	**sw** = swarm	

SAT 14: JP-04/2-R6: (2006S)
SAT 17: JP-04/2-BF 12: (2006S)
SAT 23: WH-04: (2006S)
SAT 28: MR-04: TS x R/BL-40: (2004) SC:(2006S)
SAT 30: MR-04: CT x MR cp-02; SH-04
SAT 34: MR-04: BL/R x SAT (2004); 2006S
SAT 65: YG-06: USBL R-05; SW-2006 x SAT; SC 2007; (2008S)
SAT 84: MR-06: up x SAT-06 (2008S)
SAT 85: MR-06: BL/R-04 (2006S) SAT 34 d x SAT-06 (2008S)
SAT 86: MR-06: CT x MR cp-02; SH-04 (2006S) SAT 30d bc (2008S)
SAT 87: MR-06: TS x R/BL-40 x SAT 2006; (2008S)
SAT 88: MR-06: Y/BL/R x RP30-2006: SC x RP 30-2006 (2008S)
SAT 93: MR-06: BL/R x SAT (2004) SAT 34 d x RP 30-06 (2008S)
SAT 96: TR-07: MR SAT 34 x RP 30-06 x B: TR-07 (2008S)

APPENDIX B

Characteristics of Saskatraz Families .These characters are subjective and based on original breeders and reselected progeny. Progeny may vary considerably in all breeders derived from the originals because of genetic processes and multiple mating. All families have good to excellent wintering ability in Saskatchewan, intermediate to excellent hygienic behavior and acceptable temperament. SAT-34 and some daughters show aggressive behavior. SAT-84 shows a VSH (varroa sensitive hygiene) phenotype.

E=Excellent; VG=Very Good; G=Good; M=Moderate; F=Fair; P=Poor.

	Honey Production	Varroa Tolerance	Tracheal Mite Resistance	Chalkbrood Resistance
SAT 14	E	F	VG	E
SAT 17	VG	F	E	E
SAT 23	F	P	E	E
SAT 28	G-VG	VG	VG	E
SAT 30	E	G	G	E
SAT 34	M	VG	E	E
SAT 65	VG	VG	VG	E
SAT 84	M-F	VG	VG	E
SAT 85	F-M	G-VG	VG	E
SAT 86	G-VG	F	G	E
SAT 87	G	G	VG	E
SAT 88	G	VG	VG	E
SAT 93	M-F	G-VG	VG	E
SAT 96	VG	G	VG	E

Saskatraz stock availability and prices 2011.

Saskatraz stock will be available to any beekeepers or queen breeders interested in 2011. Collaborating queen breeders with certified breeders ,or queen cells purchased from the breeding program will be encouraged to out cross, re-select and return selections back to the program (initiated in 2006) .New selections for testing in the Saskatraz are also welcomed .New selections or stock returned for evaluation will be purchased or traded with new Saskatraz selections ,and evaluation results will be shared with contributors.This process was started in 2004 with Saskatchewan and Manitoba queen breeders to initiate the Saskatraz project

Prices for breeding stock in 2011 will be as follows:

1.Out crossed Saskatraz breeder queens $80,sold with four frame nuc $250.
2.Closed population mated Saskatraz breeder queens $300.
3.Queen cells from extensively tested Saskatraz breeding lines $20.These breeder queens are never sold ,just queen cells derived from them. Many of these families are described in the report.

We began re-current selection procedures in 2007 to help maintain selected Saskatraz families. The re-selected colonies were set up for closed population breeding. We have established over the last three years apiaries with re-selected Saskatraz colonies for closed population mating procedures. We consider these valuable gene pools and will provide custom breeding services to interested queen breeders. We will close population mate virgin queens to the drones from these apiaries . The queen breeder could supply either queen cells or virgin queens less than six days old.

We suggest any one interested in adding Saskatraz breeding stock to their operation purchase at least 10 queen cells from several families of interest .Out crossing this stock to your drone population and evaluating the daughters will give you an idea of the cross ability and what families work best with your bee population .Some queen breeders re-select some of these daughters as breeders for their own operation .Others have re-constructed their own Saskatraz apiaries for multiplying queens by closed population mating procedures .Please contact us for advice on re-constructing Saskatraz breeding families.

In 2010 we tested 16 breeding lines from an Australian breeder for honey production and varroa tolerance, and re-selected 3 of the best performing lines for re-evaluation in Canada this

year. We will not know about wintering ability until April. Some commercial production queens are being offered in May from these breeding lines ,at competitive prices.

Proceeds from all stock sales are used to support the Saskatraz breeding program.

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