SUMMARY OF SASKATRAZ RESEARCH PROJECT ACTIVITES 2010-11

THE SASKATRAZ RESEARCH TEAM : Tom Robertson1, Neil Morrison1, Mohammad Mosterjeran1, Syed Qasim Shah1, Wayne Connor2, Sanjie Jiang3, Philip Griebel2, Xiao Qiu3, Albert Robertson.*1

1. Meadow Ridge Enterprises Ltd, Box 1 GS 602, RR6, Saskatoon, Saskatchewan, Canada. S7K 3J9.

2. Veterinary Infectious Disease Organization, 120 Veterinary Road, University of Saskatchewan, S7N 5E3

3. Food and Bioproduct Sciences,51 Campus Dr.,University of Saskatchewan ,Saskatoon ,Saskatchewan ,S7N 5A8

*Author for correspondence: a.j.robertson@sasktel.net ; Website: saskatraz.com

INTRODUCTION

The objective of this project is (i) to continue development and distribution of productive, gentle, honey bee colonies with tolerance to mites and brood diseases. (ii) to identify new types of molecular markers by comparing high and low honey producers, varroa sensitive and tolerant lines and viral sensitive and resistant (immune) lines (using infection models), for differences in expression of key genes (microarrays) and molecules (kinome arrays).

This report will be divided into three sections.The first section will deal with: (1) continued progress on the Saskatraz breeding program at Meadow Ridge Enterprises LTD,in collaboration with cooperating queen breeders: (2) the second with progress on microarray design and printing for identification of informative molecular markers for economic traits in collaboration with Dr. Xiao Qiu and graduate student Sanjie Jiang at Food and Bioproduct Sciences, University of Saskatchewan: (3) the third with the effect of varroa on expression of pathogenic honey bee viruses and screening of Saskatraz breeding lines for susceptibility and resistance to viruses in collaboration with Dr. Philip Griebel, Wayne Connor and associates at the, Veterinary infectious Disease Organization, University of Saskatchewan.

SECTION 1: SASKATRAZ HONEY BEE BREEDING PROGRAM, MEADOW RIDGE ENTERPRISES LTD.

SUMMARY OF STOCK DISTRIBUTION.

The Saskatraz breeding program distributed 887 queen cells from Saskatraz breeders and 17 breeder queens to Saskatchewan and other Canadian queen breeders in 2010. This returned $15,950 to the Saskatraz breeding program and $3200 to the Saskatchewan Beekeepers Association in 2010. Since 2006 Saskatraz breeding program stock sales has returned approximately $138,150.This represents distribution of a total of 4977 queen cells and 82 breeder queens . Many of the queen breeders purchasing Saskatraz breeding stock multiply this stock not only for use in their own operations, but for resale and distribution to commercial honey producers. A number of queen breeders continue to return outcrossed and new selections back to the breeding program for evaluation in exchange for certified Saskatraz

stock. Saskatraz stock is becoming wide spread in Saskatchewan, and now present in BC, Alberta, Manitoba, Ontario, Quebec and New Brunswick.The stock is also part of breeding programs in BC , Manitoba,Ontario and New Brunswick. Considerable interest has also been expressed by queen breeders in the United States. Some progress has recently been made in the export of breeder queens to Chile. An official document prepared by Drs. Pierre Lafortune and Albert Robertson in consultation with the Hive Health Commitee (Canadian Honey Council), and CAPA, the Canadian Association of Professional Apiculturists was completed in January and has now been sent to SAG, CFIA's counterpart in Chile. Australia has also expressed interest in importing Saskatraz stock, and they are eventually looking at importation through their quarantine facilites.

Current Saskatraz Breeding Program Activities, Meadow Ridge Enterprises LTD.

Personnel. Mohammad Mosterjeran research associate (varroa analyses, selection, stock multiplication), Tom Robertson, Neil Morrison (honey production, stock selection, distribution and multiplication), Dr. Syed Shah (visiting scientist, varroa analyses and selection), Dr Albert Robertson.

In 2010 three Saskatraz natural selection yards were operated. The original saskatraz-Q yard site is used to test pre-selected colonies (honey production, wintering ability, temperament, etc.) for varroa tolerance. Many of the colonies tested at the original Saskatraz yard site have been outcrossed and subjected to recurrent selection.The second (Saskatraz-PW) and third (Saskatraz–D) are used for progeny analyses of breeders selected at the Saskatraz–Q apiary. The best daughters are re-selected (recurrent selection) from each breeding line at these two yard sites, for outcrossing, back crossing and closed population breeding. During the summer months selection involves measuring honey production, temperament, varroa populations on adult bees, varroa death rates (sticky board analyses), brood diseases (chalk brood, etc.), checks for virus infections (visual and molecular) and microsporidia (Nosema apis and ceranae) by microscopic analyses and PCR (polymerase chain reaction). In the fall of 2008 we began brood analyses for percent varroa infection. Both the percent brood infected and the number of varroa per cell were scored. During the summer of 2010 Meadow Ridge Enterprises Ltd, hired a full time student to score brood for varroa infestation to test for Varroa Sensitive Hygiene phenotypes. Fourteen breeding lines and over 100 frames were thoroughly analysed.

Honey production is our primary selection criteria, and all Saskatraz colonies are assessed for honey production every year. Honey is harvested and weighed from each colony at least 3 times during the summer, and net honey production at each time period compared between colonies. In 2010,112 colonies from Saskatraz breeding lines were assessed and seven (S113, 114, 23A, 84C, 86C, 28A, BG1F) were selected for honey production and further multiplication in 2011.

The same colonies were subjected to intensive tracheal and varroa mite analyses, as well as our varroa nursery and all closed population mating yards (4 apiaries, 156 colonies). In addition, outcrosses of Saskatraz lines were assessed at 50 different apiaries at Meadow Ridge. Varroa infestations of adult bees were determined by alcohol washes as well as natural drop on sticky boards. Sticky boards were scored by counting the number of mature and immature mites

every 7 to 10 days. Samples of varroa mites as well as adult bees and pupae were also collected for virus and nosema analyses. Tracheal mite analyses was performed by the provincial apiculture laboratory, by standard protocols, in Prince Albert, Saskatchewan and reported by Geoff Wilson. Most, if not all, Saskatraz breeding lines are now showing excellent resistance to tracheal mites without treatment. In 2010 the following colonies showing good honey production and suppression of varroa mite reproduction were selected for further analyses and distribution in 2011 (S23A, 65C, 84C, 86C, 88 and 113). Sat 84 showed a VSH phenotype in 2008, and a daughter of S84, 84C showed a similar phenotype in 2009. We are still analysing large amounts of data collected in 2010 for VSH phenotypes. Sat 88 is now the longest surviving colony at Saskatraz-Q, existing for 42 months without synthetic miticide treatment.

The figure presented below summarizes our current Saskatraz field breeding program logistics. A novel feature of this breeding initiative involves the application of natural selection recycling after outcrossing, back crossing and recurrent selection. Letters A to E represent sources and F to G reservoirs of selected breeding lines or familes. A genetically diverse gene pool was established by preselecting stock from unrelated populations showing economic traits (honey production, wintering ability, good temperament and mite tolerance [Russian stock]). Selections from these populations were assembled at the original Saskatraz yard site and re-selected for honey production and mite tolerance for three years. Re-selected outcrosses and back crosses of selected Saskatraz familes are recycled through a natural selection process at each of the three natural selection yard sites (Saskatraz–Q, PW, D). Saskatraz PW and D are used for progeny analyses. This process functions to continually enrich for genes producing the most benefical phenotypes,combining the most fit drones and queens. For example back crossing virgin queens from colonies showing the best honey production and varroa tolerance under high varroa infestation selects for the most fit drones under natural selection conditions. Twelve back crosses made in 2010 at Saskatraz will be evaluated in 2011.

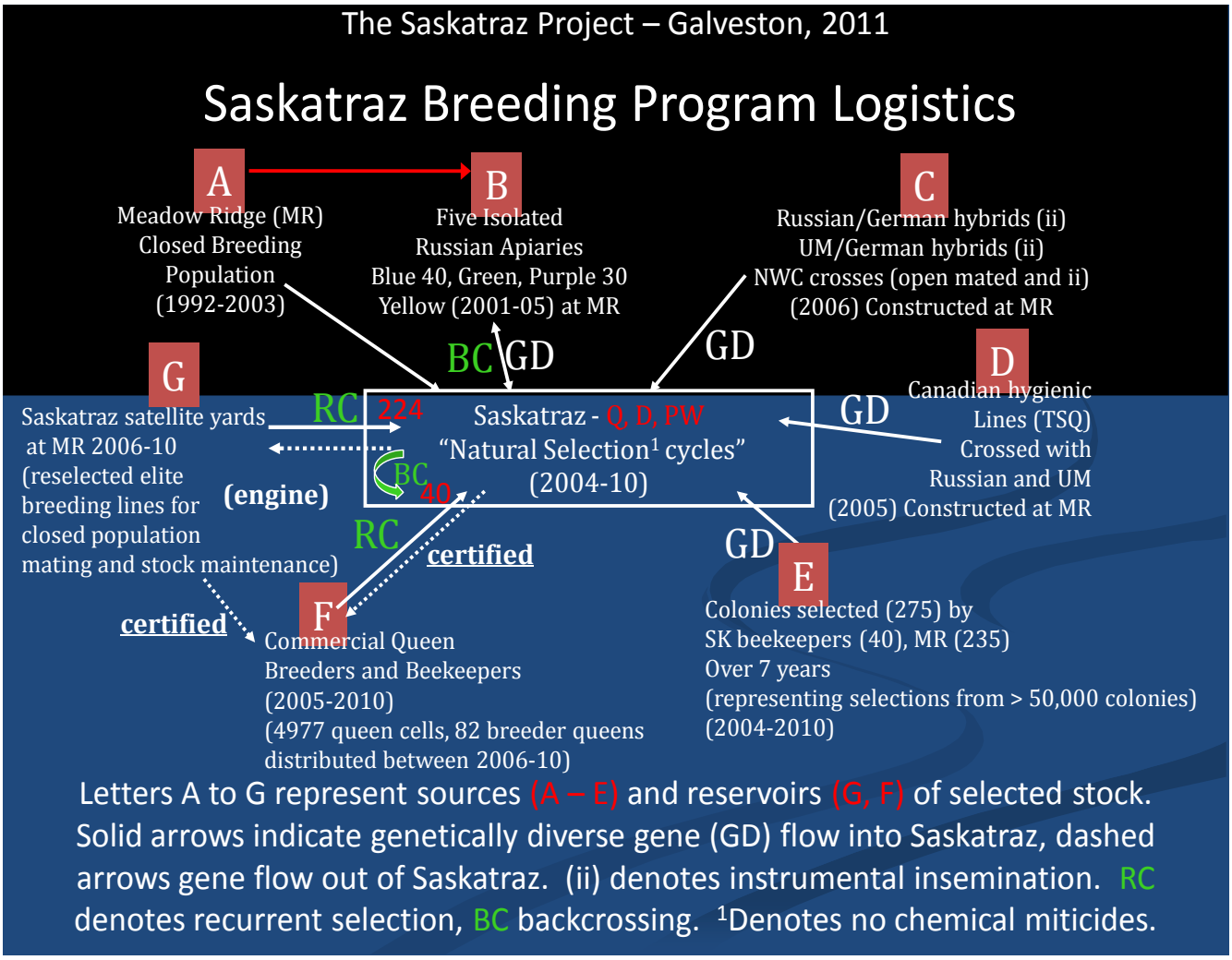


Figure 1.Saskatraz Breeding Program Logistics.

In 2008 we initiated indoor wintering experiments focused on grooming behavior, morphometric measurements, varroa population growth, virus and Nosema suceptibility (2009-2010) on the progeny of selected breeding lines.This allowed us to select for certain traits during the winter months. In 2007 we introduced selection for varroa sensitivity, removing colonies showing higher varroa populations from our closed population breeding yards, and commercial apiaries.In 2010 these colonies (17) were moved to an isolated apiary and used as a varroa nursery for molecular analyses (microarrays and viruses). No treatments were made and these colonies are left to die of varrois and associated pathogens.

Morphometric analyses (Mohammad Mosterjeran) of body parts on 21 Saskatraz breeding lines was performed in an attempt to correlate morphological traits with honey production, suppression of varroa population growth and grooming behaviour. "Morphometric analyses involved sampling 30 worker bees in to a preservative from each selected Saskatraz colony (43) and measuring body parameters and appendages (legs, wings, proboscis, etc,) with a stereo microscope using the Ruttner Standards. Twelve colonies remain to be assessed and statistical

correlations made between phenotypes (honey production, varroa tolerance, grooming behaviour, etc.). Preliminary observations indicates Sat 65 and 88 have the longest legs and show good grooming behaviour.

Whole colony grooming assays continue with 64 colonies from 8 different Saskatraz families being assayed. These assays are performed by normalizing the varroa mite populations in each colony by miticide treatments, and then adding equal amounts of varroa mites to each colony and assaying for varroa drop on sticky boards at frequent intervals over several weeks. Eight daughters from each family are used in progeny analyses, to identify the best groomers for selection and propagation. Sat 84C is an example of a selection with good grooming behaviour that also showed a VSH phenotype, good varroa suppression in the field and excellent honey production at Saskatraz PW in 2010. Daughters from this family are undergoing further multiplication and recurrent selection.

The 64 colonies undergoing grooming assays are also being monitored for viruses, and microsporidia (*Nosema apis* and *ceranae*). Virus monitoring was initiated to screen for colonies showing possible resistance to pathogenic viruses (Deformed Wing Virus [**DWV**]; Kashmir Bee Virus [**KBV**]; Israeli Acute Paralytic Virus [**IAVP**]). Sampling consists of collecting dead and live bees and dead and live varroa mites from test colonies every 14 days. These samples are assayed in collaboration with VIDO, and will be discussed in the next section. All colonies are monitored for Nosema infections according to standardized procedures cf http://www.ars.usda.gov/is/np/honeybeediseases/honeybeediseases.pdf., which involves measuring spore levels in the gut by microscopic analyses. Typing species involves PCR procedures at VIDO, University of Saskatchewan

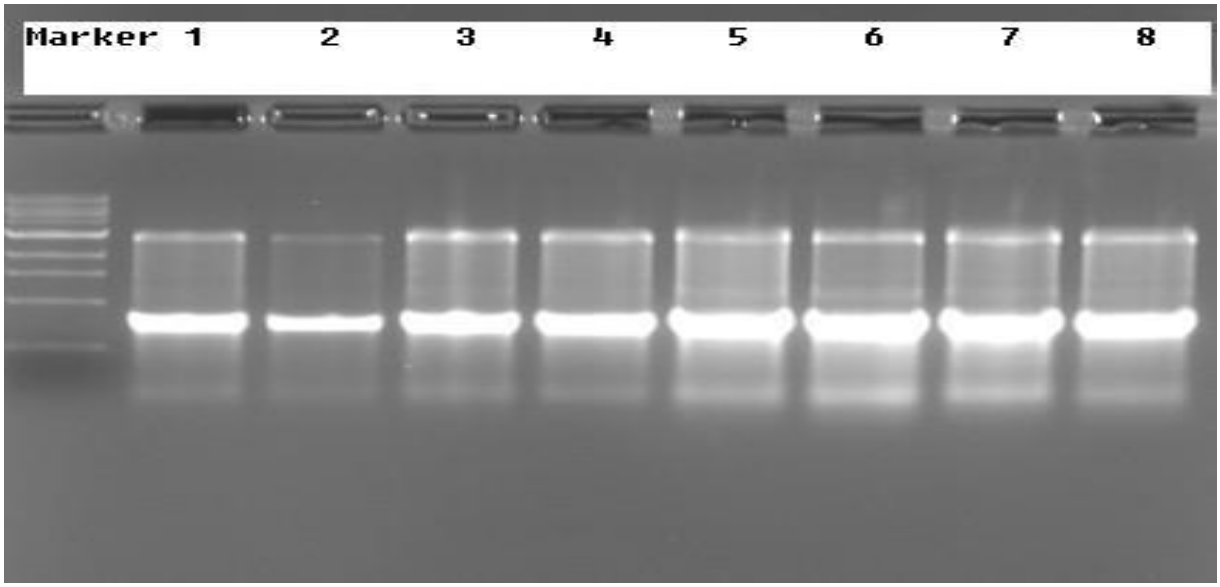
Section 2: Microarray analyses for identification of genes involved in the expression of varroa tolerance and honey production.
Personnel involved. This part of the project is being carried out in collaboration with Dr. Xaio Qui, and graduate student Sanjie Jiang at Food and Bioproduct Sciences, University of Saskatchewan and Dr. Albert Robertson, Meadow Ridge Enterprises Ltd.

This project was initiated in August,2010, with the arrival of a graduate student, Sanjie Jiang to work on as part of his master's thesis. The following information was provided by Sanjie Jiang as a progress report on the microarray project.
Investigation of possible molecular mechanisms involved in conferring tolerance to varroa mites in domestic honey bees is being approached by comparing gene expression profiles (microarrays) of sensitive and tolerant Saskatraz breeding lines. Honey bee pupae at different developmental stages were collected , as well as from adult worker bees in the fall of 2010. All bees were collected from Meadow Ridge apiaries by Sanjie Jiang with the assistance of the Saskatraz field research team. Brood frames were removed from sensitive and resistant colonies, and brought to the field laboratory for harvesting pupae. They were stored at 32 C and 80% humidity until collection was complete. Collection involved carefully opening capped brood cells, and removing pupae at the described stages under a 10× stereo microscope. Pupae with and without varroa were collected from both sensitive and tolerant colonies. Adult honeybees (approximately 200 per sample) were collected from sensitive and tolerant colonies by hand catching live worker bees with and without varroa infestations. Rubber surgical gloves were

worn to prevent contamination with nucleases and keratin, etc. Samples were frozen in liquid nitrogen before being stored at −80°C.

Total RNA was extracted from the heads of two bees (dark-eye pupae) using RNeasy kits (Qiagen, Valencia, California) as described by manufacturer and treated with DNase (Rnase free DnaseI, also Qiagen). RNA purity and integrity were checked by electrophoresis (1% agarose gels) as shown in Figure 2.

FIGURE 2.



The RNA extracted from the bees heads shows excellent quality, and is being used for microarray analyses.

Section 3. The effect of varroa on expression of pathogenic honey bee viruses and screening of Saskatraz breeding lines for suceptibility and resistance to viruses and Nosema; in collaboration with Dr. Philip Griebel and associates at VIDO.

Personnel: Wayne Connor, PCR technical specialist and Dr. Philip Griebel (scientist) and associates, VIDO, University of Sask.and Dr. Albert J.Robertson, Meadow Ridge Enterprises Ltd.

In the spring of 2007 all of the original Saskatraz colonies died during the winter, after varroa infestations reached high levels in the fall of 2006. Although 6 colonies were selected over a three year period, which showed good honey production and suppression of varroa population growth, none showed resistance to varroa. We began an extensive and thorough post mortem analyses of colonies which collapsed during the 2007 winter at Saskatraz, as well as tests on colonies from Saskatchewan beekeepers having higher than normal loses.These studies are still in progress. Briefly, in addition to over 30 Saskatraz colonies, we investigated viral and nosema pathogens in more than 17 case history studies for commercial beekeepers since 2007.

We tested for viruses by RT-PCR (DWV, IAPV, KBV, Sac brood, Black Queen cell virus and microsporidia (*Nosema apis* and *ceranae*) by PCR. Methods were developed to test a variety of samples from collapsed colonies. We tested adult bees, pupae, bee feces, varroa, varroa feces, hive products (bee bread and honey), and commercial pollen. It was found that the presence of more than two viruses (DWV + IAPV or KBV) and microsporidia (either and or both) results in high colony losses. We also observed that high spore counts of both apis and ceranae causes high colony mortality and continued colony dwindling in the spring. Fumidil B was an effective treatment with ceranae infection disappearing often before apis in follow up testing.The discovery that hive products (bee bread and pollen) can be infected with viruses and microsporidia in colonies showing high levels of infection is of concern.This indicates reuse of equipment may cause infection of new colonies. However, we do not yet know the infectivity of pathogens in hive products,and more experiments are required to answer these questions.The detection of pathogens in dead bees, pupae and feces has proved useful in determining what factors are involved in colony losses, and in determining treatment protocols. A search for viral and microsporidia in natural reservoirs was also investigated. Wasps in the vicinity of bee hives in some, but not all cases carried DWV and IAPV. Bumble bees showed DWV, and KBV, but we did not identify any with IAPV. A sample of flys at the Saskatraz apiary showed high levels of DWV and IAPV, where as samples from another area did not. Floral sources (clover blossoms, canola, wild flowers, etc.) were sampled around the Saskatraz yard site, and all tested negative for viruses. PCR analyses of bumble bees in the Saskatoon area for Nosema species was negative.

Some of our investigations on the effect of varroa infestations on the incidence of virus infection at Saskatraz in 2005 and 2006, detected three pathogenic honey bee viruses (DWV, KBV and IAPV) showing up over time, by Rt-PCR analyses of varroa sampled from bottom boards (natural drop). As varroa infestation levels increase more virus was detected in the varroa. However, colonies selected for suppression of varroa population growth (SAT 28, 30, 34) showed less virus infection in the varroa collected, than non-selected colonies (Sat-01, 24) showing higher varroa infections earlier. In addition,varroa sensitive colonies died quickly after virus infection, where as varroa tolerant colonies survived longer, even after detection of viruses in the varroa infecting these colonies. This led us to look at the susceptibily of selected Saskatraz colonies to viruses.

A report by Maori et al. 2007. Virology, 362: 342-349 showed certain lines of Israeli honey bees showed resistance to IAPV. We assayed some of our Saskatraz breeding lines, exposed to all three pathogenic viruses (Sat-65,84), which did not show any detectable IAPV sequences (infection) by RT-PCR,for genomic sequences of DNA homologous to IAPV. The presence of genomic sequences in these lines would suggest that retrotransposition of recombinant RNA from host and pathogen may have occurred to confer immunity to IAPV. Except for one weak signal in genomic DNA from a Saskatraz selection we have not been able to dulpicate the Israeli results. Further testing of Saskatraz breeding lines showing some resistance to viruses is in progress.

In 2010 we established cell culture lines of several Saskatraz breeding lines (SAT-28, 30, 65, 84, G4abp, etc.) using honey bee pupae (Hunter, W.B. 2010 In vitro cell. Dev.Biol,- animal

46:83-86.). These cultures are being developed as an assay system for screening the infectivity of honey bee viruses, and to determine whether breeding lines showing some immunity to viruses maintained the immunity in vitro. We succeeded in establishing honey bee cell cultures from Saskatraz breeding lines and cultures established from pupae infected with virus maintained the virus infection, but no amplification of the virus was detected. Attempts at infecting the cultures with dead varroa containing identifiable virus sequences failed, and experiments with live varroa are planned.The cell cultures were slow growing and died after several months of incubation. Repeats of these experiments, with some modifications, are planned in 2011.

In our current breeding trials we have added varroa to 64 colonies (8 daughters from 8 SAT breeding lines) for grooming assays. These colonies are also being monitored for the presence of viruses (DWV, KBV, IAPV) by RT-PCR and Nosema species by PCR in live and dead bees and varroa at two week intervals during the winter. Preliminary results indicate that viruses are detected in varroa before they show up in the worker bees. Although varroa are thought to spread viruses through out the honey bee population, it is curious that virus multiplication appears to begin in the varroa. These experiments will assist us in identifying Saskatraz breeding lines with virus immunity and some resistance to Nosema. Variabilty within and between lines has already been identified, but the analyses is preliminary.

ACKNOWLEDGEMENTS

The financial support of Saskatchewan Agriculture and Food, Agriculture Development Fund, Agriculture Council of Saskatchewan and Meadow Ridge Enterprises Ltd in 2010 is gratefully acknowledged. We thank Wink Howland ,Secretary Treasurer of the SBA for administering grant funds. All proceeds from the sale of Saskatraz breeding stock is used to support the breeding program. We gratefully acknowledge the support of all queen breeders purchasing Saskatraz breeding stock ,and collaborating by contributing re-selected outcrosses and new selections back to the program for evaluation.