

ACAAF FINAL REPORT FOR THE SASKATRAZ PROJECT SK0234CO, File #S74-A.  
Report prepared by Dr Albert J. Robertson, Principle Investigator.

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## 1. PROJECT OBJECTIVES AND BACKGROUND.

### (i) How was this project conceived?

Saskatchewan beekeepers in cooperation with the Ontario Beekeepers Association imported Russian stock from the USDA breeding program in 2001 to 2003. Dr. Albert Robertson proposed research directed towards improving the resistance and tolerance of Canadian honeybees to tracheal and varroa mites, while maintaining productivity, and other desirable traits. Saskatchewan beekeepers supported the proposal and a number donated breeding stock to the program. Robertson wrote several grant applications outlining breeding procedures and molecular analyses to ADF, CBRF and ACAAF between 2005 and 2009 .

### (ii) Project time frame.

This project was initiated as a three year project (2007-2009), but was extended until March 31, 2011, because of surplus funds associated with molecular analyses. ACAAF funding ended on March 31, 2011. The project continues at Meadow Ridge Enterprises Ltd, with stock sales helping to maintain the breeding program and ADF funding until April 2012.

### (iii) Project Objectives and relation to ACAAFS Program objectives.

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 bio-markers (microsatellites, microarrays and proteins). Natural selection has been used to select superior genotypes with mite tolerance, avoiding the use of synthetic chemical miticides. This approach will continue to increase the food safety of honey and other products using honey and provide honey bee stock with improved productivity and disease resistance to Saskatchewan and Canadian queen breeders and commercial beekeepers. These goals are in line with ACAAFS program objectives.

## 2. PROJECT DESCRIPTION.

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). Most breeding and research programs have focused on selecting for hygienic behaviour, a trait correlated with varroa tolerance. The Saskatraz project 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, Robertson made a request 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. Several putative bio-markers for varroa tolerant phenotypes have recently been characterized in collaboration with University of Saskatchewan scientists, technicians and graduate students. A 50 page booklet (Robertson, Albert J. 2010). The Saskatraz Project-A Review (2004-2009) is available from the author, and can be accessed at the following website: [www.saskatraz.com](http://www.saskatraz.com) . The website also contains recent power point presentations and several articles on the

project, containing details on how this project has been conducted.

**Project Partners:** This project partnered with Saskatchewan and Canadian (British Columbia, Alberta, Manitoba, Ontario, New Brunswick and recently Quebec) queen breeders for stock acquisition, evaluation and out crossing of Saskatraz breeding lines. Several collaborators have out crossed Saskatraz breeding lines and selected the best daughters to return to the program for re-evaluation, natural selection recycling, and re-current selection and closed population mating. This serves to enrich for important traits and continues to improve Saskatraz breeding stock performance, while maintaining genetic diversity.

Dr. Rob Currie at the University of Manitoba and Members of the Manitoba Queen Breeders Association have been long time collaborators on the Saskatraz project. Manitoba queen breeders continue to access Saskatraz stock to incorporate into their breeding program.

In 2010 Meadow Ridge was approached by Australian breeders to evaluate Australian breeding stock for varroa tolerance and performance (honey production, wintering ability, chalk brood resistance, etc) in Saskatchewan. In 2011, Dr. Max Whitten, former head of entomology at CSIRO, currently working with the Wheen Foundation requested we test several Consortium lines developed by a long term government breeding program in Australia, and salvaged by a Consortium of Australian queen breeders. This work is in progress and is currently being funded by Meadow Ridge. The Australians do not have varroa yet, but are working toward setting up a quarantine system to import Saskatraz stock for multiplication in Australia. If successful the infrastructure is in place to multiply Saskatraz commercial queens in Australia for distribution in Canada. A similar initiative is still in progress with Chile, a summary of progress can be found in Robertson, T. et al. 2011. Summary of Saskatraz Research Project Activities 2010-2011. Hivelights, 24; 41-46. Articles have also been posted on the Saskatraz website.

In the spring of 2011 we were in contact with a queen breeder in Israel, Dr. Tzachi Goldenberg who wishes to collaborate on the Saskatraz breeding program and is making efforts to import Saskatraz stock in to Israel. We have established an excellent collaboration with Dr Xiao Qiu, at Food and BioProduct Sciences and Dr. Philip Griebel, VIDO, University of Saskatchewan. In collaboration with Dr Xiao Qiu we are supporting a graduate student, who is working on microarrays, to study gene expression in Saskatraz varroa sensitive and tolerant lines, to develop bio-markers. Funding to initiate microarray screening was provided by ACAAF. In collaboration with Dr Philip Griebel and Wayne Connor, at VIDO, we are working on viruses and microsporidia and the susceptibility of Saskatraz breeding lines to infection. With other members of Dr. Griebel's team we are looking at kinome arrays to further characterize sensitive and tolerant Saskatraz breeding lines. ACAAF funding was used to initiate kinome array construction.

This spring a collaborative project was initiated with Drs. Randy Purves and Sue Abrams at the NRC at PBI to start protein analyses of Saskatraz breeding stock. Dr. Robertson has received visiting scientist status and in exchange for training the proteomic staff on 2D SDS-PAGE and preparing bee proteins for proteomic analyses. We will be looking at some new funding in collaboration with university collaborators to start this project.

### 3. RESULTS AND CONCLUSIONS.

#### (i) Saskatraz project results.

The Saskatraz project was initiated in 2004. No synthetic chemical miticides were applied and natural selection procedures were 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 were also out crossed and subjected to recurrent selection to preserve the selected gene pool, to maintain genetic diversity, and to enrich for economic traits. Re-selected colonies were returned to the Saskatraz apiary and the natural selection process is repeated (natural selection re-cycling) in the search for genotypes with increased expression of mite tolerance and

honey production without the use of chemical miticides. Detailed project results are available on our website [www.saskatraz.com](http://www.saskatraz.com). Only a brief summary is provided here.

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 is required to breed lines with improved varroa tolerance.

Since 2006, the Saskatraz breeding program has released more than 14 families (SAT-14, 17, 23, 28, 30, 34, 63, 65, 84, 86, 87, 88, 96, 98.) to queen breeders for multiplication. As of June 25, 2011, about 5617 queen cells and over 90 breeder queens been released to Canadian queen breeders.

(ii) Meeting project objectives and timelines.

Saskatraz selections show excellent honey production, good wintering ability, tracheal mite and chalk brood resistance and varying degrees of varroa tolerance. None show complete resistance to varroa mites and continued efforts will be required to improve varroa tolerance. The breeding process we have developed in honey bees to improve varroa tolerance shows promise, but will take time. It involves enrichment of traits for varroa tolerance, and continued selection. Details on this procedure, which involves natural selection re-cycling, re-current selection and progeny analyses, will soon be available on our website. An article is in press as an SBA news letter showing the increased varroa tolerance of Saskatraz stock compared to imported commercial stock (cf attachment Robertson et al. 2011.), and several articles will be published in the American Bee Journal.

(iii) Extent project was successful in relation to original objectives.

The Saskatraz project has been very successful at breeding for increased honey production, wintering ability, tracheal mite and chalk brood resistance. Our stock demonstrates some tolerance to varroa, but can be improved by further breeding efforts, particularly with our discovery of several putative bio-markers for varroa tolerance.

(iv) Project Modifications.

Alterations in molecular analyses were required because of availability of contract services. Our original work at SRC could not be pursued because of other priorities by SRC. We began collaborative work with University researchers in 2008-2009, but lagged behind in our molecular analyses. We are now making good progress in this area. It was also necessary to develop diagnostic procedures for pathogens (viruses and microsporidia) to screen our breeding lines for susceptibility to disease. We also provided considerable diagnostic services to industry.

(v) Recommendations for further development.

The Saskatraz project has developed some families with good honey production and varroa tolerance. Maintenance and distribution of this stock is critical, and further improvement is possible. Stock maintenance and distribution has been carried out by Meadow Ridge Enterprises Ltd. Sales revenue from the breeding stock has assisted in this process. In 2010 an agreement was made with the SBA board of directors that Meadow Ridge collect the sales revenue to assist in stock multiplication, maintenance and distribution. Meadow Ridge continues to work with Saskatchewan and Canadian queen breeders as in the past, and is taking full responsibility for the project. Further development will depend on what revenue can be obtained from stock sales, and funds available from Meadow Ridge and supportive agencies. Meadow Ridge has covered all infrastructure costs and most operating and principal investigator costs from the beginning of the project. ADF currently supports our University collaborators (graduate student, technician assistants) and our research associate Mohammad Mostajeran's salary.

#### 4. COMMUNICATIONS

Three SBA newsletters, four reports in Hivelights (national bee journal, Canadian Honey Council) and a booklet have been published on the Saskatraz project since 2007. Meadow Ridge developed a website in 2011, and publications and reports are regularly added to the site. Invited presentations have been made in Mexico at the Second World Symposium on Queen Breeding 2008, and at three National conferences in the United States (Houston, Texas 2007, Orlando, Florida 2010, Galveston, TX, 2011), and numerous Canadian Symposiums and international meetings.

#### RECENT ARTICLES ON BEE BREEDING.

Robertson, Albert J. 2007. HiveLights, 20; 15-19 Robertson, Albert, J. 2005. Evaluation of Varroa and Tracheal Mite Tolerance in Selected Honey Bee Lines and Attempted Correlation of Tolerance with DNA Markers. HiveLights, 18:13; Robertson, Albert J. 2007. HiveLights, 20; 15-19. Teerawanichpan, Prapapan. Robertson, Albert J. and Qiu, Xiao. 2010. A fatty acyl-CoA reductase highly expressed in the head of honey bee (*Apis mellifera*) involves biosynthesis of a wide range of aliphatic fatty alcohols. Insect Biochemistry and Molecular Biology 40;641-649.

In the Saskatchewan Beekeepers Association Newsletters: Vol. 7, Number 4, 2002, Page 15, Vol. 8, Number 1, 2003, page 6, Vol. 8, Number 3, 2003, page 13, Vol 8, Number 4, 2003, page 13, Vol. 9, Number 1, 2004, page 7, Vol 9, Number 4, 2004, page 15, Vol 10, Number 2, 2005, page 13, Vol , 10, Number 3, 2006, Volume 11, Number 2, 2007, Volume 12, Number 3. Robertson, Albert J. 2008. The Saskatraz Project. In proceedings of 2<sup>nd</sup> World Symposium of Queen Breeders and Artificial Insemination, Nayarit, Mexico Oct. 15-20, Page 121-125. Robertson, Albert J. 2008. The Saskatraz Project: Selection of Productive Honey Bee Genotypes with Tolerance to Varroa and Tracheal Mites, HiveLights. 2008 Supplement. Page 13. Robertson, Albert J. 2009. Saskatraz-Chile-Agrovivo. SBA Newsletter Vol. 14, Number 5. Robertson, Albert J. 2008. The Saskatraz Project: Selection of Productive Honey Bee Genotypes with Tolerance to Varroa and Tracheal Mites, HiveLights. 2008 Supplement. Page 13. Robertson, Albert J. 2009. Saskatraz-Chile-Agrovivo. SBA Newsletter Vol. 14, Number 5. Robertson, Albert J. 2010. The Saskatraz Project: Selection of Productive Honey Bee Genotypes with Tolerance to Varroa and tracheal mites. HiveLights; vol. 23, p. 22. Robertson, T.R., et al. 2011. Summary of Saskatraz Research Activities 2010-2011. HiveLights, in press.

#### CONFERENCES, MEETING AND SYMPOSIUM PRESENTATIONS: (27).

Robertson, Albert J. 2005, The Saskatchewan Beekeepers Honey Bee Breeding Program. Proceeding of the 64<sup>th</sup> Annual CHC-CCM Meeting, Feb. 4, Sheraton Cavalier, Saskatoon, Sask. Robertson, Albert J, 2006. The Saskatchewan Beekeepers Honey Bee Breeding Program. American Honey Producers Association 37<sup>th</sup> Annual Convention (January 10-14, 2006) Houston Texas, USA-invited speaker; Canadian Honey Council Meetings (January 24-28, 2006), Quebec City, Canada; Annual SBA Convention (February 1-4, 2006); SBA Field Day June 17, 2006; SBA Annual Meeting, November 17, 2006; Canadian Honey Council Meeting (Jan, 2007) Langley, BC; Annual SBA Convention, (Feb 1-4, 2007 ); SBA Field June 2007; Meetings with Minister of Agriculture, Sept. 2007 and Research presentation; SBA general Meeting, November 29, 2007. Canadian Honey Council, Calgary, Alberta January 24-26, 2008. SBA Field Day June, 2008; 2<sup>nd</sup> World Symposium of Queen bee breeders and Artificial Insemination, Nayarit Mexico Oct 15-20, 2008. SBA Annual Meeting, Saskatoon, Sk., December 2-4, 2008. Ontario Beekeeper's Association, December 9- 13, Niagara Falls, Ont., 2008. Santiago, Chile meeting with AgroVivo; January 10-22<sup>nd</sup>, 2009., SBA Field Day, June 20, 2009, Invited speaker; The Saskatraz Project. North American Beekeepers Conference, Orlando, Florida, USA. Jan. 2010; SBA Field Day, Manitoba Beekeepers Association, March, 2010, Brandon, Manitoba June. 25, 2010. The Importance of Apiculture. Agriculture Biotechnology International Conference, Sept 15 2010, Saskatoon, Saskatchewan, Canada-invited speaker. Robertson, Albert J. 2011. SBA Annual Meeting Dec. 3, 2010. Invited Speaker; The Saskatraz Project. The

Saskatraz Breeding Program. North American Beekeeping Conference, January 4-8, 2011, Galveston, Texas, USA.

In 2010 Robertson was invited to be a CANADIAN REPRESENTATIVE FOR APIMONDIA WORKING GROUP 7 (AWG-7). INTERNATIONAL REPRESENTATION OF BEEKEEPING FEDERATION.

Queen rearing practices and impact on the genetic diversity and fitness of honey bee colonies.

The Apimondia Working Group (AWG 7) was created on October 25, 2010 as a Scientific Commission of Apimondia. The aim of the WG is to collect information on honey bee queen rearing practices, and their impact on the genetic variability and general health of honey bee colonies. The WG consists of 21 members from 14 different countries. The world wide survey being conducted is focused on gathering information on selection methods, large scale commercial queen rearing practices, use of instrumental insemination and damage to feral colony populations due to *Varroa infestation*. The information collected will contribute on an international level, to our understanding of how apiculture practices affect honey bee productivity and health issues.

Maria Bouga<sup>1</sup>; Gérard Arnold<sup>2</sup>; Malgorzata Bienkowska<sup>3</sup>; Ralph Büchler<sup>4</sup>; Lionel Garnery<sup>5</sup>; Fani Hatjina<sup>6</sup>; Evgeniya Neshova Ivanova<sup>7</sup>; David De Jong<sup>8</sup>; Meral Kence<sup>9</sup>; Nikola Kezic<sup>10</sup>; Per Kryger<sup>11</sup>; António Murilhas<sup>12</sup>; Benjamin Oldroyd<sup>3</sup>; Randy Oliver<sup>14</sup>; María Alejandra Palacio<sup>15</sup>; Plamen Petrov<sup>16</sup>; Alice Printo<sup>17</sup>; Albert Robertson<sup>18</sup>; Peter Rosenkranz<sup>19</sup>; Pilar de la Rua<sup>20</sup>; Damir Šekulja<sup>21</sup>

(18Meadow Ridge Enterprises Ltd. Box 1, Group Site 602, Saskatoon, Saskatchewan, Canada. S7K 3J9. (306)373-9140; a.j.robertson@sasktel.net. Robertson participated in drafting the abstract and described some details of the Saskatraz Project to be presented as a poster at Apimondia, in Brazil in Sept. 2011. These communications have been reached by many thousands of people on an international scale. When we first posted the website we had to increase the band width to accommodate the traffic. We do not know the number of people reached by the communications.

## 5. PROJECT REACH.

(i) The project target audience and beneficiaries have been primarily Saskatchewan and Canadian queen breeders and commercial beekeepers.

(ii-v) The Saskatraz Project has both national and international recognition. Canadian beekeepers have been the beneficiaries, but there is worldwide interest in the breeding procedures and the breeding stock. I have received inquiries from around the world with interest in the program. Our website currently provides access to interested individuals around the globe.

## 6. PROJECT IMPACT.

(i) The Saskatraz Project has developed and distributed families of Honey bees across Canada with improved honey production, wintering ability, mite tolerance and chalk brood resistance. This has and will continue to provide economic benefits to beekeepers using the stock. New opportunities exist for commercial queen breeders to multiply this stock in southern hemispheres to distribute production queens in North America in the spring for pollination and honey production purposes.

(ii) Short Term Results:

-Release of improved honeybee stock for honey production and mite tolerance on a limited basis.

As of June 25, 2011 we have released 5617 queen cells from selected (honey production, mite tolerance) and intensively tested breeding lines. By the end of this season we will have released more than 100 breeder queens. This was more than anticipated and the demand continues to be strong with increased interest from Alberta, Manitoba, BC, and Quebec. We have not been able to meet the demand for stock in the USA, partly because of our short season.

-Identification of some molecular markers to improve selection methods.

We previously identified 20 informative molecular markers for identifying different bee populations (genetic diversity) with CARDS funding. Progress in this area was delayed until we established collaborative research activities with the University. We now have a number of molecular markers correlated with varroa tolerance, but more development of these markers is required.

-Expansion of gene pool to improve long term selection results.

We have tested many new selections provided by collaborating Canadian queen breeders, US queen producers and Australian breeders. We are now assembling a new Saskatraz apiary for back crossing and out crossing under close population mating conditions. We believe this apiary harbors the best gene pool constructed to date by continual progeny analyses and re-current selection. It may be one of the most diverse and valuable in the world.

Long Term Results:

-Construction of improved stock by combining desirable traits through instrumental insemination using single and mixed drone semen and progeny evaluation will identify honeybee lines with increased honey production, mite tolerance and other desirable traits. Identification of a powerful set of molecular markers for marker assisted breeding.

We are continuing with this goal, and addressed this point under short term results.

-Extensive release of breeding stock to queen breeders should result in a marked reduction in chemical usage, improved, quality, honey production and protection against massive losses of honeybees due to CCD or varroa resistance to chemical miticide treatments.

Extensive release of honey bee stock has already occurred .We have initiated experiments with organic miticides (naturally occurring compounds-Thymol, formic acid ,oxalic acid and hops)and varroa tolerant lines. Some of these results are encouraging, and it should be possible to use organic treatments to complement genetic tolerance to varroa, avoiding the use of toxic synthetic miticides. Synthetic miticides are toxic to the environment, leave possible residues in wax and honey, and mites become resistant to them quickly.

-New market development will occur for Saskatchewan and Canadian queen breeders for improved genetic stock in both the pollination and honey industry in North America.

Many Canadian queen breeders are using Saskatraz stock in their operations after purchasing breeder queens from the Saskatraz breeding program. This stock is becoming widely distributed to commercial beekeepers in Saskatchewan. New opportunities will arise for Canadian queen breeders in South America and Australia when breeding stock is exported for multiplication. This would allow production of stock adapted to Canadian environments in the Southern hemisphere for export to Canada in the spring. Queen producers in Chile and Australia have expressed in forming joint queen breeding initiatives to export to Canada.

(iii) This project has been monitored by Saskatchewan and Canadian beekeepers and strong demand for the stock continues. It is evident by the extensive use of breeding stock from the program for production of queen cells and queens for internal use as well as marketing to commercial producers that the beekeepers find this stock beneficial to their programs. The demand is increasing for breeder queens, making it difficult to meet market requirements. We cannot meet the market demand in the US, because of our short season

(iv) We would be willing to work with ACS to track the long term impacts of this project.

## 7. PERFORMANCE STORY.

The Saskatraz project is significant because of the unique approach of attempting to use natural selection re-cycling and genetic solutions to improve the health and productivity of the domesticated honey bee. The unique approach involved assembling, at an isolated apiary called Saskatraz, the best genetic stock (honey production, overwintering ability) available from the Canadian industry and combining these selections with Russian and German genetics. The Russian and German stock was previously selected for varroa tolerance.

This diverse gene pool was used to initially select 6 breeding lines with the best honey production, varroa tolerance and resistance to brood diseases. This stock was first released to the industry in 2006, and by the end of 2011 we will have released over 6000 queen cells and 100 breeder queens to Canadian queen breeders. Many of these breeders are distributing stock to commercial beekeepers. The demand for breeder queens is more than we can supply, and we have not yet met the US demand for breeding stock. This project was not only successful at selecting improved honey bee stock, but at distribution of it to industry. An added benefit to industry was the development of diagnostic services for beekeepers in our collaborative work with VIDO, at the University of Saskatchewan. In the last year we have identified some putative markers for varroa tolerance using microarray analyses, but this work is not complete. Further longer term efforts with university collaborators are required to complete the molecular work, and Meadow Ridge will need to maintain and further improve the breeding stock using the methods developed by the Saskatraz project. Industry will continue to be informed of our progress through our website [www.saskatraz.com](http://www.saskatraz.com)