The Saskatraz Project

Objective: To develop productive, gentle honeybees with tolerance to mites and brood diseases

By: Albert J. Robertson
SBA Honeybee Breeding Program

Summer 2004-05

The Saskatraz Project – Galveston, 2011

The Saskatraz Project
SBA Honey Bee Breeding Program

- Established in 2004 in collaboration with Saskatchewan and Manitoba queen breeders.
- Involved assembling a large diverse gene pool at an isolated apiary called Saskatraz.
- Aimed at using natural selection (no synthetic chemical miticides) to select for honey bee genotypes with increased honey production and tolerance to parasitic mites.

Primary Selection Criteria:

1. Honey Production
2. Wintering Ability
3. Mite Resistance and Suppression
4. Resistance to Brood Diseases (chalk brood etc.)
5. Viruses and Nosema Susceptibility

Breeding methods used to select and enrich for important traits (natural selection, out crossing, back crossing, recurrent selection, progeny analyses and closed population mating).

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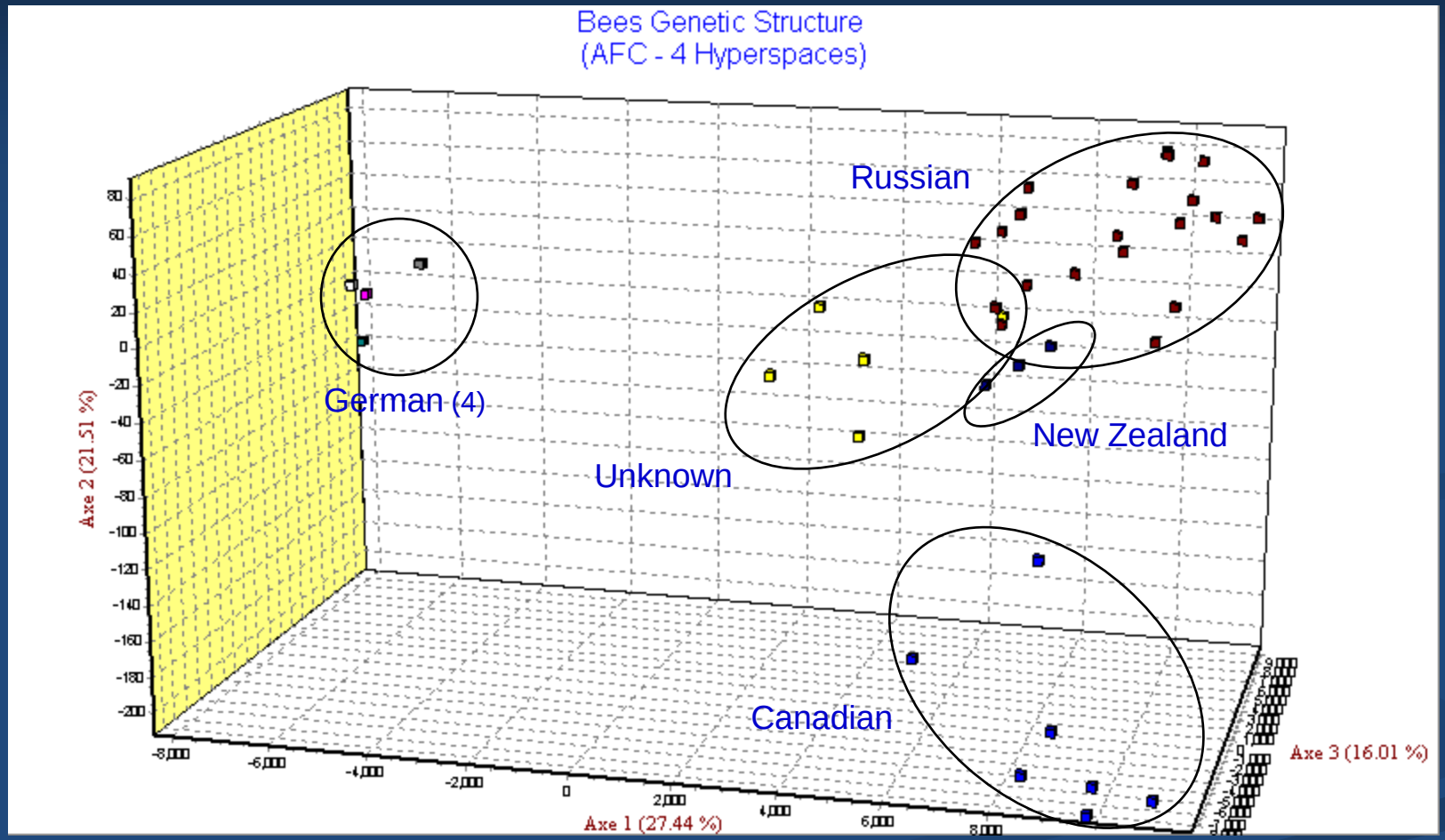

A three dimensional plot showing the grouping of 5 different honeybee populations using 20 informative microsatellite markers.

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Saskatraz natural selection yard site fall 2006 – fenced.
Selection for this Saskatraz yard site is a death sentence.

September 2006

These bees look good, but they are dying.

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Saskatraz Survivors May 2007

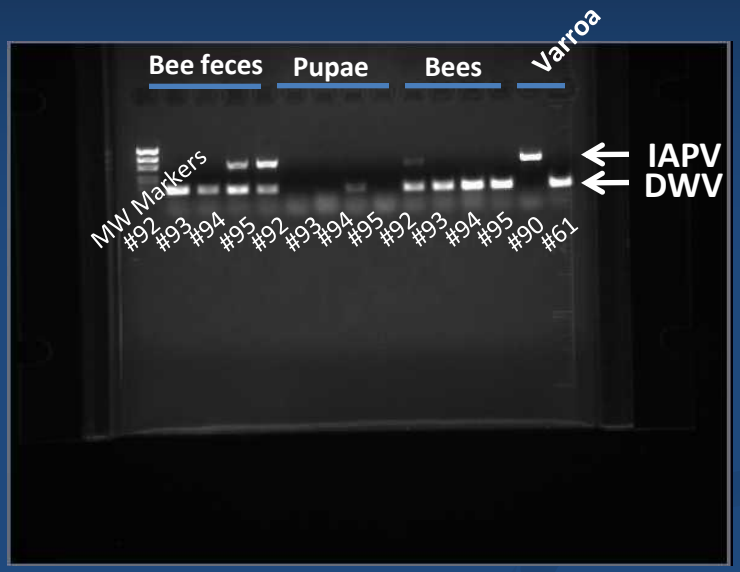
M = 32 months

Honey Bee Health Issues

- In the media since 2007, Colony Collapse Disorder (CCD). Increased and continued decline in honey bee populations throughout the world is of serious concern.
- Possible Causes:
  - Parasitic mites (Varroa Destructor)
    - Apis cerana → Apis mellifera
    - Pathogens (viruses and microsporidia) associated with the mites
  - Synthetic chemical miticide treatments
    - Residues, mite resistance, decreased natural immunity and suppression of the development of natural resistance to mites
  - Lack of genetic diversity in the managed bee population.
  - Compounded by poor apicultural practices and agricultural pesticide uses (nicotinoids)

Varroa Mites in Pupae

PCR Detection of IAPV and DWV



Material was gathered from individual hives (#61, 90, 92, 93, 94, 95)
Bee feces: RNA extracted from 50-100 mg
Pupae: RNA pooled from 3 pupae
Bees: RNA pooled from 3 bees
Varroa: RNA pooled from 5-10 mites

Viruses can be detected not only in adult bees, pupae and Varroa, but also in bee feces. Feces collected from the hive can be used to diagnose virus infections, bee and varroa feces may be a source of colony infection by viruses and microsporidia.

Varroa (ND 2005-2006) Were Sampled From All Saskatraz Colonies To Monitor Virus Infection Status of the Varroa Population and Host Colony.

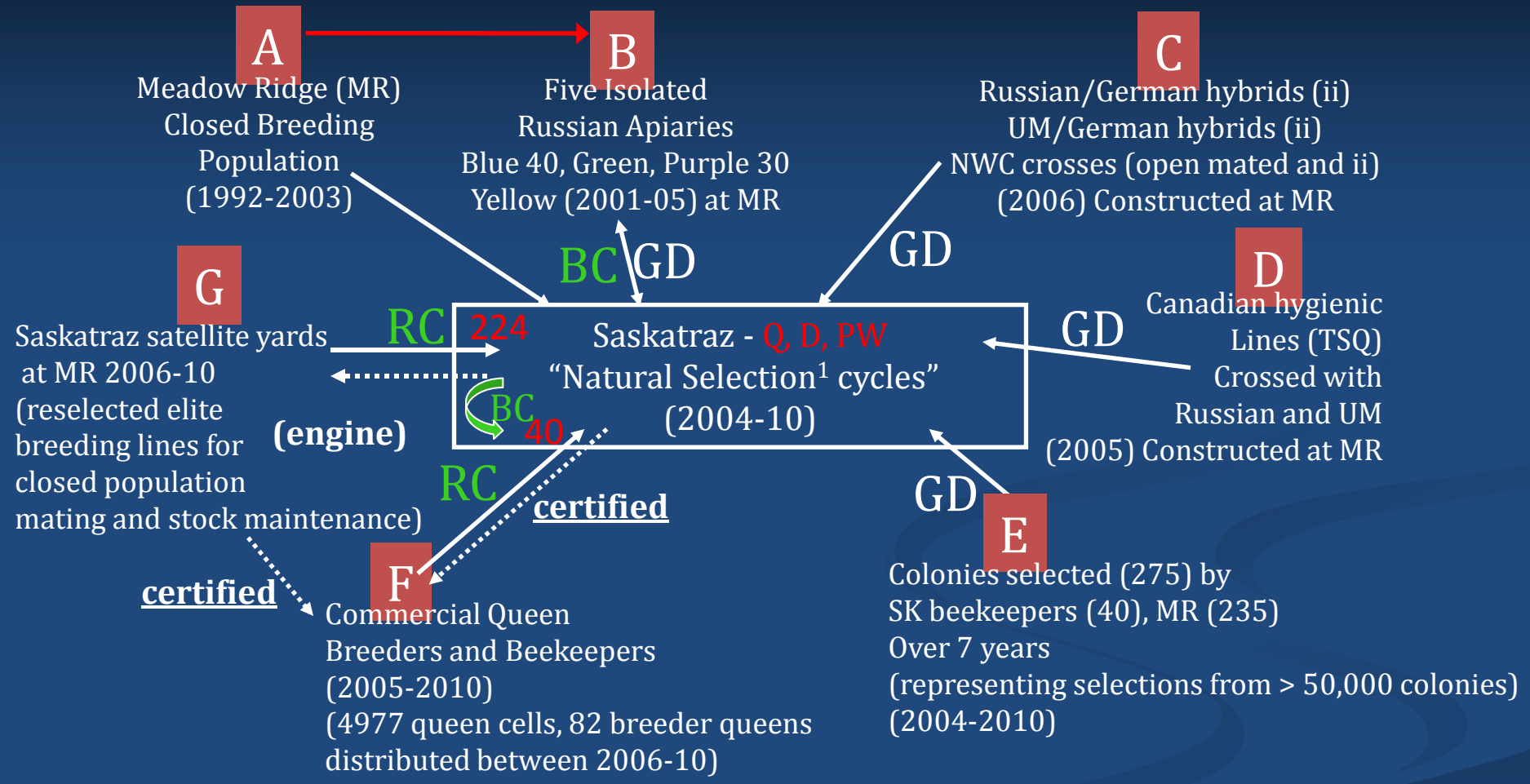
Pandemic

Colony (2004)	Virus	May 2005	June 2005	July 2005	Aug. 2005	Sept. 2005	Oct. 2005	May 2006	June 2006	July 2006	Aug. 2006	Sept. 2006	Oct. 2006
SAT 01	DWV	–	–	+	+	+	–	+	+	+	–	+	Dead
	IAPV	–	–	–	–	–	–	+	–	+	+	+	
	KBV	–	– – –	3 – –	–	– – 1.2	–	– – 7	– – 1	+ 9	– + 31	+	
SAT 24	DWV	+	–	+	+	+	+	–	–	+	Dead	Dead	Dead
	IAPV	–		–	–	–	–	–	–	+			
	KBV	–	– – –	1 – –	–	– – 1.3	–	3 – –	1 – 17	7 – 3	2 33		
SAT 28	DWV	+		–	+	–	+	+	+	–	+	–	+
	IAPV	–		–	–	–	–	+	–	–	+	+	+
	KBV	–		– – –	–	– – –	–	– – –	–	–	1 + 12	9 + 46	+
SAT 30	DWV	–	+	+	+	+	–	–	–	–	+	–	–
	IAPV	–	–	–	–	–	–	–	+	–	+	+	+
	KBV	–	– – –	– – –	–	– – 0.9	–	2 – –	– – 3	– – 10	3 + 26	4 + 95	+
SAT 34	DWV			+	+	+	–	–	+	–	–	+	–
	IAPV			+	–	+	–	–	+	–	+	+	+
	KBV			– – –	–	– – 0.75	–	– – –	+	– – 5	– + 11	– + 26	+
Saskatraz Apiary	%T		0.33	0.64		1.5		0.5	0.3	0.72	0.9	0.75	
	%V		–	0.06		1.6		1	3.3	3.9	15	32	

Outline of Current Research Activities

1. Honey bee health issues
   - Diagnostics of honey bee pathogens (viruses and microsporidia)
   - Varroa mites (interactions with bees)
2. Saskatraz breeding program (Industry Collaboration)
   - Breeding logistics (natural selection, back/out crossing, recurrent selection)
   - Progeny analysis (grooming, VSH phenotyping)
   - Microsatellites (genetic diversity and relatedness)
   - Biomarkers (microarrays, proteomics and kinome peptide arrays)
   - Culturing breeding stock cell lines for infectivity assays and molecular analyses
   - Screening selected families for virus and nosema susceptibility
3. Management of varroa populations by bee breeding and organic varroa treatments.

Saskatraz Breeding Program Logistics



Letters A to G represent sources (A – E) and reservoirs (G, F) of selected stock. 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, BC backcrossing. [1]Denotes no chemical miticides.

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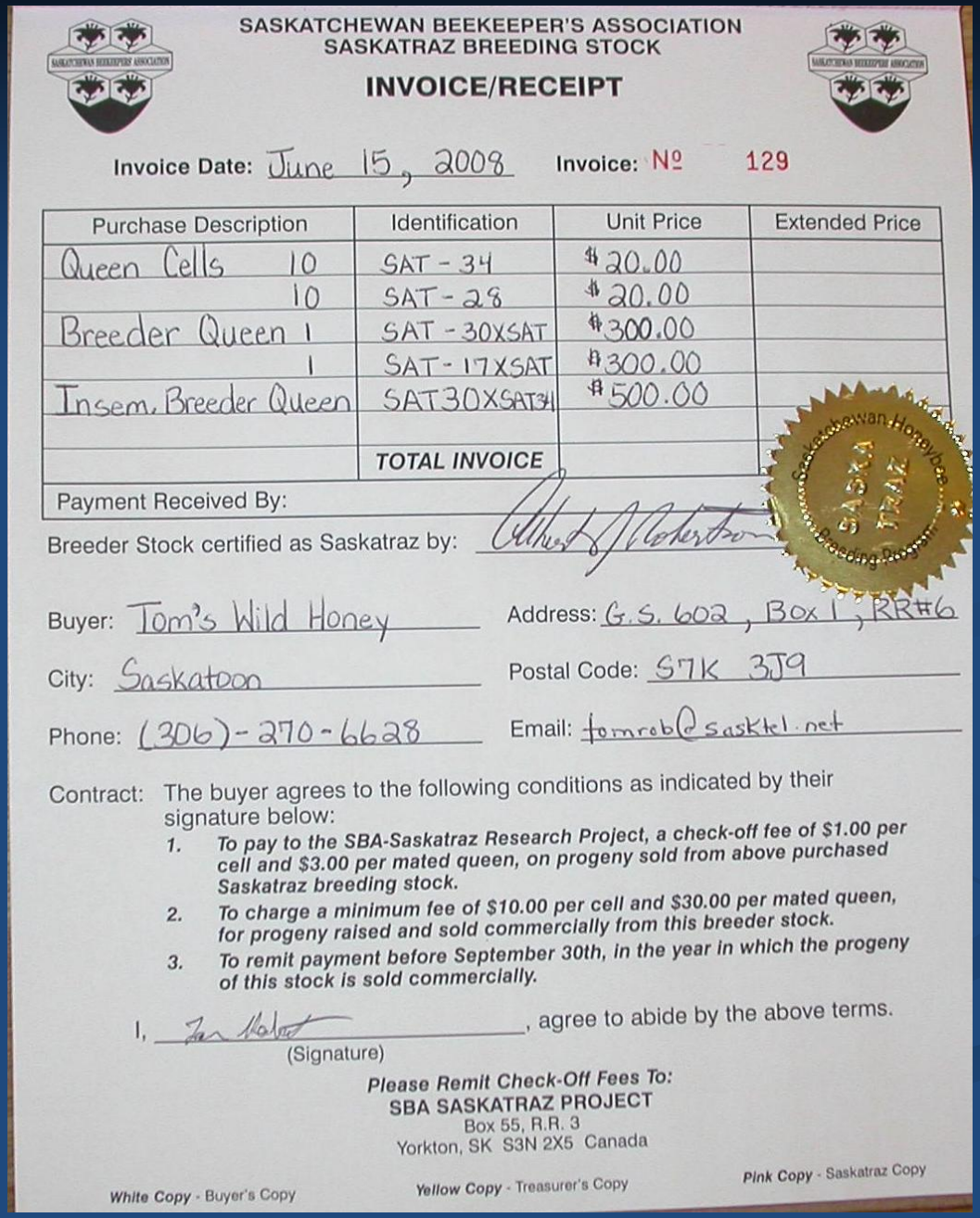

SASKATCHEWAN BEEKEEPER'S ASSOCIATION
SASKATRAZ BREEDING STOCK

INVOICE/RECEIPT

Invoice Date: June 15, 2008 **Invoice:** Nº 129

Purchase Description	Identification	Unit Price	Extended Price
Queen Cells 10	SAT - 34	$20.00	
10	SAT - 28	$20.00	
Breeder Queen 1	SAT - 30XSAT	$300.00	
1	SAT - 17XSAT	$300.00	
Insem. Breeder Queen	SAT30XSAT34	$500.00	
	TOTAL INVOICE		

Payment Received By:

Breeder Stock certified as Saskatraz by: Arthur J. Robertson

Buyer: Tom's Wild Honey Address: G.S. 602, Box 1, RR#6

City: Saskatoon Postal Code: S7K 3J9

Phone: (306) - 270 - 6628 Email: tomrob@sasktel.net

Contract: The buyer agrees to the following conditions as indicated by their signature below:

1. ***To pay to the SBA-Saskatraz Research Project, a check-off fee of $1.00 per cell and $3.00 per mated queen, on progeny sold from above purchased Saskatraz breeding stock.***
2. ***To charge a minimum fee of $10.00 per cell and $30.00 per mated queen, for progeny raised and sold commercially from this breeder stock.***
3. ***To remit payment before September 30th, in the year in which the progeny of this stock is sold commercially.***

I, _____, agree to abide by the above terms.
(Signature)

Please Remit Check-Off Fees To:
SBA SASKATRAZ PROJECT
Box 55, R.R. 3
Yorkton, SK S3N 2X5 Canada

White Copy - Buyer's Copy ***Yellow Copy*** - Treasurer's Copy ***Pink Copy*** - Saskatraz Copy

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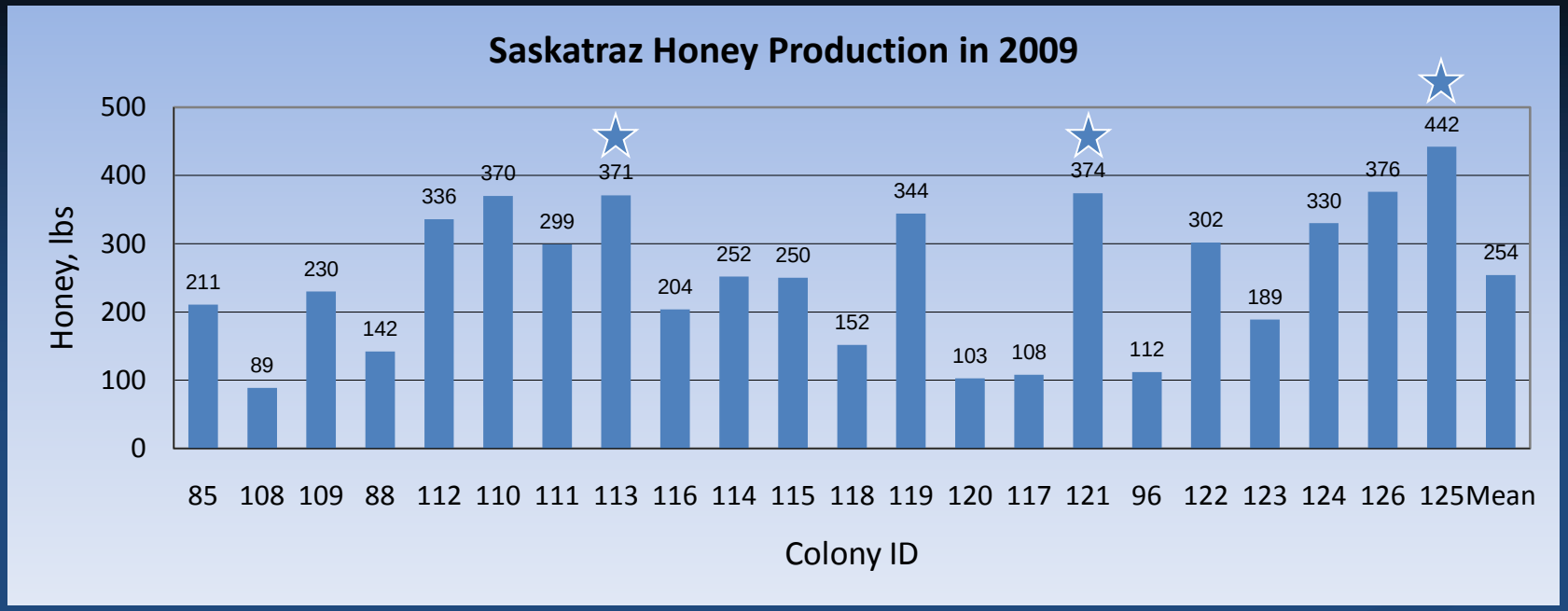

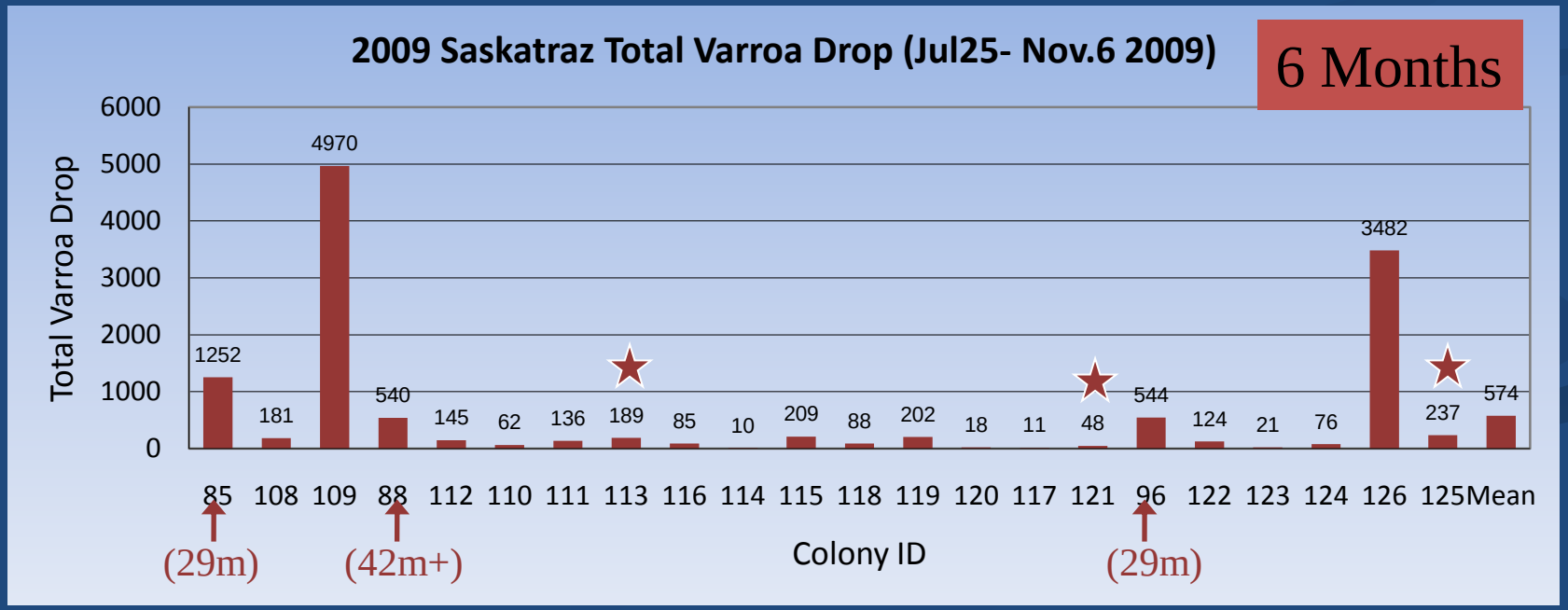

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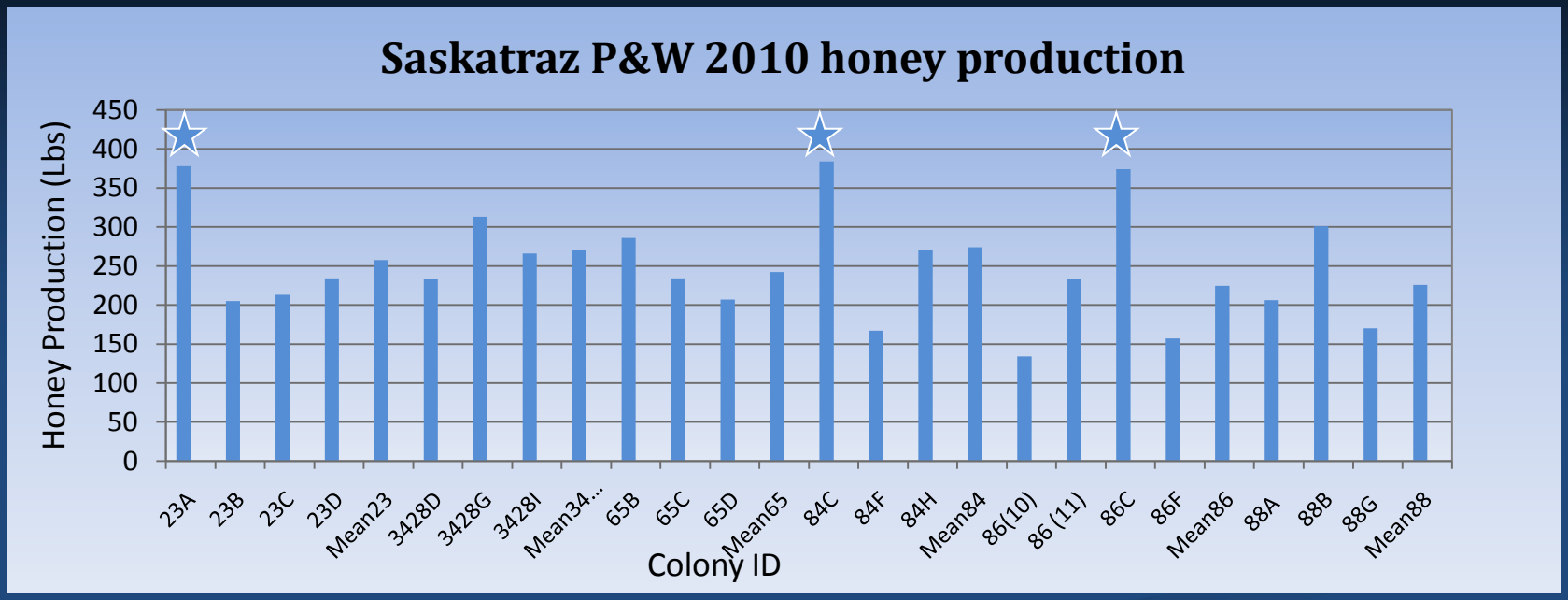
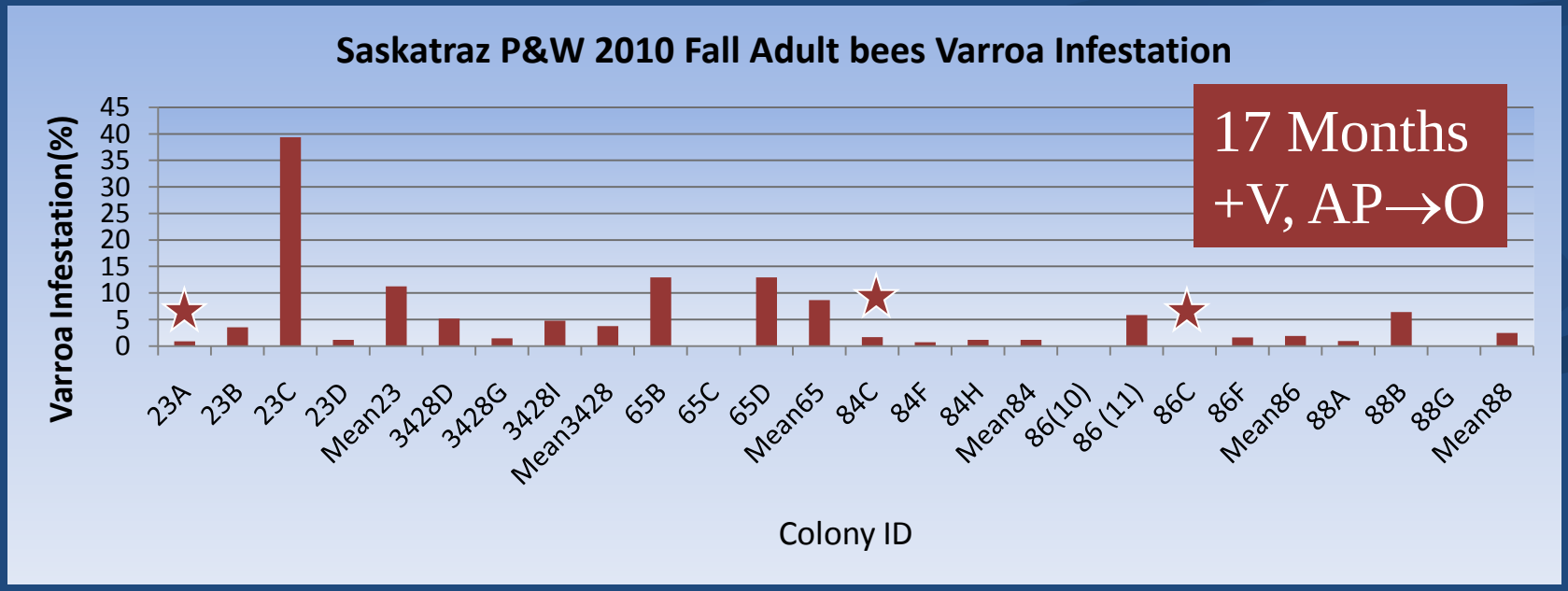
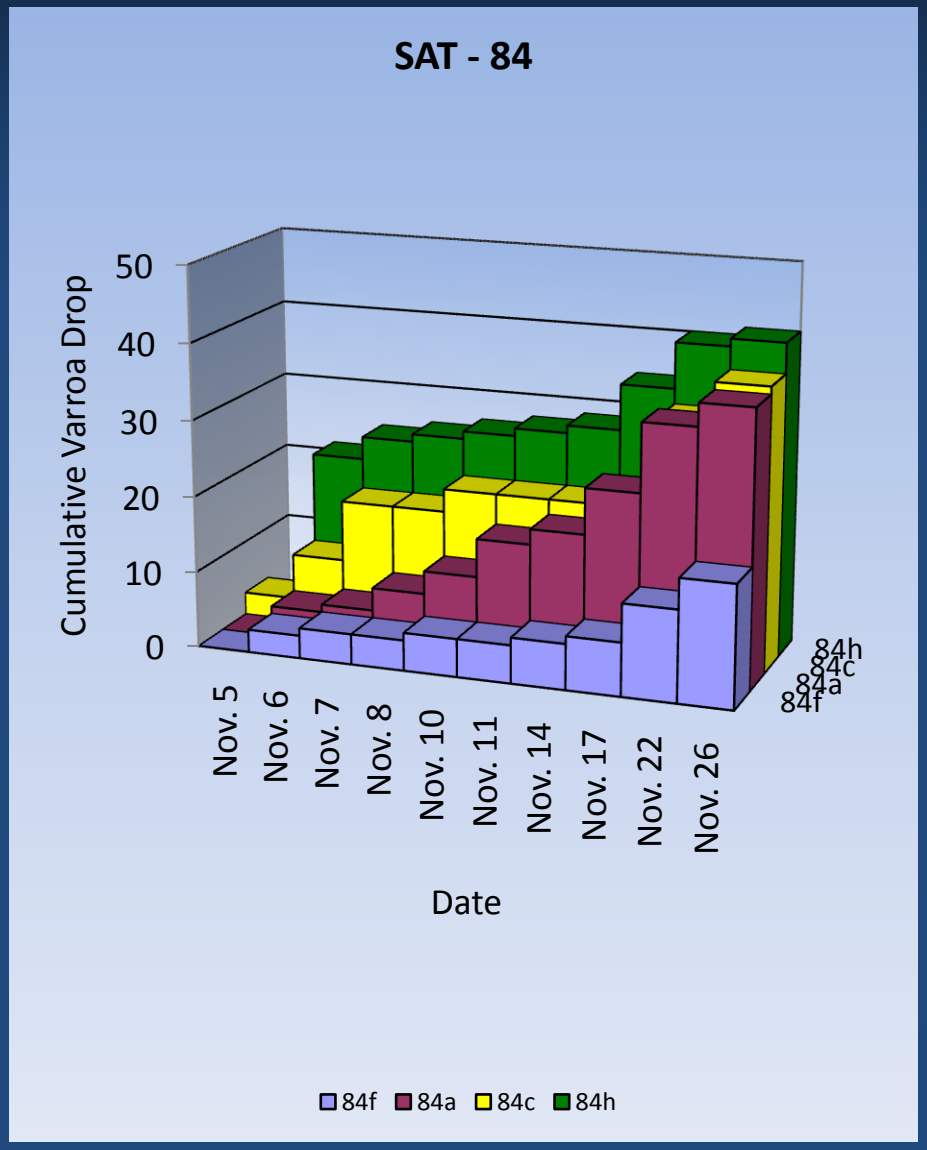


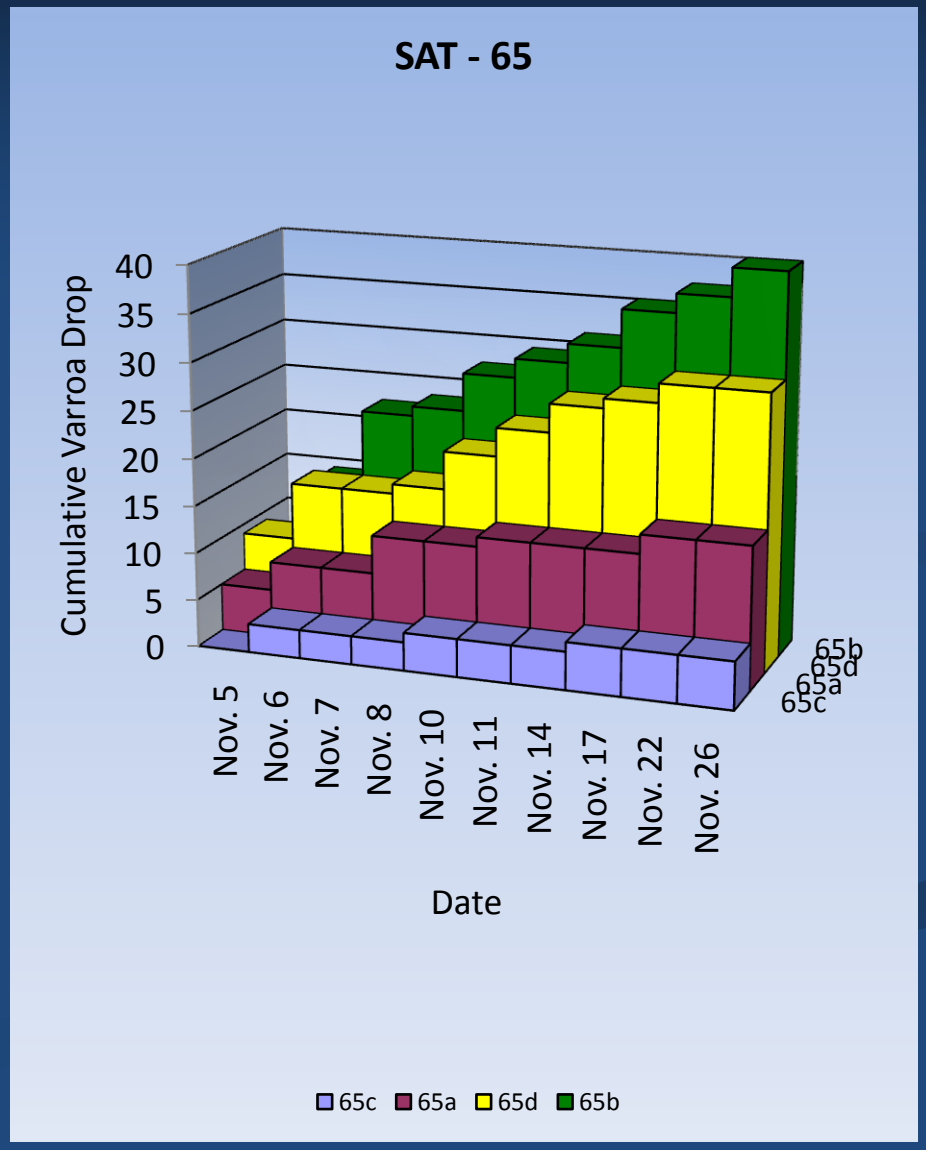


Progeny Analyses of Selected Saskatraz Breeders (14 families)

- Grooming Assays (whole colony)
- VSH phenotyping
- Morphometric Analyses
- Molecular Marker Analyses (microsatellites)
- Selecting for variability in virus susceptibility
- Screening Saskatraz breeding lines for virus and nosema susceptibility/resistance

Grooming Assay





Morphometric Analyses (22 Saskatraz Colonies)

SAT-65 (n = 20)

- Showed longest leg length (8.46 mm, SE = 0.036), corbicular area (pollen sac) (2.59 mm^2, SE = 0.029) and forewing length (10.01 mm, SE = 0.022).
- Second longest proboscis (6.39 mm, SE = 0.031).
- Showed largest difference in microsatellite analyses, increased honey production, and good suppression of varroa mite population growth at Saskatraz. Good grooming behaviour.

SAT-88 (n = 20)

- Longest proboscis (6.40 mm, SE = 0.034).
- Second largest corbicular area (2.57 mm^2, SE = 0.031) and leg length (8.41 mm, SE = 0.034).
- Saskatraz survivor.

Grooming Behaviour:

- Does leg length have an effect?

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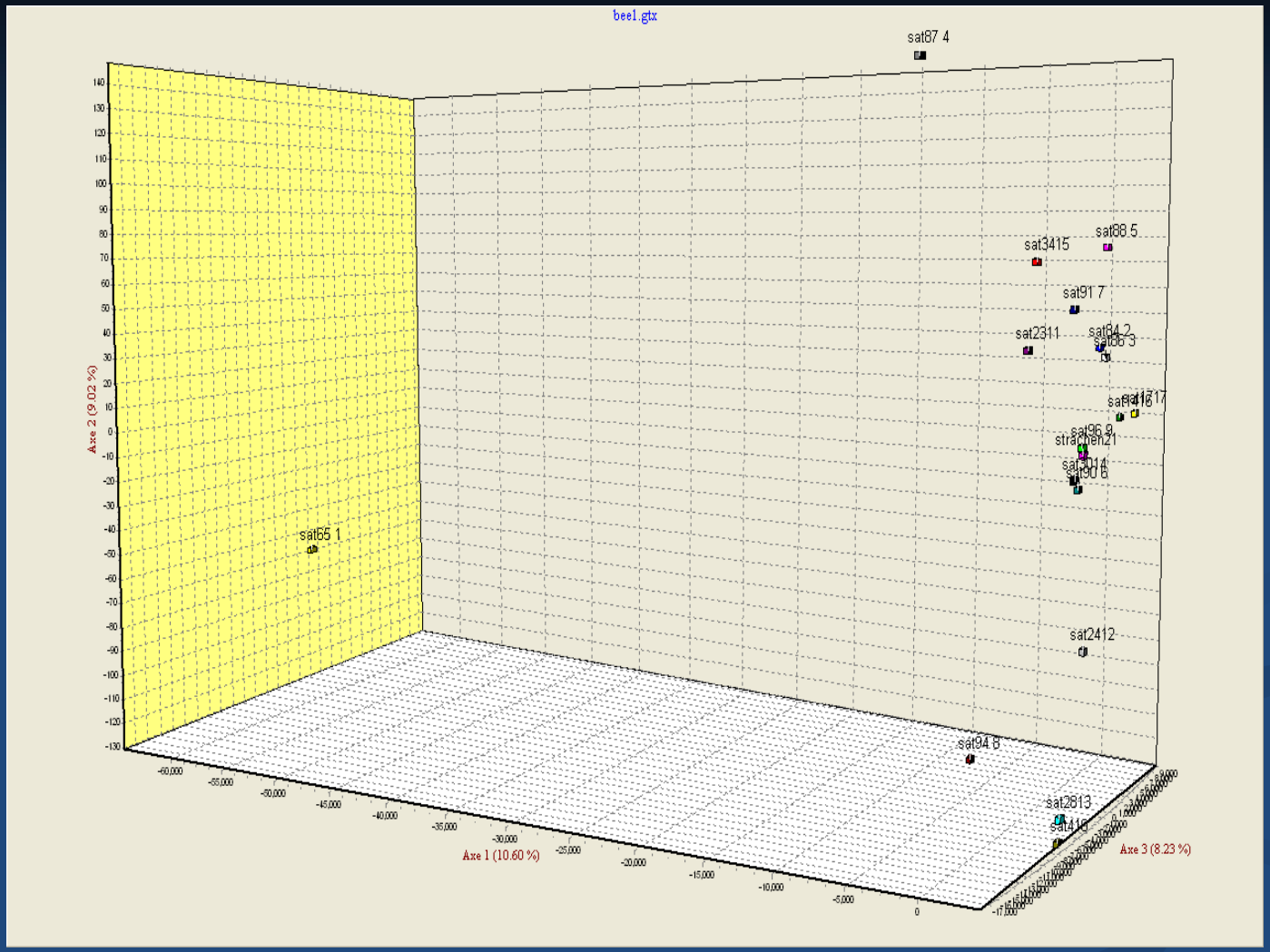

Genetic relatedness (genotyping) between Saskatraz breeding lines and siblings. Loci (Ap53 and A107) effective for genotyping siblings (Frank, P. et.al., 1999, Insect Molecular Biology 8: 419-421) recently added to our other 20 informative markers.

Screening Saskatraz Breeding Lines for Virus and Nosema Susceptibility/Resistance

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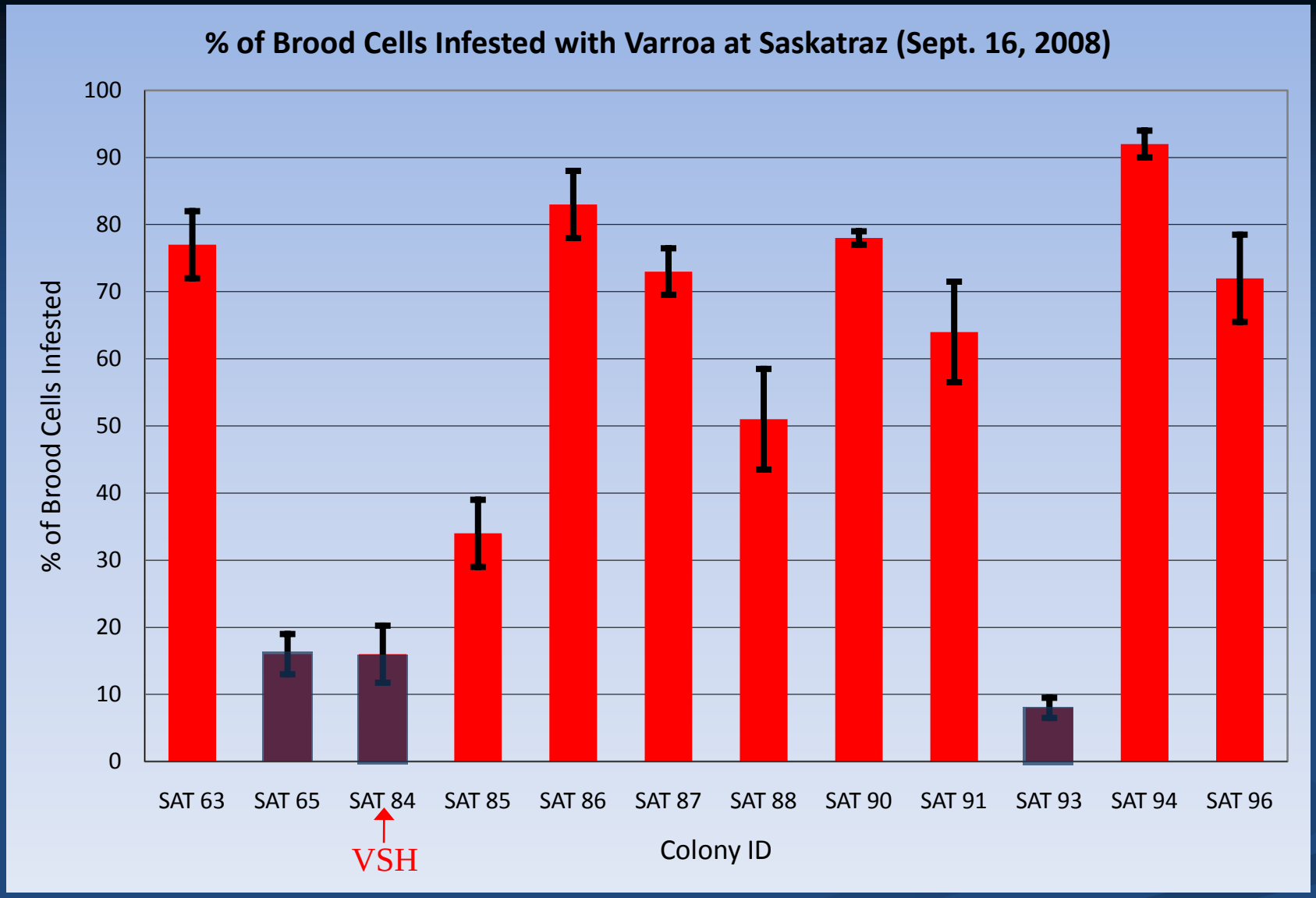
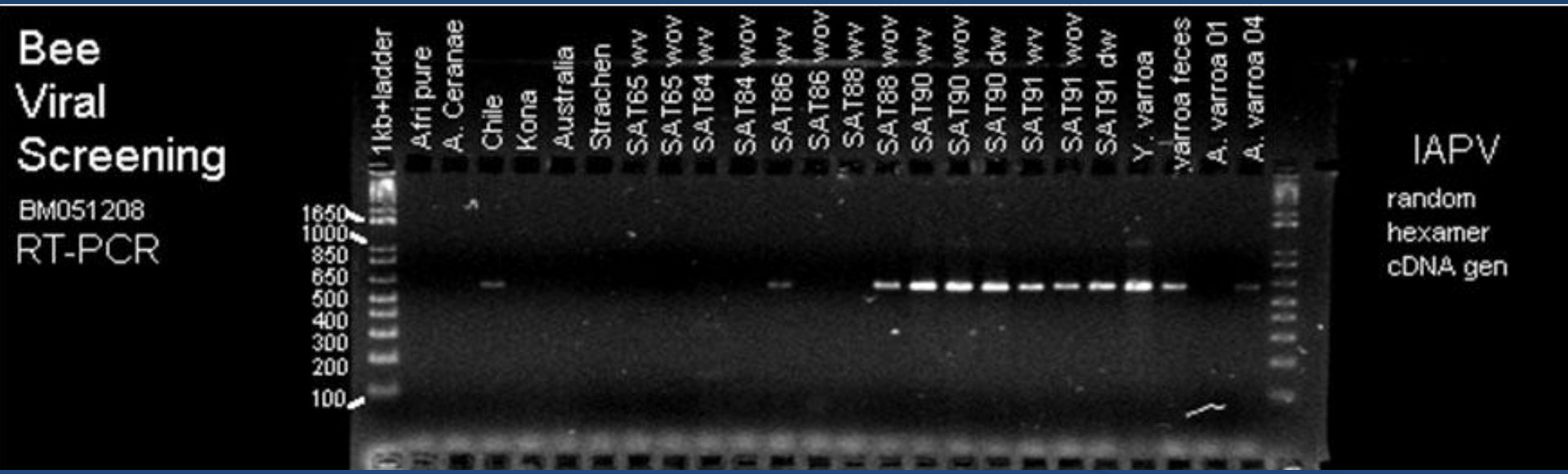


Percent of brood cells infested with varroa at Saskatraz on Sept. 16, 2008.
(Red bars indicate colonies showing virus infections)
Values plotted are mean, error bars are SE.

Screening of Pre-Emergent Pupae From Varroa Tolerant and Sensitive Saskatraz Breeding Lines for IAPV using RT-PCR



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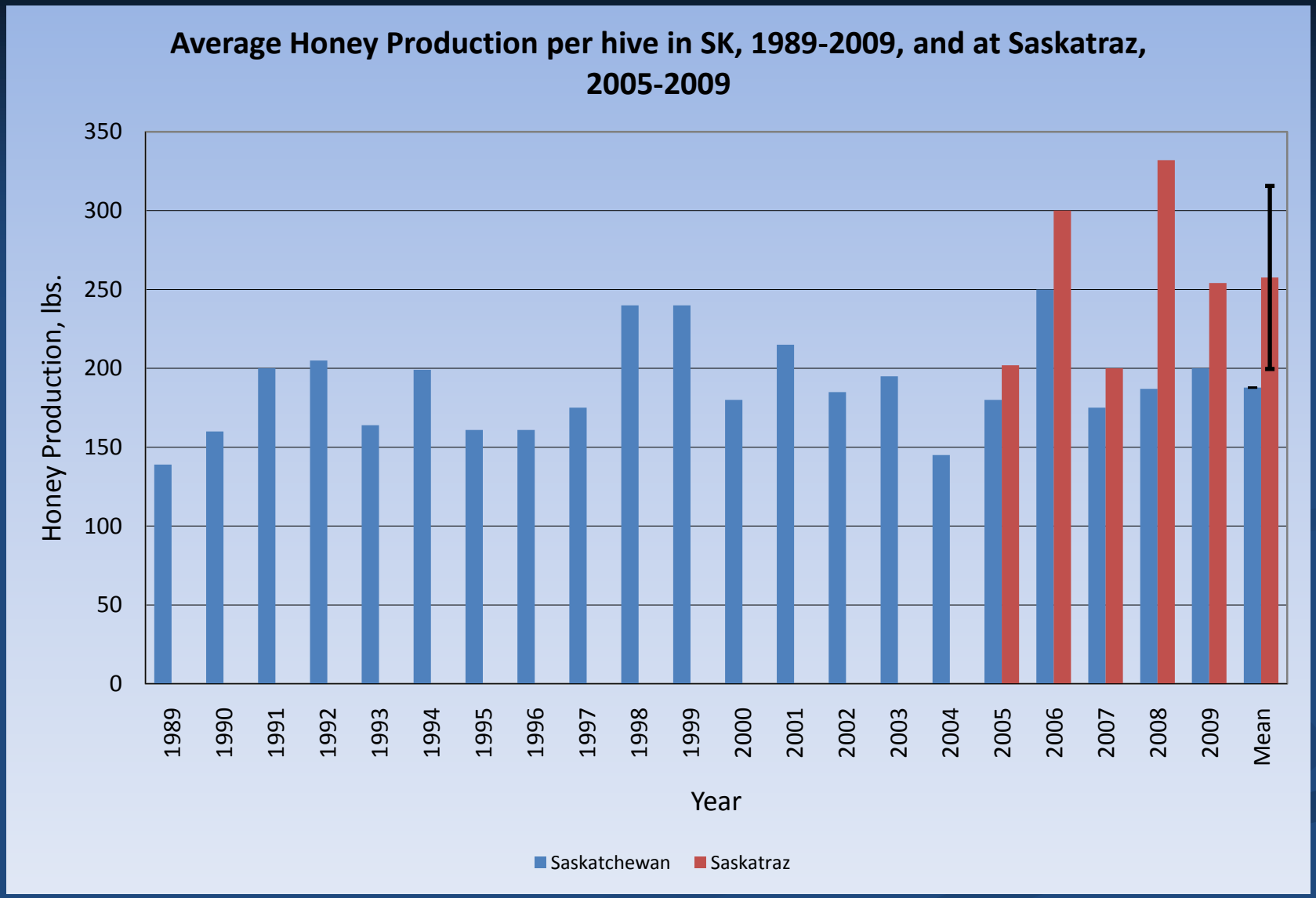

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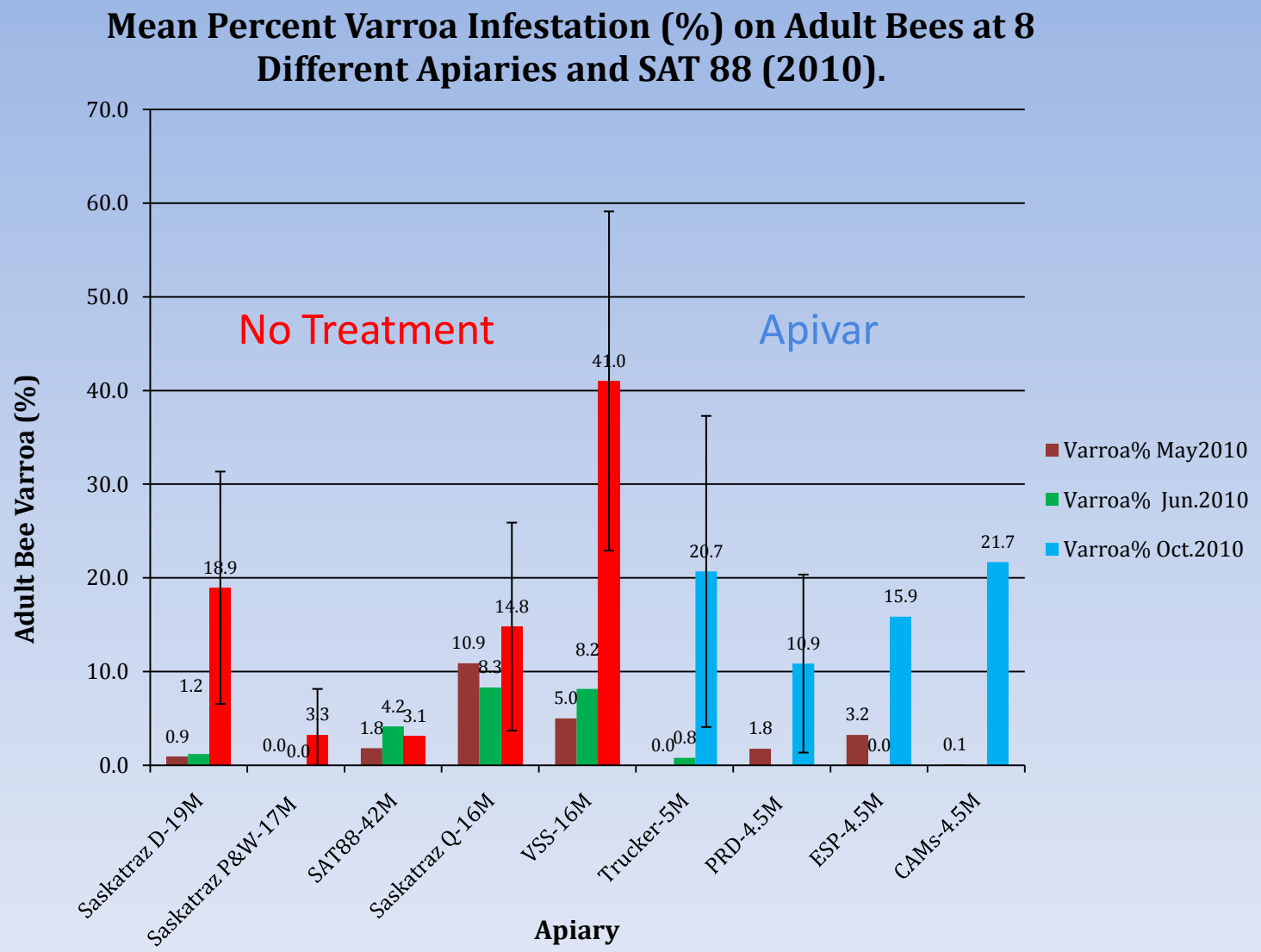

Conclusions

- Natural selection cycles and breeding procedures (backcrossing, outcrossing, recurrent selection) without the use of synthetic chemical miticides, should allow genetic processes to improve tolerance to mites and other pathogens (viruses, bacteria, fungi).
- Use of some synthetic miticides may result in decreased tolerance to varroa infestations in both selected and non-selected populations.
- Fourteen Saskatraz families released to industry since 2006 (5000 queen cells/82 breeder queens) showing increased honey production, good tracheal mite and chalk brood resistance and varying degrees of varroa tolerance.

Acknowledgements

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