LACBA Presentation – November 6th, 2023

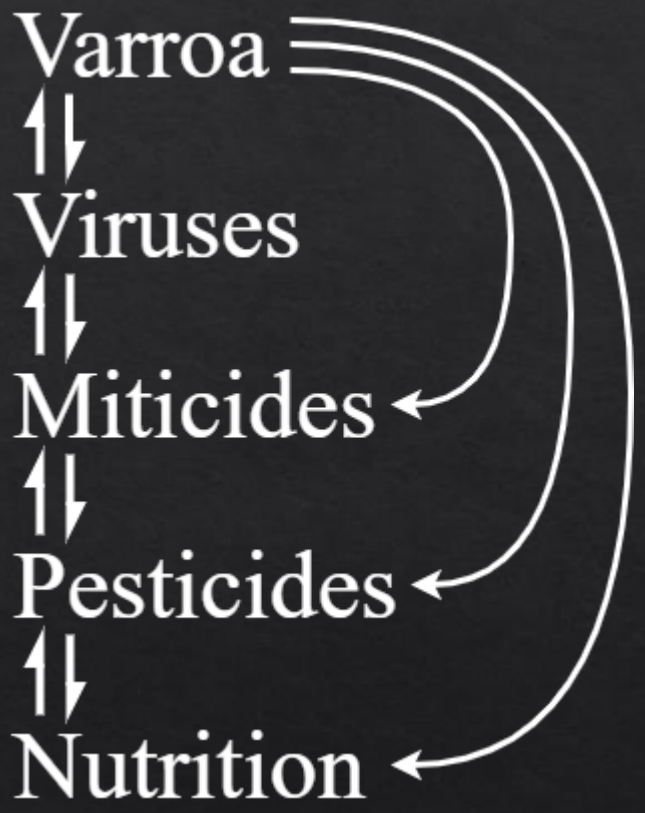
Meadow Ridge Enterprises Ltd

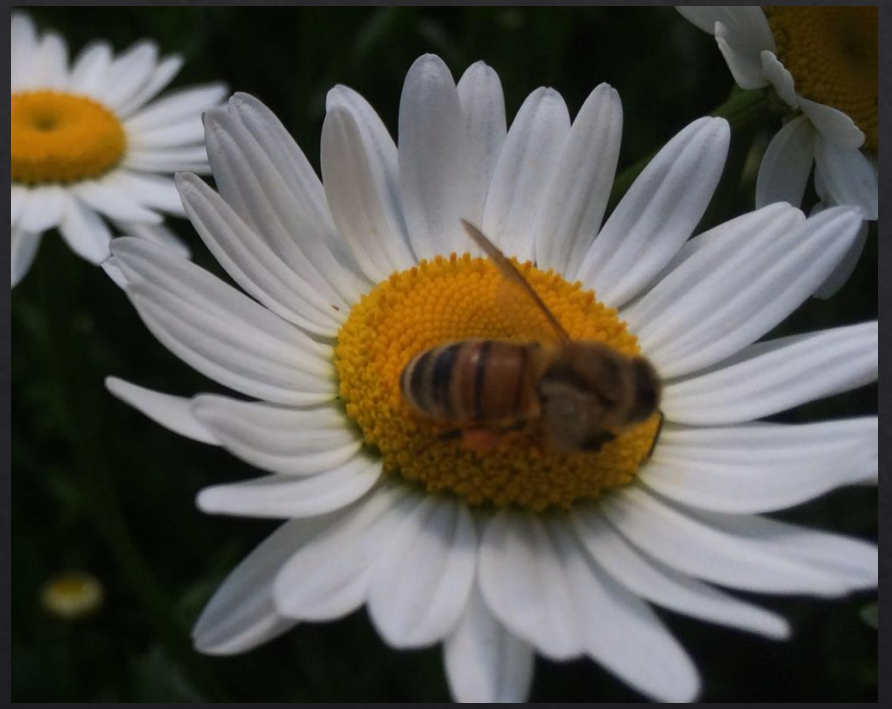
The Saskatraz Project

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

By: Albert J. Robertson
The Saskatchewan Honeybee Breeding and Selection Program

Current Honeybee Health Issues





Outline

- Saskatraz Breeding and Selection Program
- Biomarker Development (Microsatellites, Proteomics, Micro and Kinome Arrays)
- Screening Saskatraz Colonies for Virus Susceptibility
- Saskatraz Hybrid Project
  - Olivarez Honey Bees Inc. - Orland, CA www.OHBees.com
- Combined Miticide Treatment Experiments with selected and unselected stock
- Please visit www.saskatraz.com for reviews and publications
- Please visit bit.ly/Saskatraz for a comprehensive review

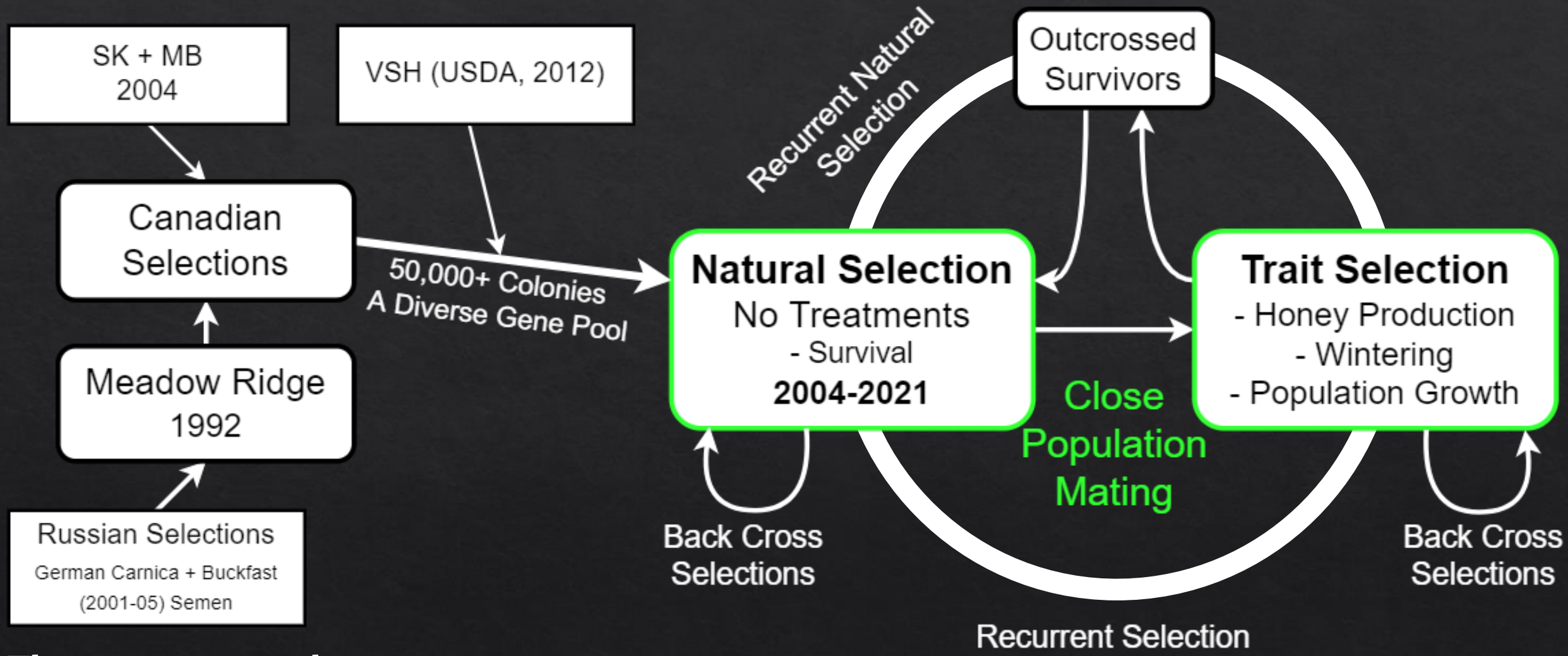
Saskatraz Breeding Program

Primary Selection Criteria:

- Honey Production
- Wintering Ability
- Spring Population Growth
- Varroa Resistance and Suppression
- Resistance to Brood Diseases
  (Chalk Brood, AFB, EFB, etc.)
- 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).

Saskatraz Breeding Program Logistics

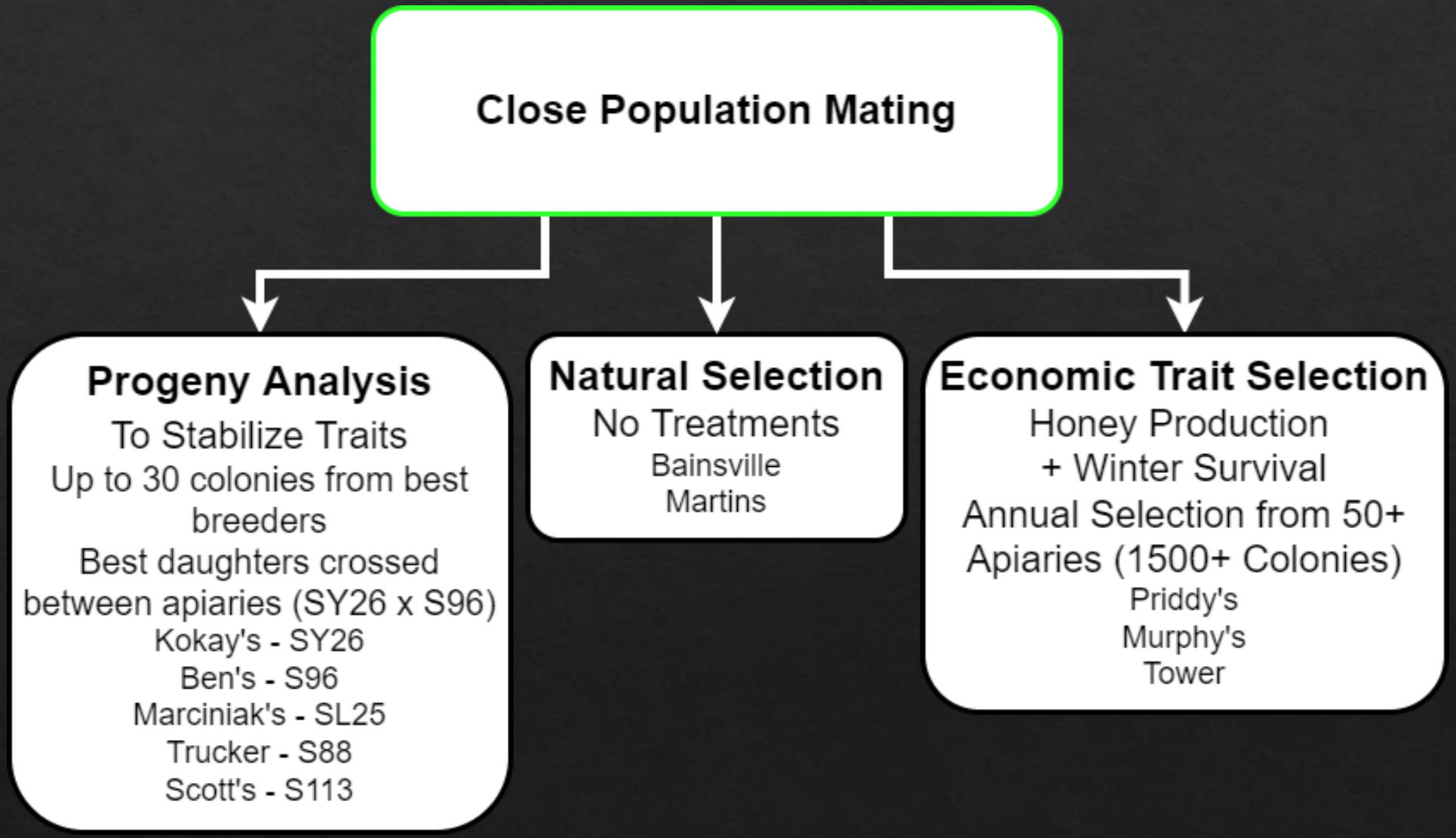


There are currently:

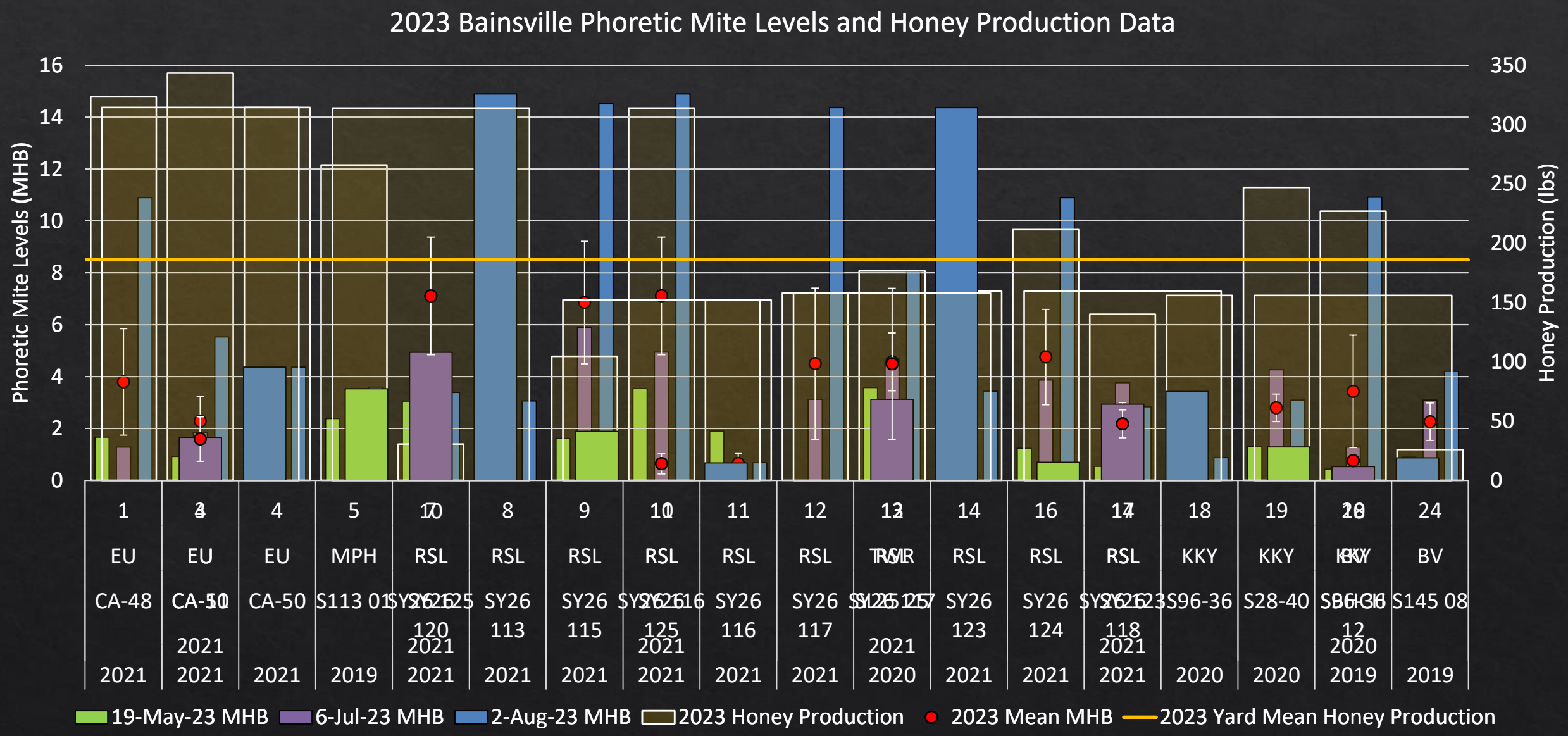
17 Saskatraz Families

Stock Distributed Yearly Since 2006

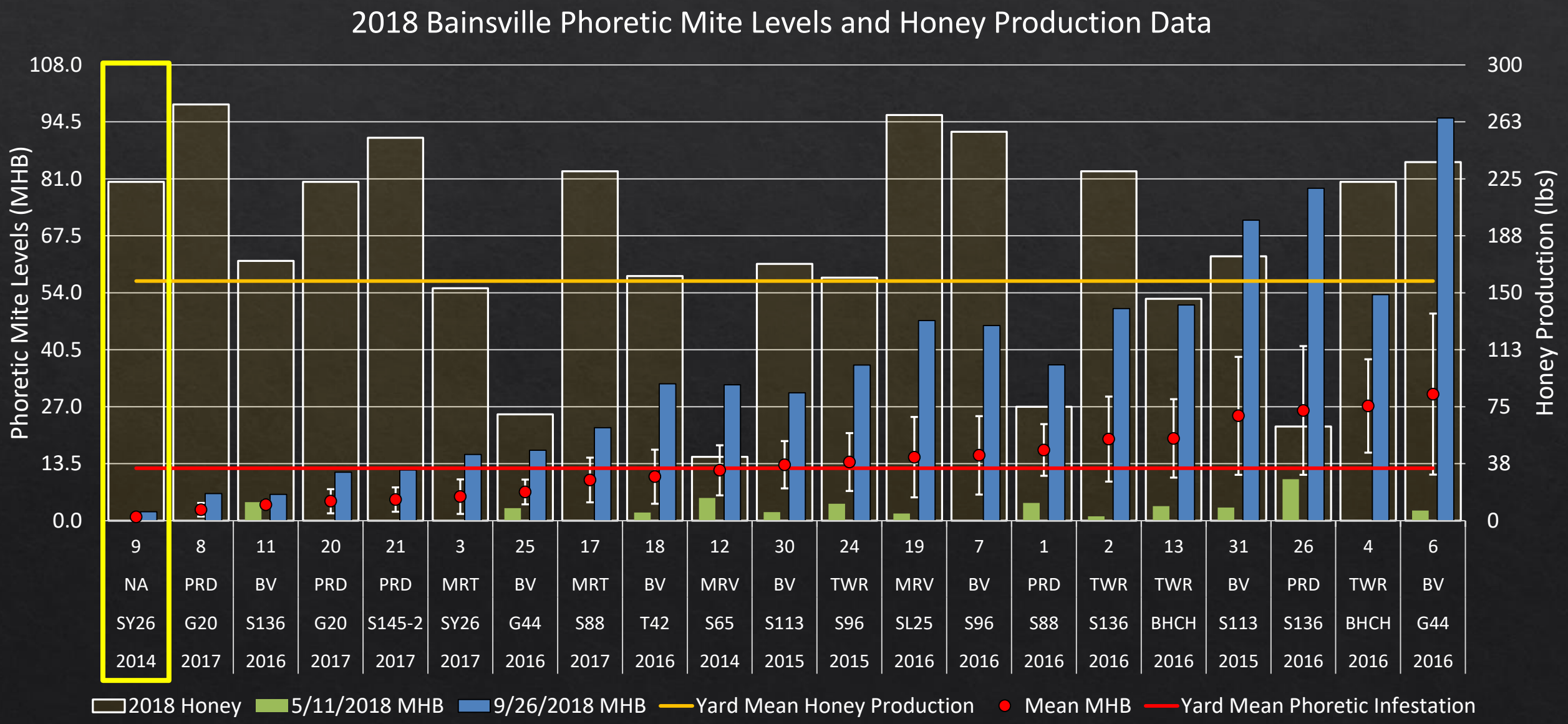
Saskatraz Breeding Program Logistics



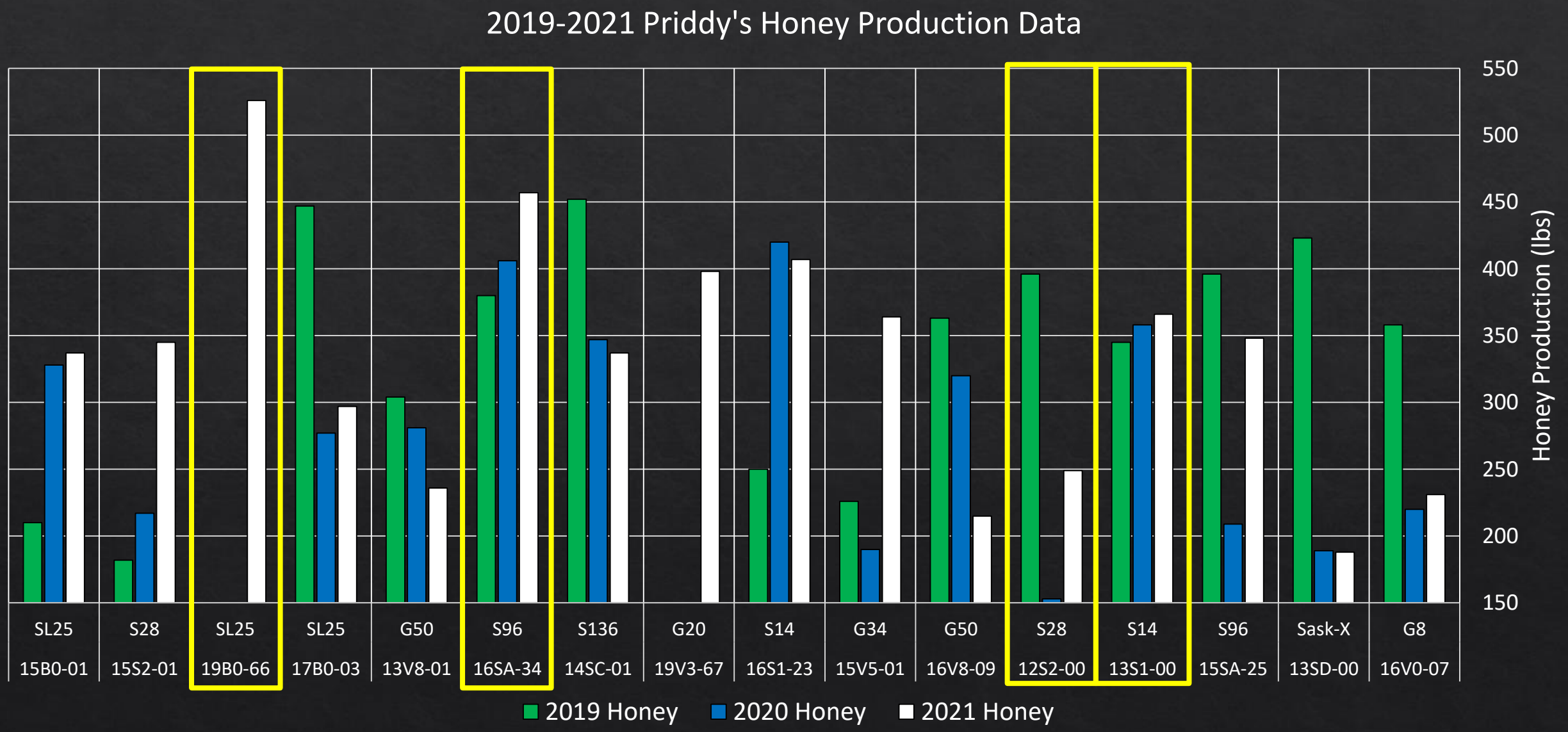
Natural Selection for Varroa Tolerance 2023



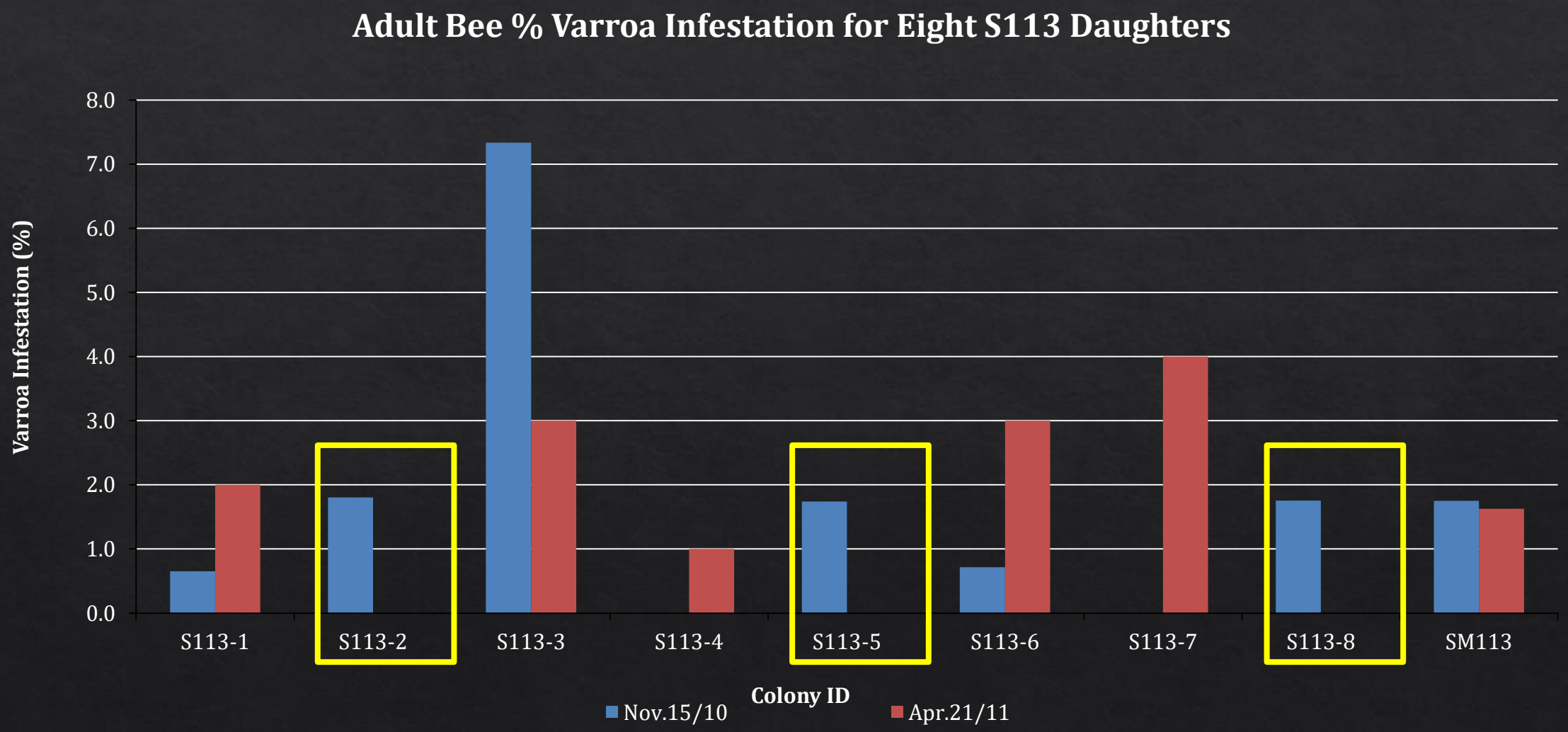
Natural Selection for Varroa Tolerance 2018



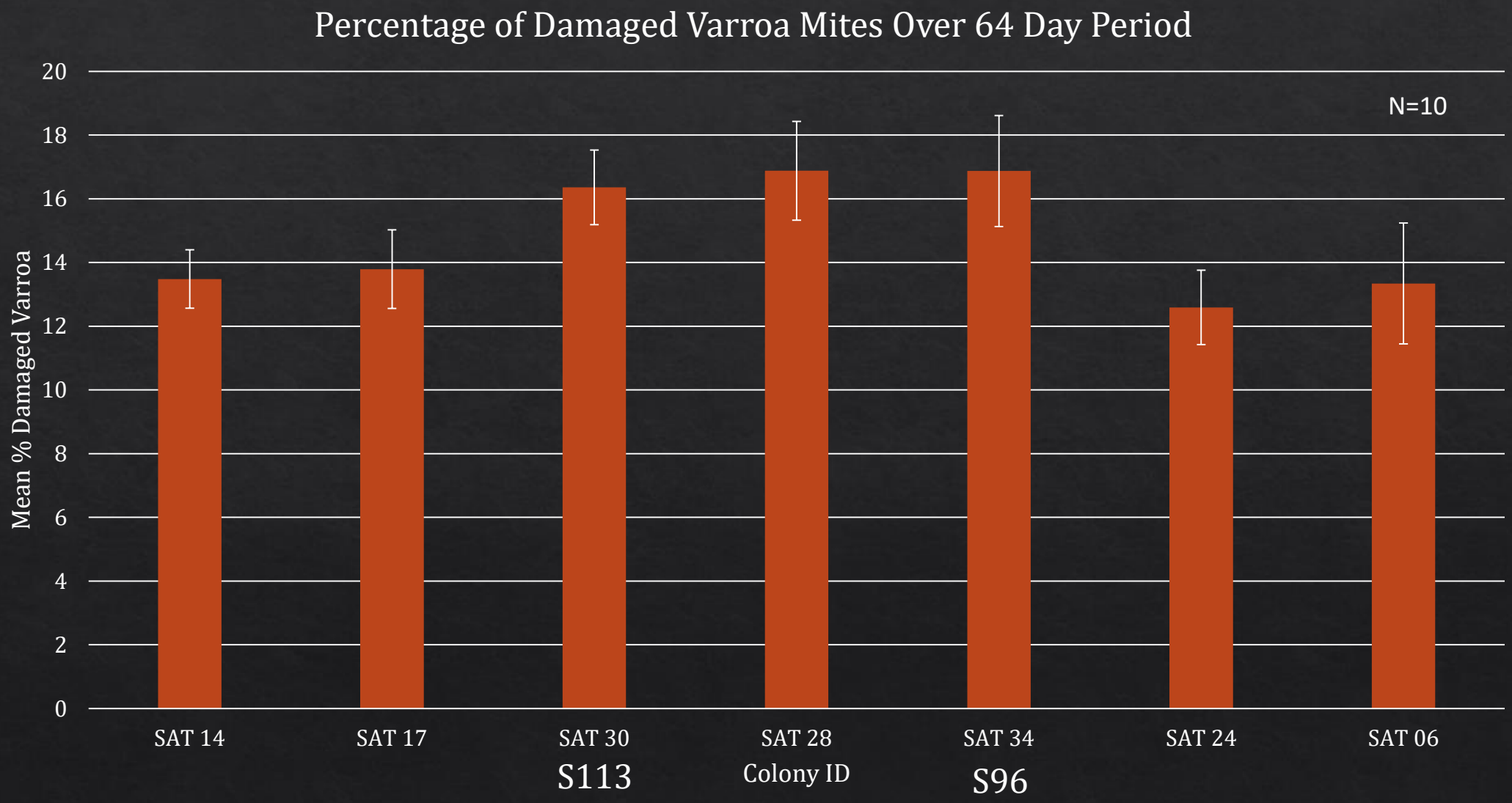
Selection for Honey Production



Progeny Analyses – S113



Mite Biting Analysis



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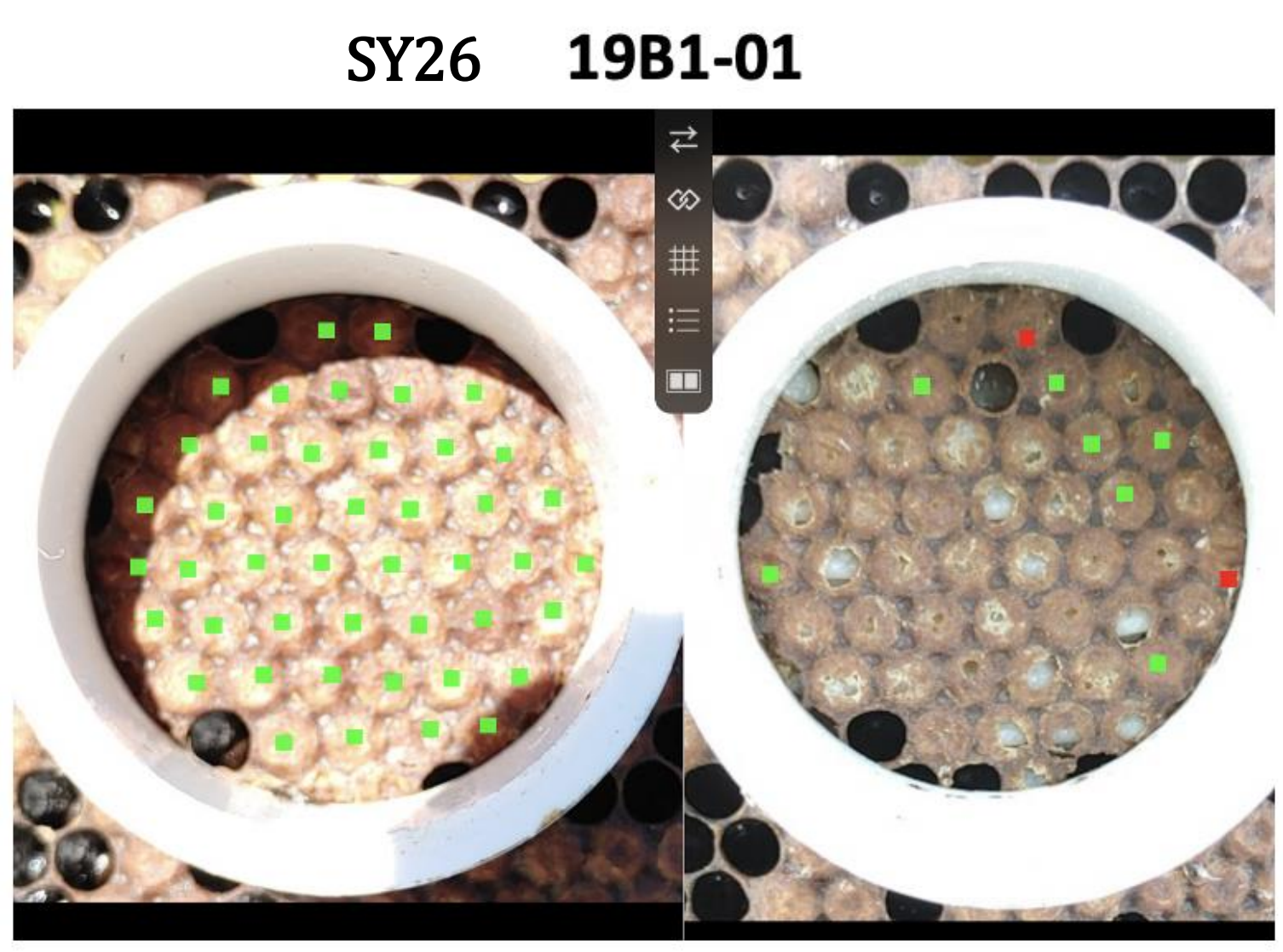
Saskatraz Progeny Analyses



VSH Analysis Using Unhealthy Brood Odor Assay

UBO Assay Developed by:
Kaira Wagoner at UNCG

UBO Assay Score:
84.4%

Project *Apis m.*

UBO Progeny Analysis – SY26 Daughters

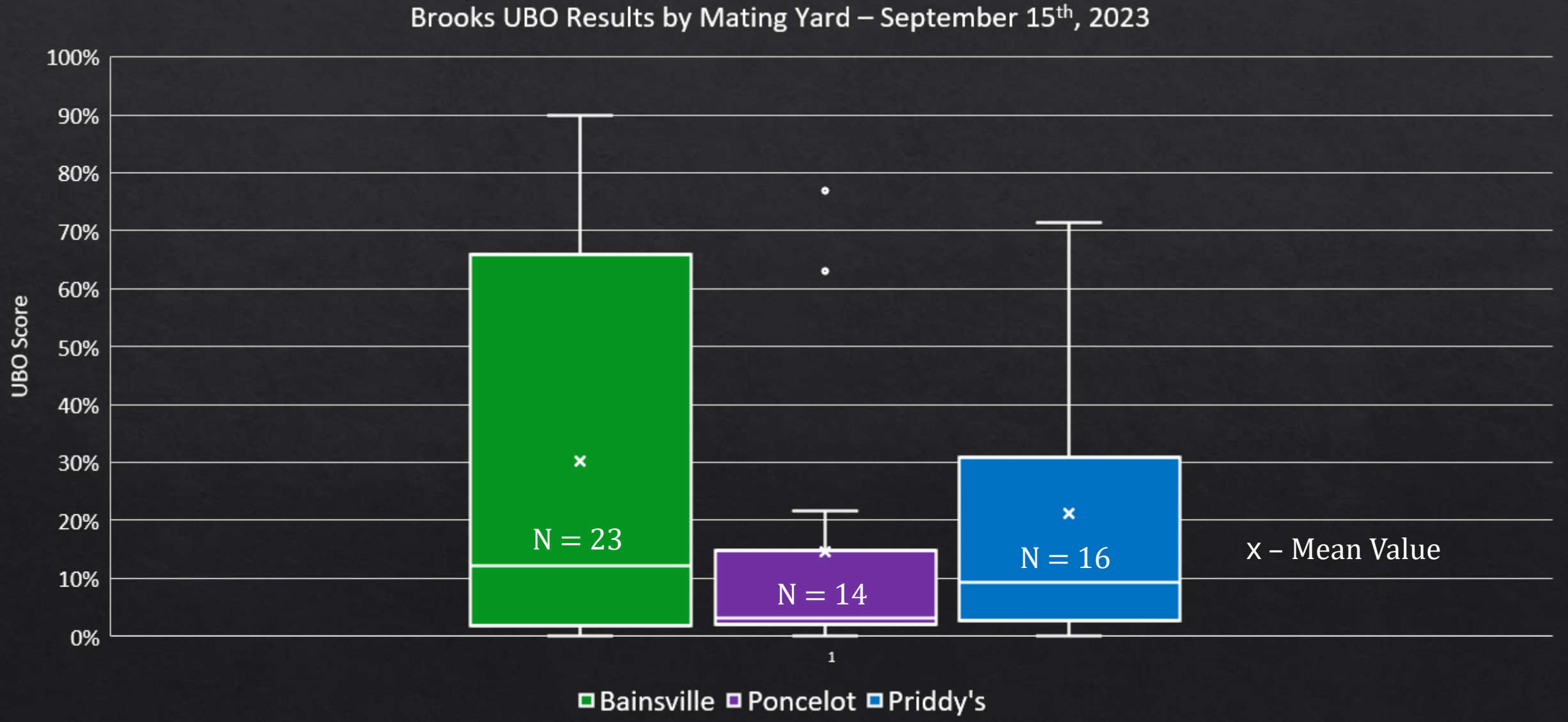
B8 (21B1-113)
Honey Production: 103%
UBO Assay Score: 55.6%

B16 (21B1-124)
Honey Production: 68%
UBO Assay Score: 79.5%

B14 (21B1-123)
Honey Production: 163%
UBO Assay Score: 64.3%

B17 (21B1-118)
Honey Production: 155%
UBO Assay Score: 7.0%

UBO Progeny Analysis – SY26 Daughters

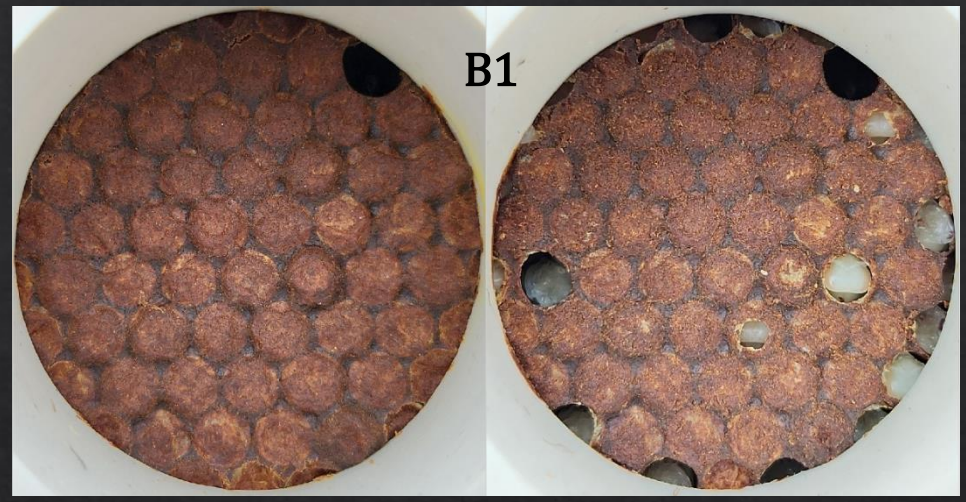


UBO Assays of Unselected Colonies

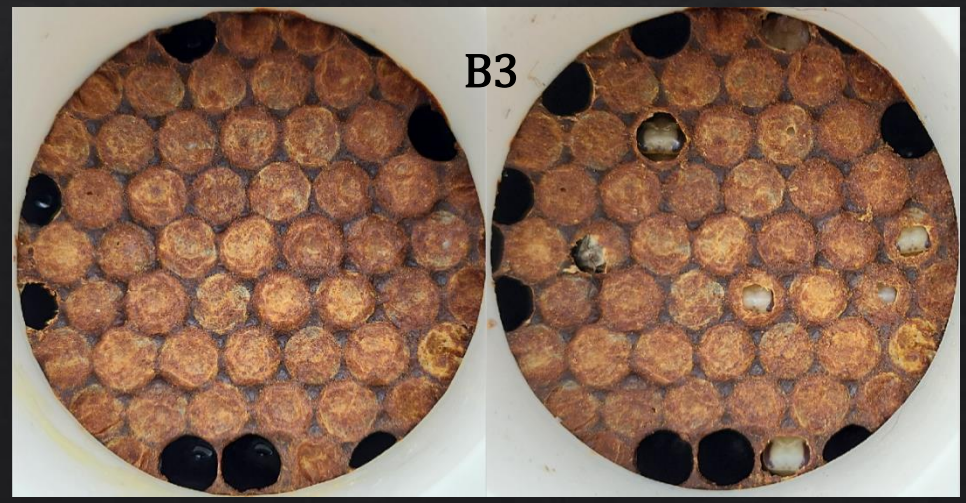
Australian Colonies

Before After 2 Hours

Caucasian Colonies



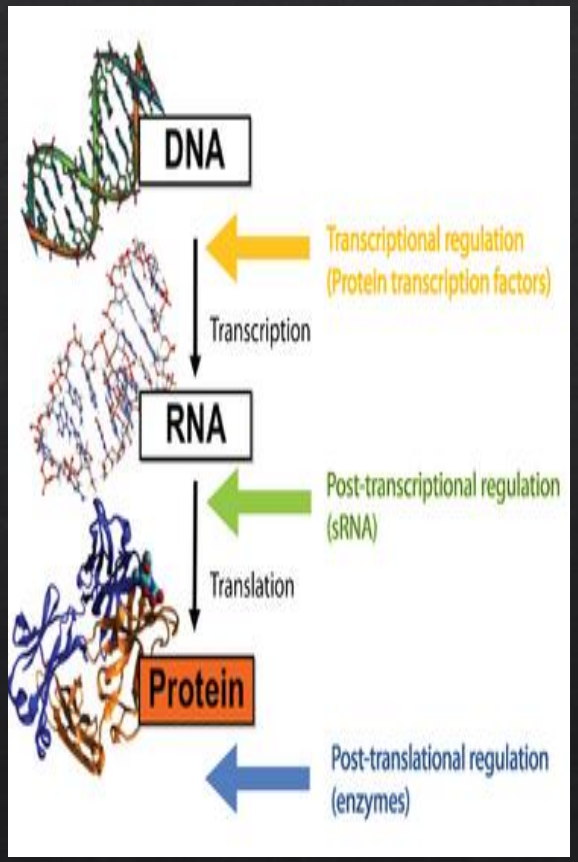
Before After 2 Hours



Biomarker Development

- Microsatellites (SNP Discovery)
- Microarrays + RNA seq (transcripts)
- Proteins
- Kinome Arrays (signal transduction)

(DNA) → (RNA) → (Protein) → (Signal Transduction)



You can find our Publications at www.saskatraz.com under the Research heading.

Differentially Expressed Transcripts in G4 and S88 In Varroa Infected and Uninfected Pupa

Category	Gene	S88-/G4-	S88+/G4+	Honey Bee Protein
Signal Transduction (Pupa)	GB17702-RA		2.40	Cadherin-87A-like
	DB777873		2.83	Neurobeachin-like
	GB14355-RA	4.45	2.69	Anosmin-1-like
Lipids (Pupa)	GB11723-RA		6.88	**Apolipoprotein D-like isoform 2**
	GB18070-RA		2.23	Acyl-CoA Delta(11) desaturase-like
	GB13246-RA		0.47	Phospholipase A1 member A-like isoform 1
	GB16889		3.41	**Esterase E4-like**
Cytochrome P450 (Pupa)	GB11754-RA		0.31	Cytochrome P450 6a14 isoform 1
	GB12136-RA		4.08	**Cytochrome P450 6A1**
Immune (Pupa)	GB13473-RA		2.07	Apidaecins type 73

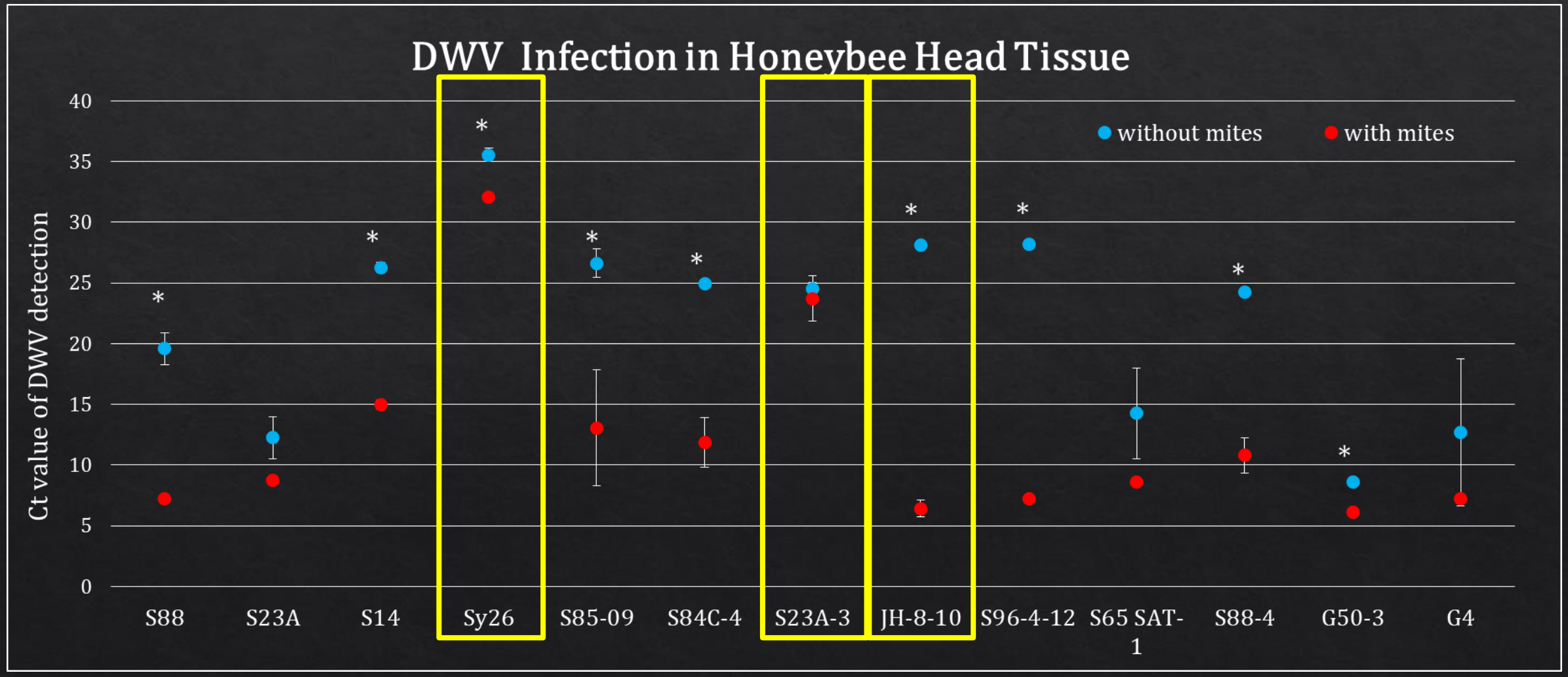
Survivor Colonies

Why do some colonies survive for extended periods in natural selection apiaries?

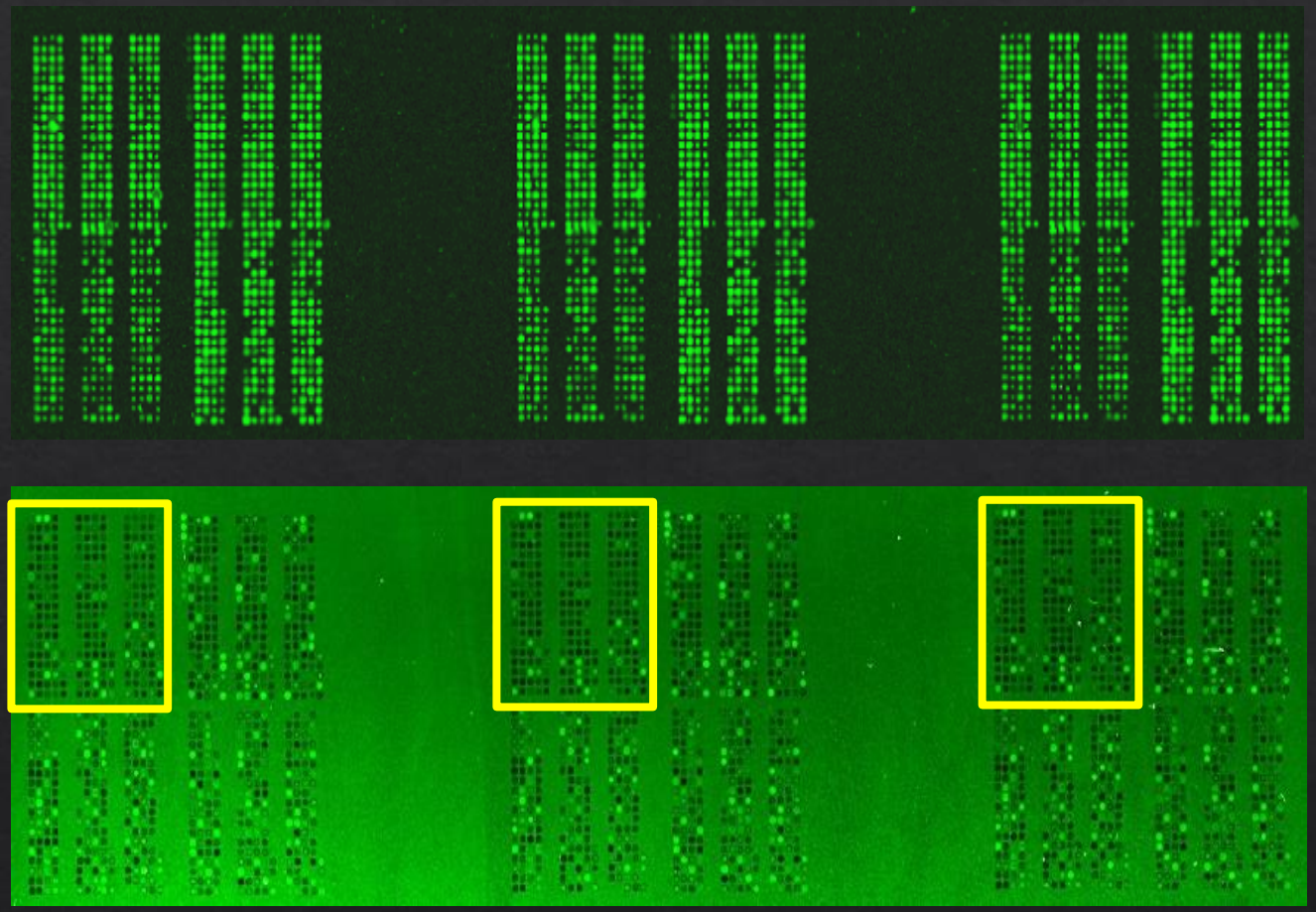
- S88 for 58 Months
- SY26 for 70 Months

- Grooming Behaviour (Mite Biters)
- Varroa Sensitive Hygiene (VSH)/Hygienic Activity
- Supersedure and Re-queening Success
- Stress Resistance – Express higher levels of detoxification factors for pesticide, miticides and environmental stressors - **Apolipoprotein D, Esterase E4, Cytochrome P450**
- Consistent Vitellogenin Transcript Levels
- Better Foraging Activity = Better Nutrition
- Virus Immunity (Innate Immunity)

DWV Analysis of Saskatraz Phenotypes with and without Mites



Kinome Analysis of Colony Phenotypes

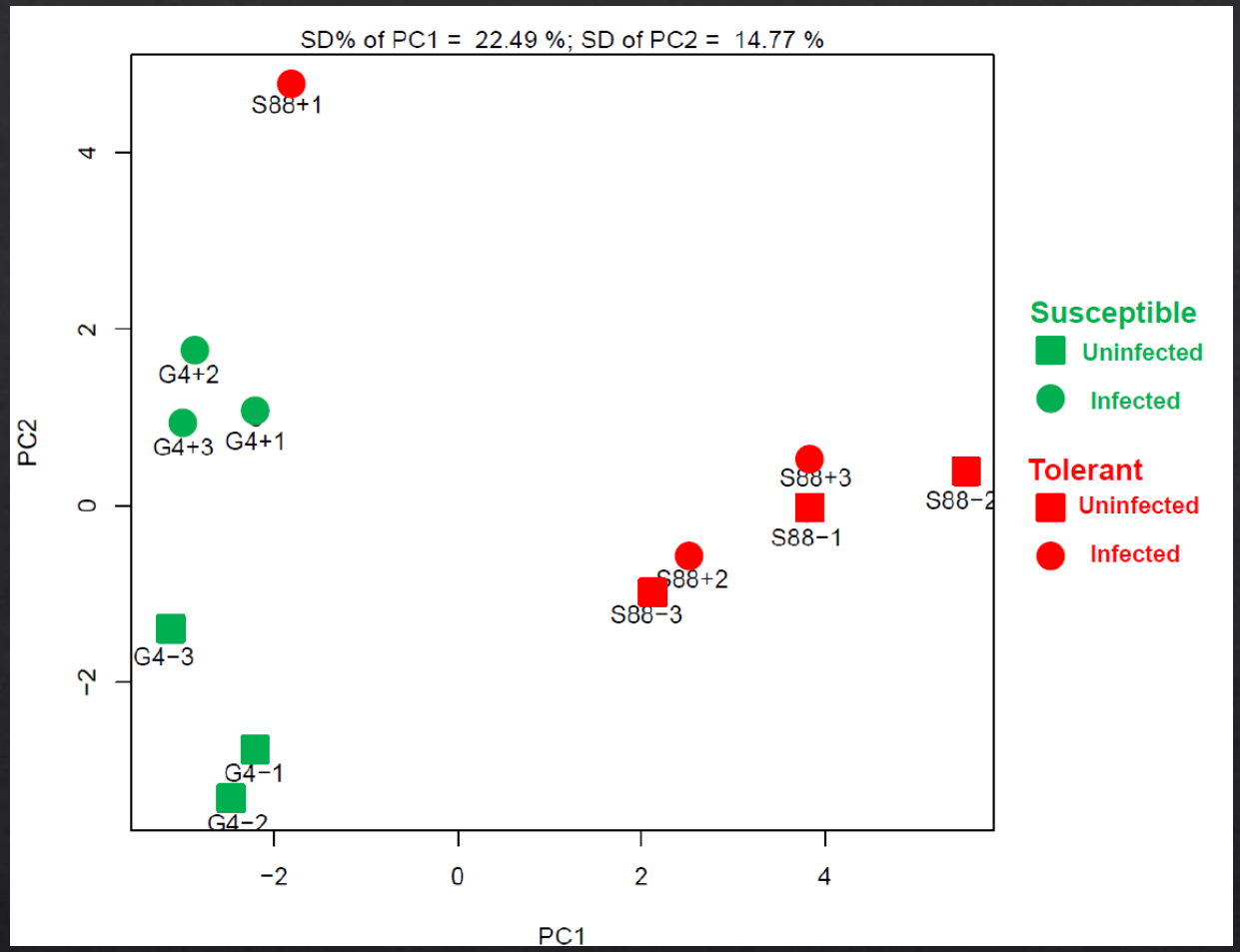


http://www.greendiary.com/hawaii-bees-infested-by-destructive-varroa-mites.html

Printing and Validation of the Bee Specific Peptide Array. A) The arrays were printed by a commercial partner (JPT Technologies). For each array each spot is printed in triplicate within each block. Each block is then printed in triplicate for nine technical repeats of each peptide. This image, taken as a quality control step in array production, illustrates the consistency and reproducibility to peptide spotting. B) An image of a data scan of a representative array that had been used for analysis of a whole bee sample. All of the arrays of this work were of comparable quality with respect to the clarity and consistency of peptide phosphorylation. A clear and consistent pattern of extents of peptide phosphorylation is apparent across the three printed blocks.

	Protein	ID	Sequence	P
Innate Immunity	TAK1 kinase	O43318	YMTNNKGSAAWMAPE	0.001
	TAK1 kinase	O43318	CDLNTYMTNNKGSAA	0.003
	Mitogen-activated protein kinase kinase kinase_5	O35099	TETFTGTLQYMAPE	0.009
	Nuclear factor NF-kappa-B p110 subunit Rel-p110	Q94527	YIQLKRPSDGATSEP	0.005
	Transcription_factor p65 Nuclear factor NF-kappa-B	Q04206	IQLKRPSDGALSEP	0.005
	Focal adhesion kinase 1 FADK1	Q05397	IVDEEGDYSTPATRD	0.005
	AP-1 complex subunit beta-1	O35643	VEGQDMLYQSLKLTN	0.008
Metabolism	ATP synthase_subunit_beta	P06576	TSKVALVYGQMNEPP	0.004
	Na-K transporting ATPase subunit alpha1	P05023	ICKTRRNSLFRQGM	0.009
	Glucose-6-phosphate isomerase	P06744	GPRVHFVSNIDGTHI	0.005
	Isocitrate_dehydrogenase subunit_beta,	O43837	TKDLGGQSSTTEF	0.006
Stress Responses	Ribosomal protein S6 kinase alpha	P51812	DSEFTCKTPKDSPGV	0.006
	Elongation factor 2 (EF-2)	P13639	KVMKFSVSPVVRVAV	0.007
	60_kDa_heat_shock_protein	P10809	ILEQSWGSPKITKDG	0.016
	Superoxide dismutase	P07895	SIFWCNLSPNGG	0.008
Other	Ephrin type-A receptor 4 EPH-like kinase 8 (EK8)	P54764	SYVDPHTYEDPNQAV	0.006
	PRKC_apoptosis_WT1 regulator_protein__	Q62627	LREKRRSTGVVHLPS	0.006
	A-Raf Kinase	P10398	QTAQGMDYLHAKNII	0.010
	Intestinal cell kinase (ICK)	Q9UPZ9	CKIRSRPPYTDYVSTRW	0.010

Biomarker Peptides: Differently Phosphorylated Peptides Between Pupae Collected from Varroa Susceptible and Tolerant Colonies.



Clustering of Kinome Data. Kinome datasets were subjected to hierarchical clustering and PCA analysis. Pupae from two colonies (G4 and S88) were selected for either the presence (+) or absence (-) of Varroa mites. Principle Component Analysis: Separation of the samples on the basis of phenotype is clearly observed with further distinction with the susceptible, but not tolerant, samples on the basis of infection status.

Saskatraz Hybrid Project

Objectives

- To commercialize and distribute Saskatraz Breeding Stock to commercial beekeepers.
- Every year colonies are selected for honey production, overwintering ability, temperament, mite resistance and brood diseases.
- This project serves to provide Saskatraz hybrid queens for reasonable prices and results in increasing the frequency of alleles associated with economic traits in commercial populations.

- Saskatraz stock distribution
  - North America
  - Iran
  - Middle East (UAE, Saudi Arabia, etc.)
  - Afghanistan
  - Ukraine
  - Turkey
  - South Korea
  - Virgin Islands, USA
- In progress
  - Australia
  - Hawaii, USA
  - Chile
  - Russia
  - Poland

Location

Saskatoon, SK

Temperature Range:
-40°C to + 40°C
Mating Season:
~3 Months

Temperature Range:
-1.0°C to + 41°C
Mating Season:
~5-6 Months

Orland, Ca USA

Saskatraz Queen Production

Saskatraz queen production in Saskatchewan is focused on production of Saskatraz breeder queens by recurrent selection and closed population mating procedures.

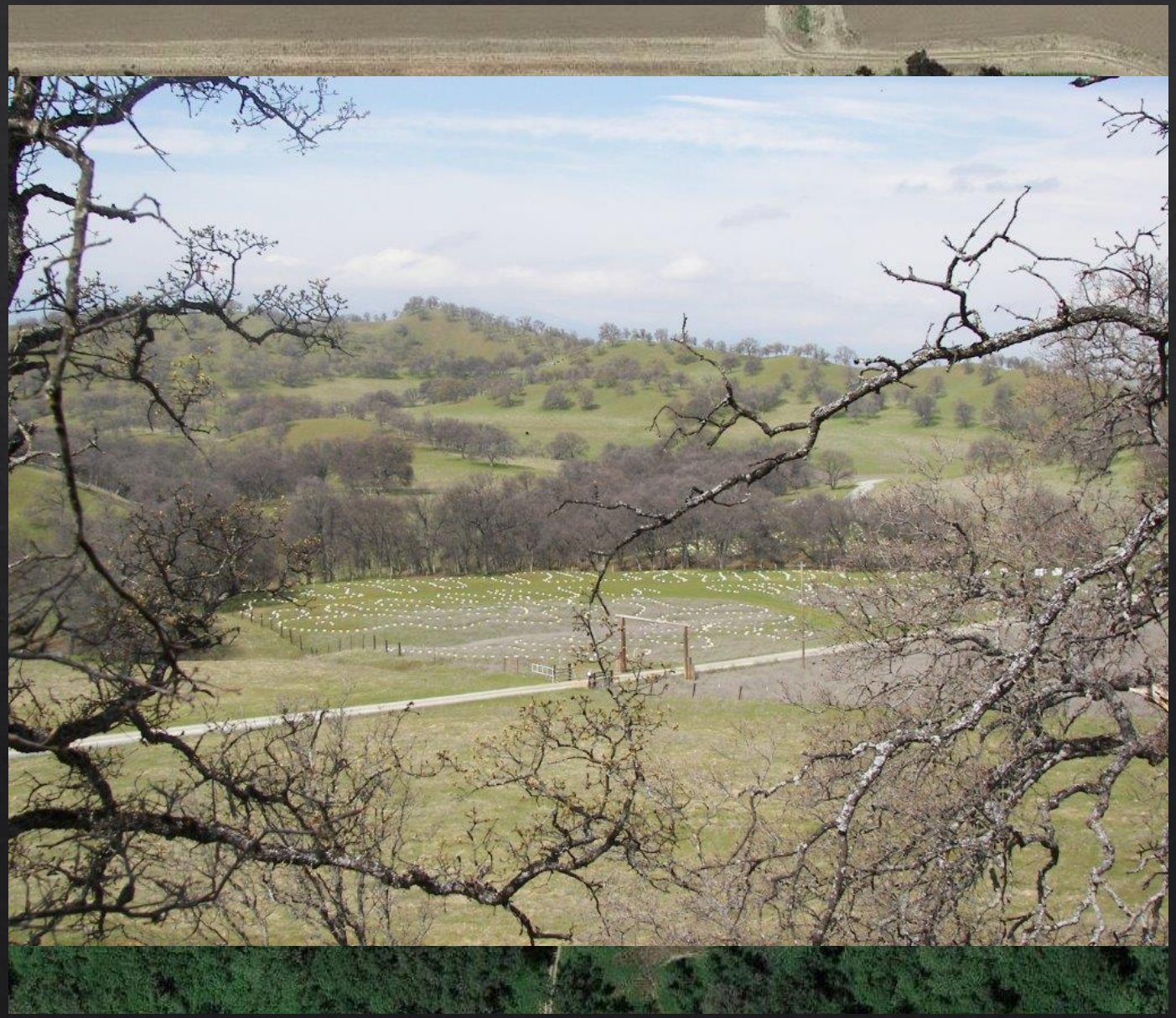
- Short queen production season
- Good location for selection criteria
- Can produce around 2000 queens/season
- Send about 150-200 breeders to be re-selected in California

Saskatraz hybrid queen production in Northern California (Orland) at OHB is focused on large scale commercial production.

- 40-60 Saskatraz breeders used after re-selection
- Ideal area and climate for large scale operations
- High populations of mature drones
- Produce several hundred thousand queens/season

California

Mating Locations



Saskatchewan

Mating Locations

Priddy's

Bainsville

Kokay's

Saskatraz Breeding Program

Behaviour Assays – Orland, CA:

1. Temperament (1 sting, 2 sting, 3 sting)
2. *Behaviour on comb (dancing, calmness, etc.)
3. Low temperature flight
4. *+Queen retinue + mating
5. +Swarming tendency and superceding success
6. +Pollen storage and propolis production
7. +Brood pattern
8. *+Worker uniformity
9. +Queen colour and markings
10. +Varroa Assays

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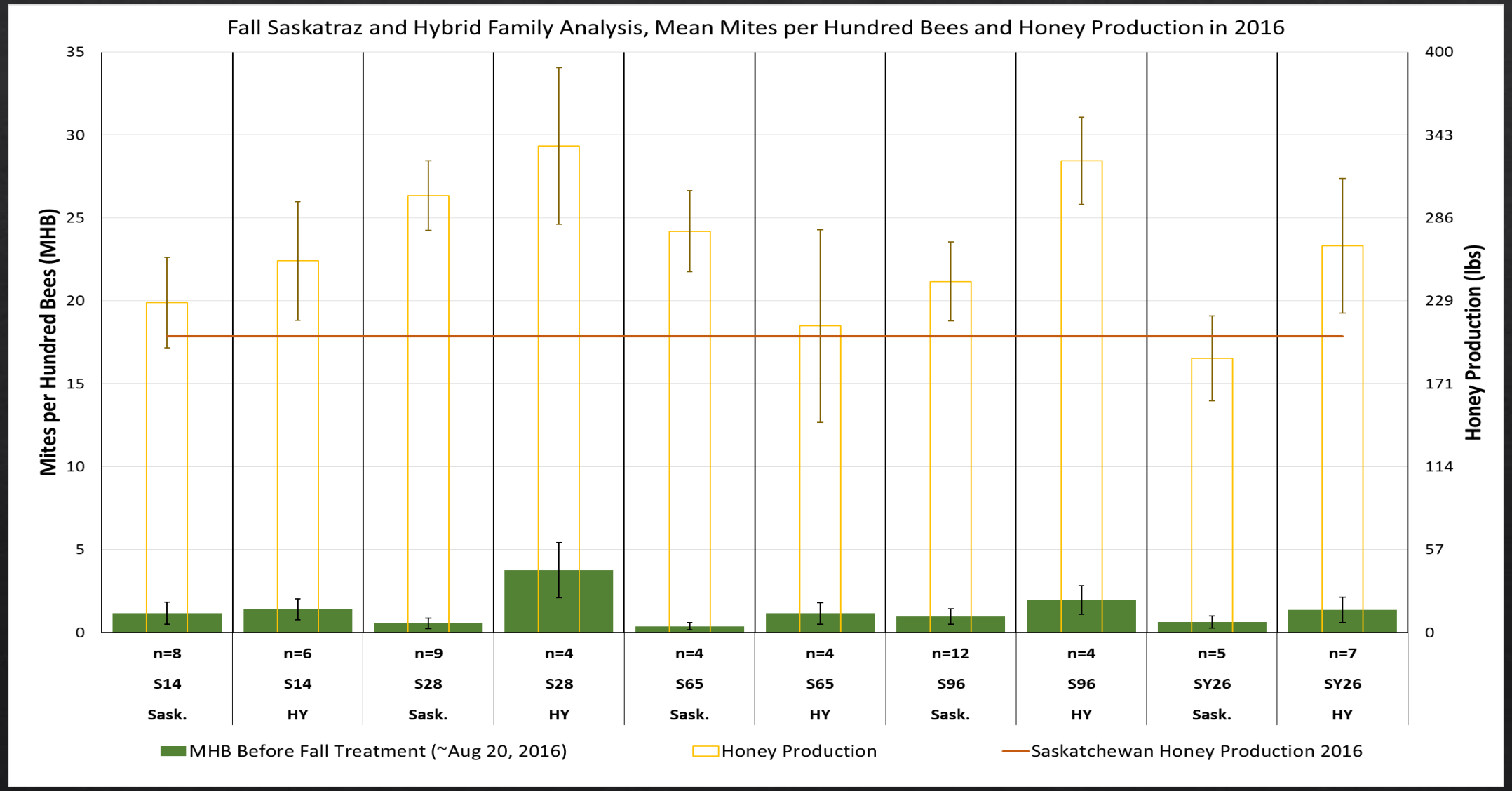
Varroa Assay


SL25 x GNS -18
The Saskatraz Project


S96 RCD~15 x BV ~18
The Saskatraz Project

Saskatraz Hybrid Performance



Summary and Work in Progress

We can select Saskatraz families with good honey production, wintering and Varroa resistance, but is difficult to balance the phenotypes. Varroa resistance is variable in the progeny because of the nature of bee genetics.

Our focus is aimed at stabilizing Varroa resistance using extensive progeny analysis with marker assisted selection and the UBO assay to speed up the selection process.

Also looking at the variability in virus susceptibility in our strains in collaboration with USDA Baton Rouge, LA.

Saskatraz Review
Presentation
Bit.ly/Saskatraz

Questions?

Acknowledgements

- Meadow Ridge Enterprises Ltd., Saskatchewan Beekeepers, and BeeMaid Honey.
- Saskatchewan Agricultural Development Fund (2009-2014); Agriculture Council of Saskatchewan (MB, AB, BC and Yukon)(2009-2014); Project Apis M (2021-2022); Project Apis M (2023-2024).
- USDA Bee Lab, Baton Rouge, LA (Dr. Michael Simone-Finstrom, Dr. Arian Avalos, Dr. Bob Danka)
- VIDO (Dr. Philip Griebel, Dr. Scott Napper and Wayne Connor).
- University of Saskatchewan Food and Bio Product Sciences (Dr. Xiao Qiu, Sanjie Jiang and Jin Wang).
- Toxicology Center, University of Saskatchewan (Dr. John Giesy, Dr. Garry Codling, Yahya Nagar).
- GenServe Labs (Bruce Mann, Dr. Yves Plante, and Dr. Steven Creighton, SRC).
- Mohammad Mostajeran (R. A. 2008-2013) and Dr. Syed Qasim Shah (2010- 2012).
- Dr. Abdullah Ibrahim (Research Associate, Summer 2007).
- John and Eric Pedersen – breeder stock multiplication and selection (2006).
- Meadow Ridge Staff; Tom, Jenny, and Cecilia Robertson, Neil Morrison, Rob Peace, Yang Tan, Colton Rutherford, Héloise Garez, Antonio and Edmundo Munoz Cerna
- Collaborators: John Gruszka (Prince Albert, Sask) Dr. Solignac (Paris, France), Dr. Ralph Buchler (Germany), Dr. Rob Currie (U of M), S. Cobey (Davis, CA), Geoff Wilson (Prince Albert, Sask).

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Saskatraz Team Members

Mohammad Mostajeran

Dr. Abdullah Ibrahim

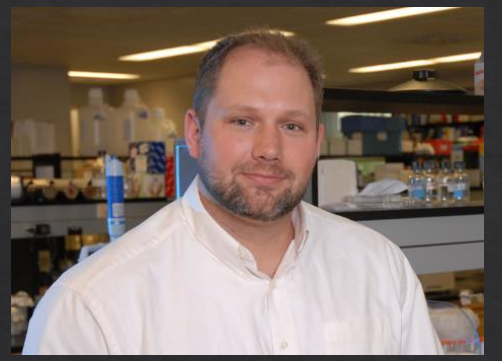
Dr. Syed Shaw

Neil Morrison Eric and John Pederson Tom Robertson

Antonio Munoz Cerna

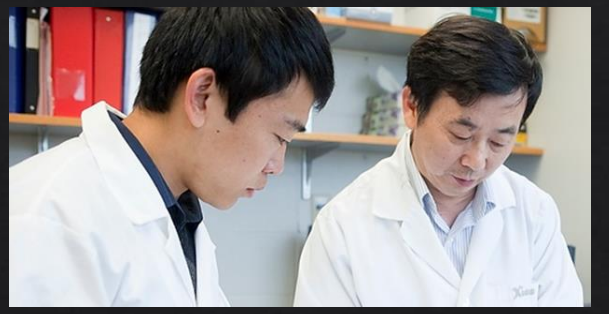
Edmundo Munoz Cerna

Colton Rutherford



Dr. Scott Napper

Dr. Philip Griebel Wayne Connor



Sanjie Jiang and Dr. Xiao Qiu