Apimondia Extended – September 7-12th, 2019

# The Saskatraz Project

Queens Produce Superior Workers

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

- Varroa
- Viruses
- Miticides
- Pesticides
- Nutrition

# Outline

- Review of Saskatraz Breeding Program
- Saskatraz Hybrid Projects
- Selection and Molecular Analysis of Extreme and Intermediate Phenotypes for Varroa Resistance and Susceptibility - Biomarker Development (Micro and Kinome Arrays) and Virus Screening
- Please find Published Papers and More Information on the Saskatraz Project at www.saskatraz.com

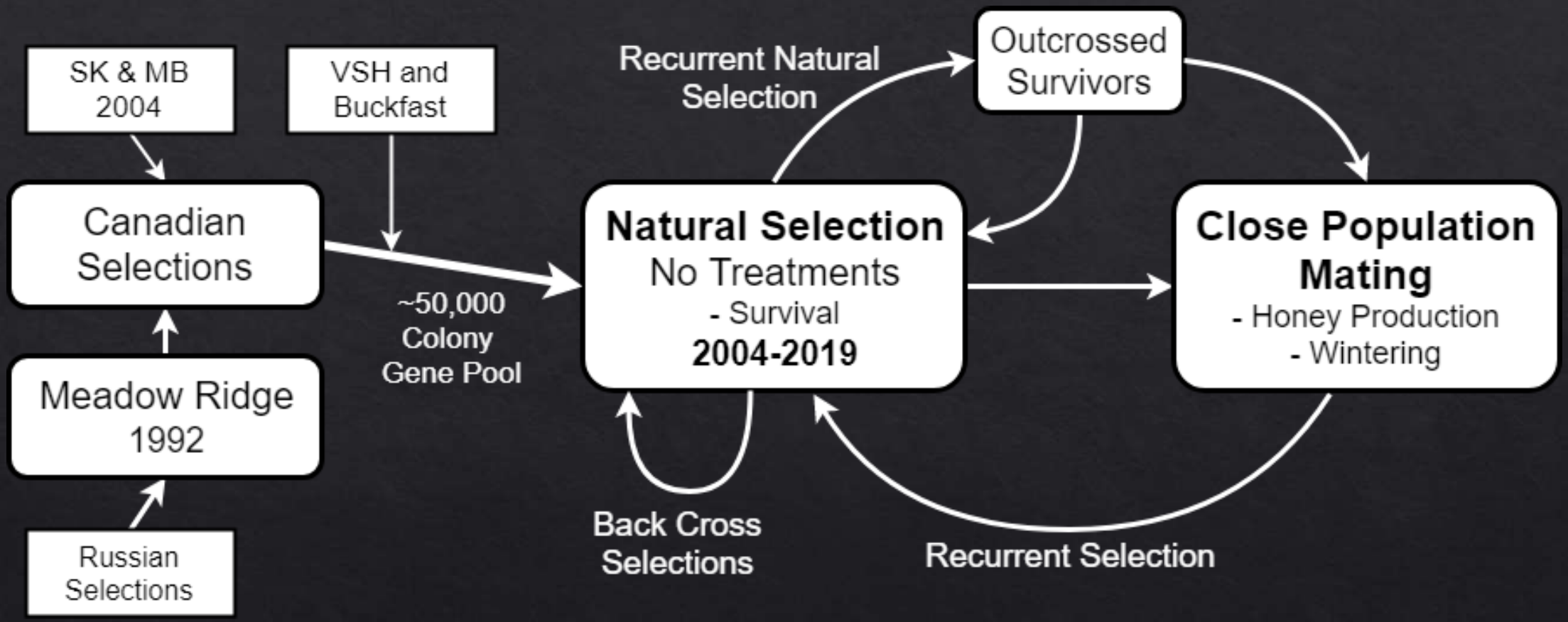
# Saskatraz Breeding Program

## Primary Selection Criteria:

1. Honey Production
2. Wintering Ability
3. Mite Resistance and Suppression
4. Resistance to Brood Diseases
   (Chalk Brood, AFB, EFB, 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).

# Saskatraz Breeding Program Logistics




There are currently:

**17 Saskatraz Families**

See www.saskatraz.com for review

# The Original Saskatraz Apiary

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

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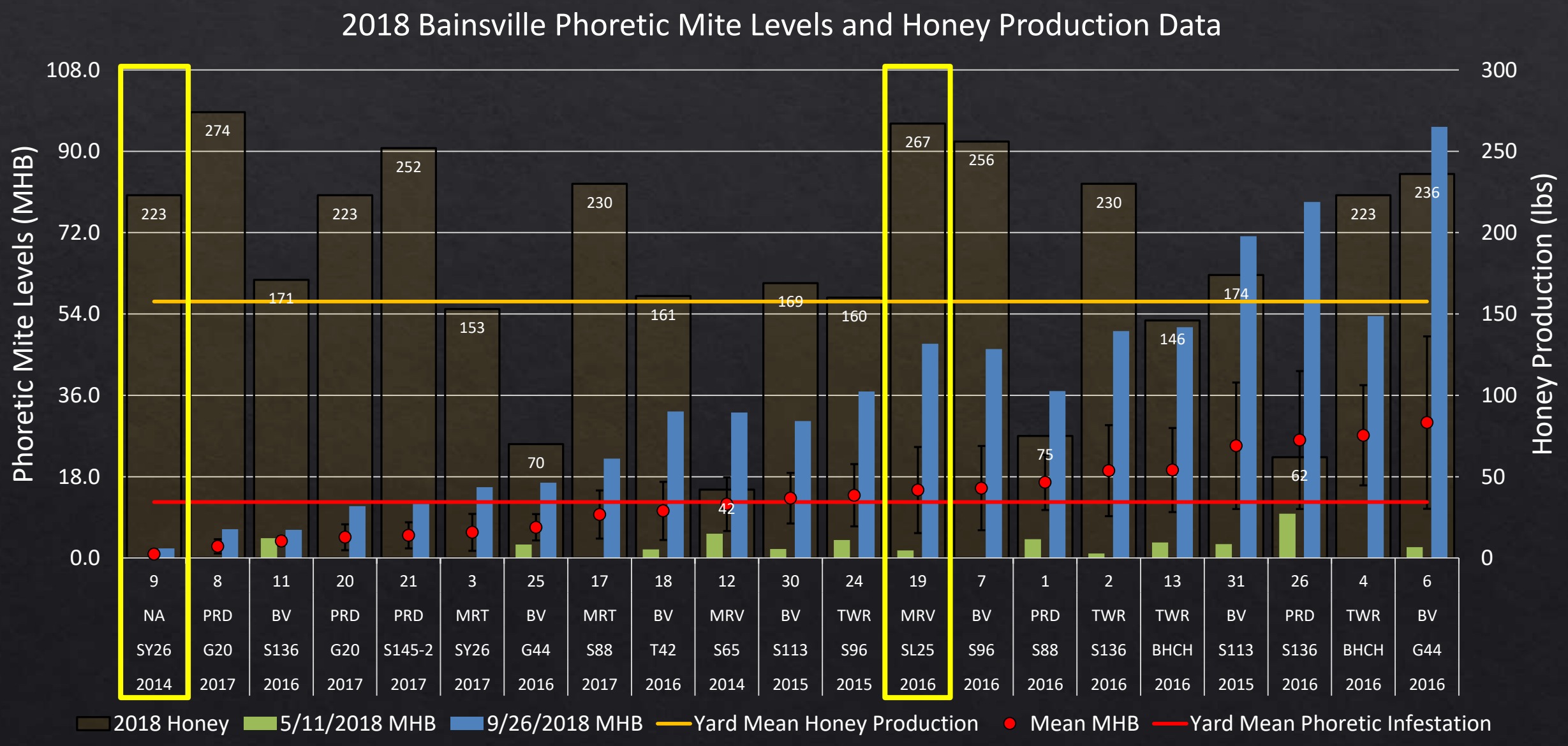
# Natural Selection for Varroa Tolerance



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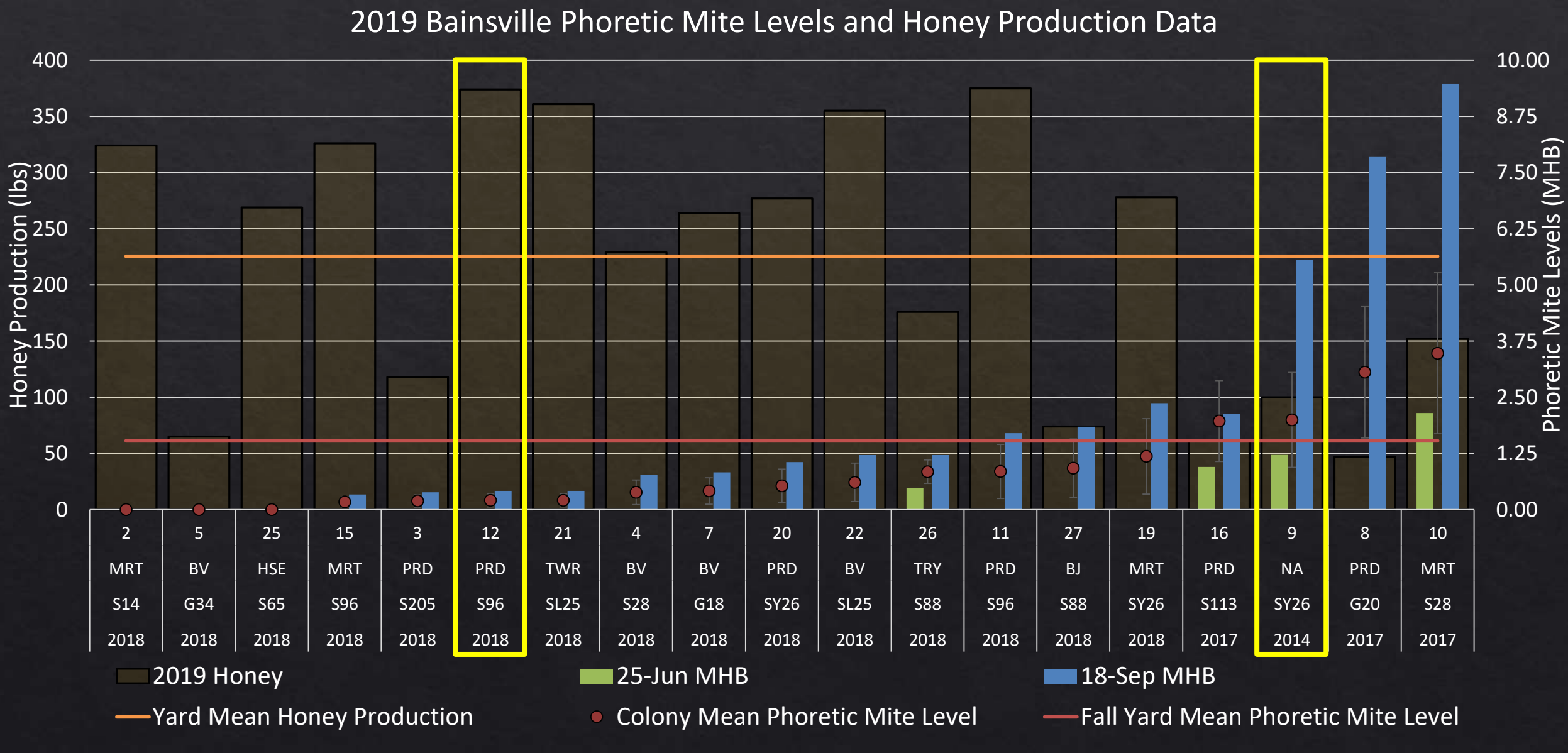
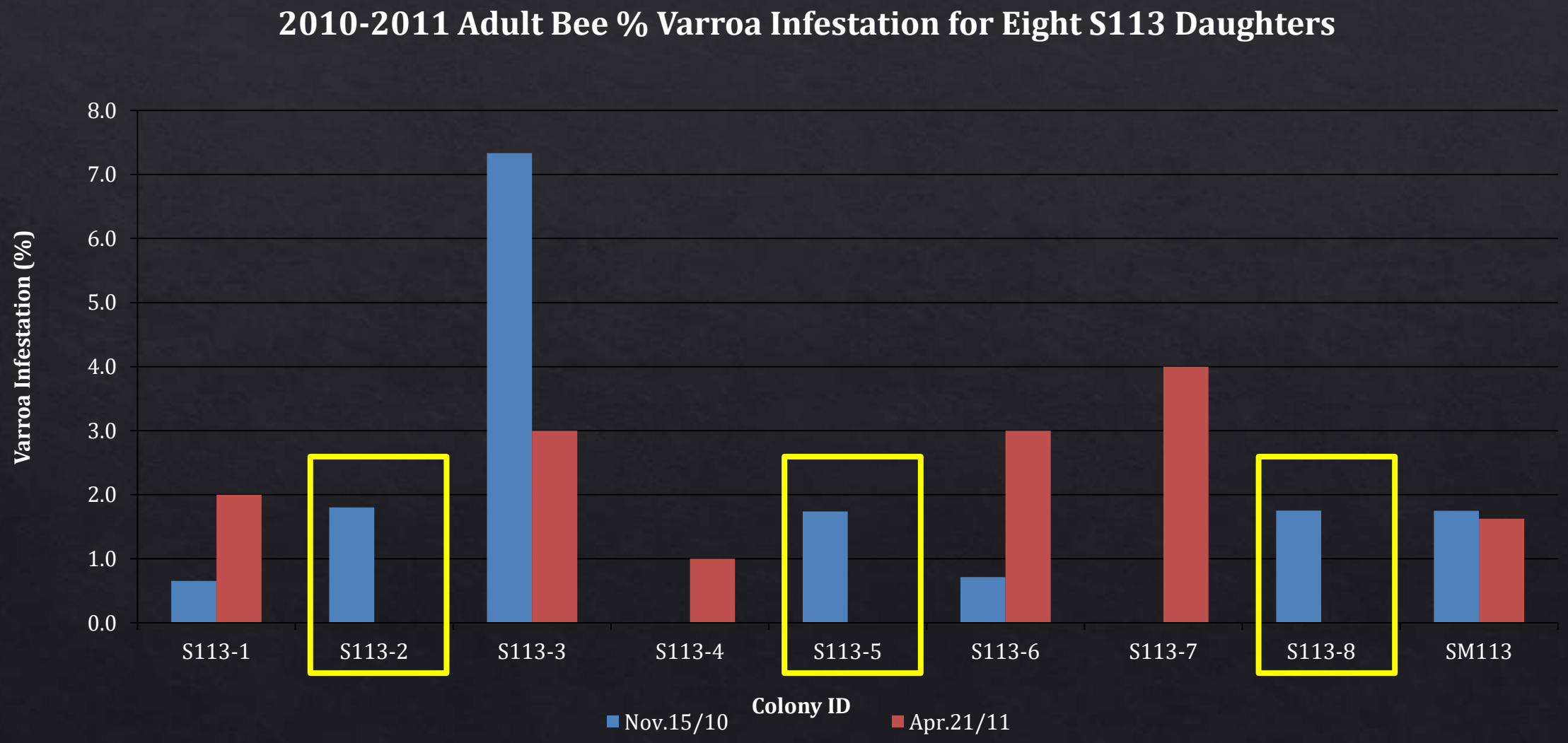
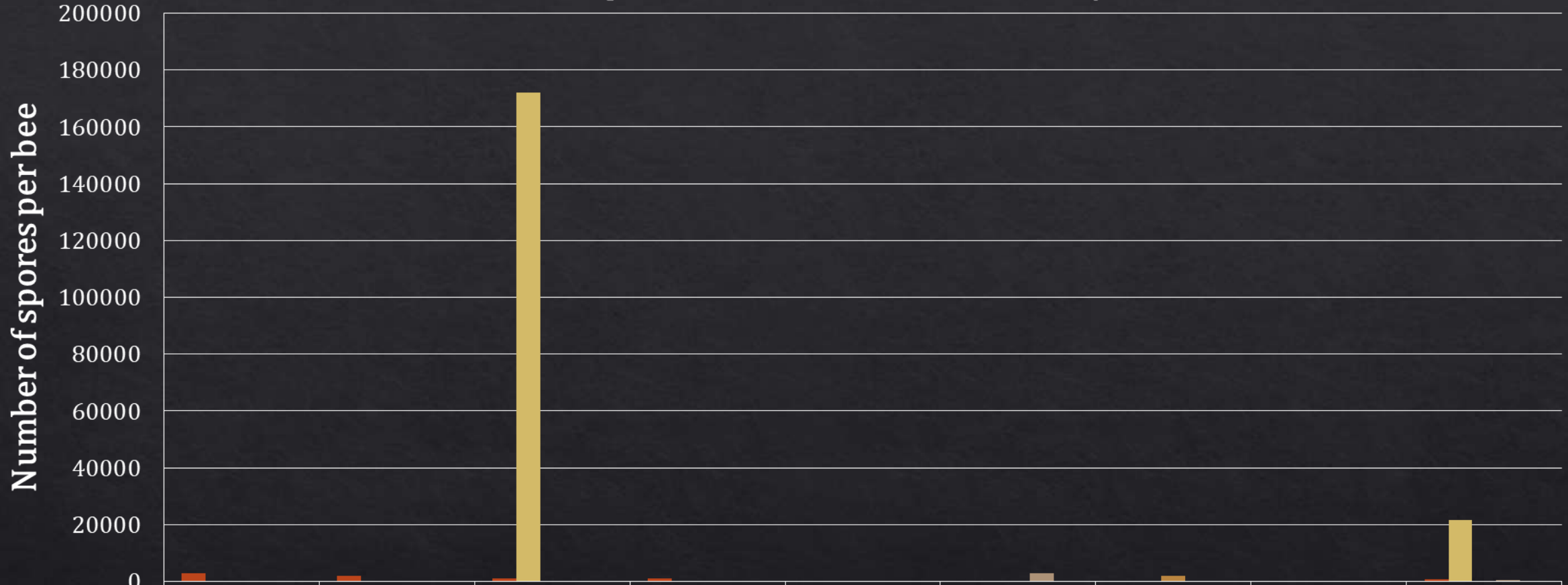
# Natural Selection for Varroa Tolerance



# Progeny Analyses – S113



## Nosema Spore counts in live bees from S88 family




| | S88-1 | S88-2 | S88-3 | S88-4 | S88-5 | S88-6 | S88-7 | S88-8 | Mean |
|---|---|---|---|---|---|---|---|---|---|
| Dec28-10 | 3000 | 2000 | 1000 | 1000 | 0 | 0 | 0 | 0 | 875 |
| Jan-13-11 | 0 | 0 | 172000 | 0 | 0 | 0 | 0 | 0 | 21500 |
| Jan-28-11 | 0 | 0 | 0 | 0 | 0 | 0 | 2000 | 0 | 250 |
| Feb-13-11 | 0 | 0 | 0 | 0 | 0 | 3000 | 0 | 0 | 375 |
| Feb-28-11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

# Saskatraz Hybrid Projects

## 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

# Saskatraz – Orland, USA

In 2019 we sent 145 pre-selected Saskatraz breeder queens to be reselected in March 2020.

The California Tech Transfer Team, Bee informed Partnership has independently evaluated our Saskatraz breeding stock in late February early March in past years. An example is shown below.

| Colony Number | Colony ID | Brood Pattern | Chalk-brood Presence (+/-) | Tempera-ment | Pollen placement | Queen Presence (+/-) | Queen Mark Presence (+/-) | Phoretic Mite Infestation (MHB) | %Mite Infestation in Worker Brood | %Mite Infestation in Drone Brood | Tech Team Hygienic Behaviour Test | Observation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **7** | S65 Robin 14 | Excellent | - | 1 | Average | + | + | 0 | 0 | 0 | 93%O / 80%R | Green mark on queen |
| **24** | Y26 x 26 Martin 14 | Good | - | 1 | Average | + | - | 0 | 0 | - | 99%O / 99%R | No drone brood; no visible mark on queen |
| **25** | Y26 x 26 Martin 14 | Excellent | - | 1 | Average | + | + | 0 | 0 | 0 | 100%O / 100%R | - |
| **37** | G44 JHN 12-9 B.V. 14 | Excellent | - | 1 | Average | + | + | 0 | 0 | - | 93%O / 75%R | No Drone |

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Y26x26 Martins (Hygienic Behavior; 100%U+100%R)

Queen

Retinue

#47-S96 CHR 14

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S96 RCD~15 x BV ~18
The Saskatraz Project

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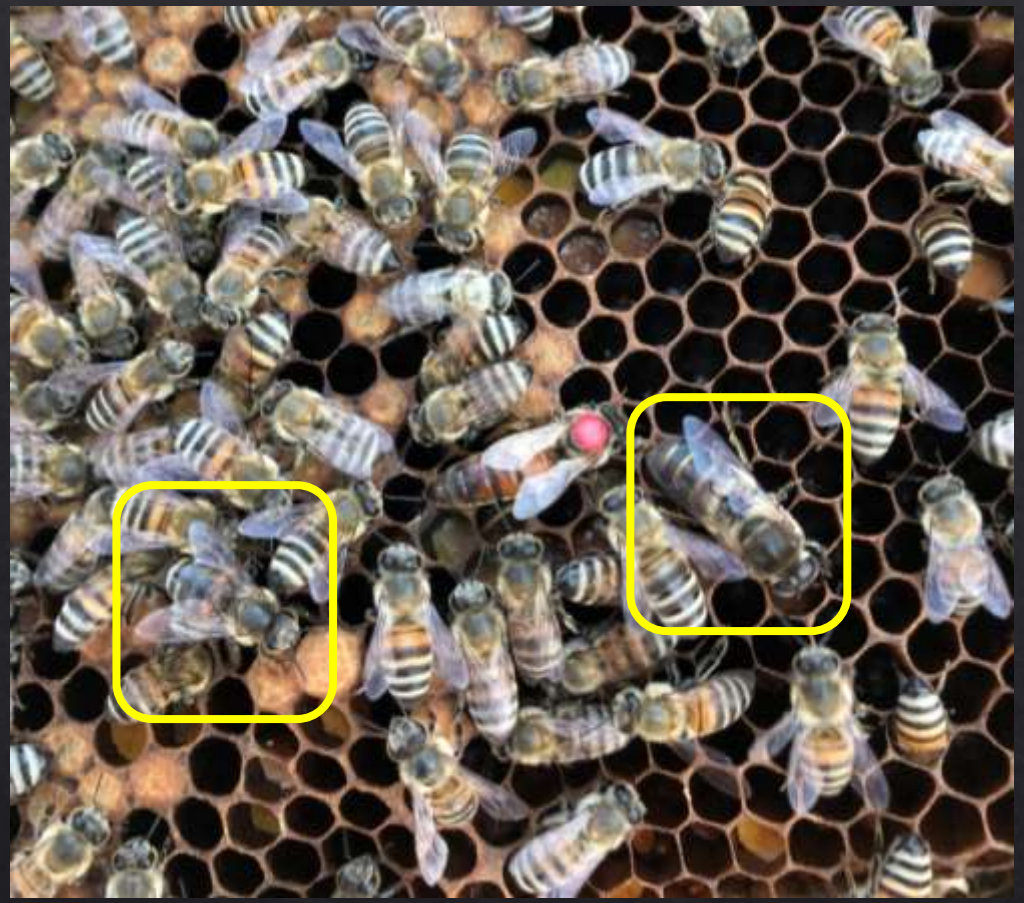
# Abu Dhabi, UAE ApiArab Expo Feb 2018

# Saskatraz – Hatta UAE

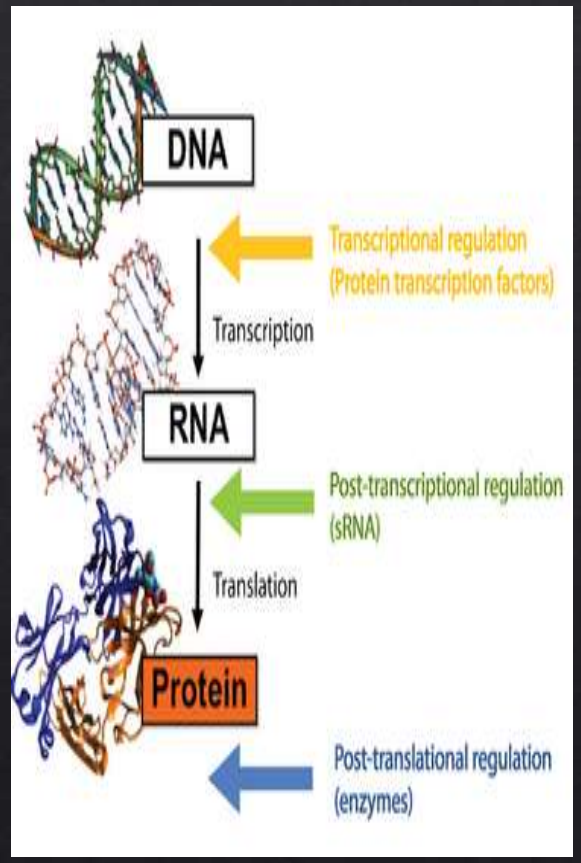


# *A. Mellifera Yemenitica*

# Biomarker Development

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

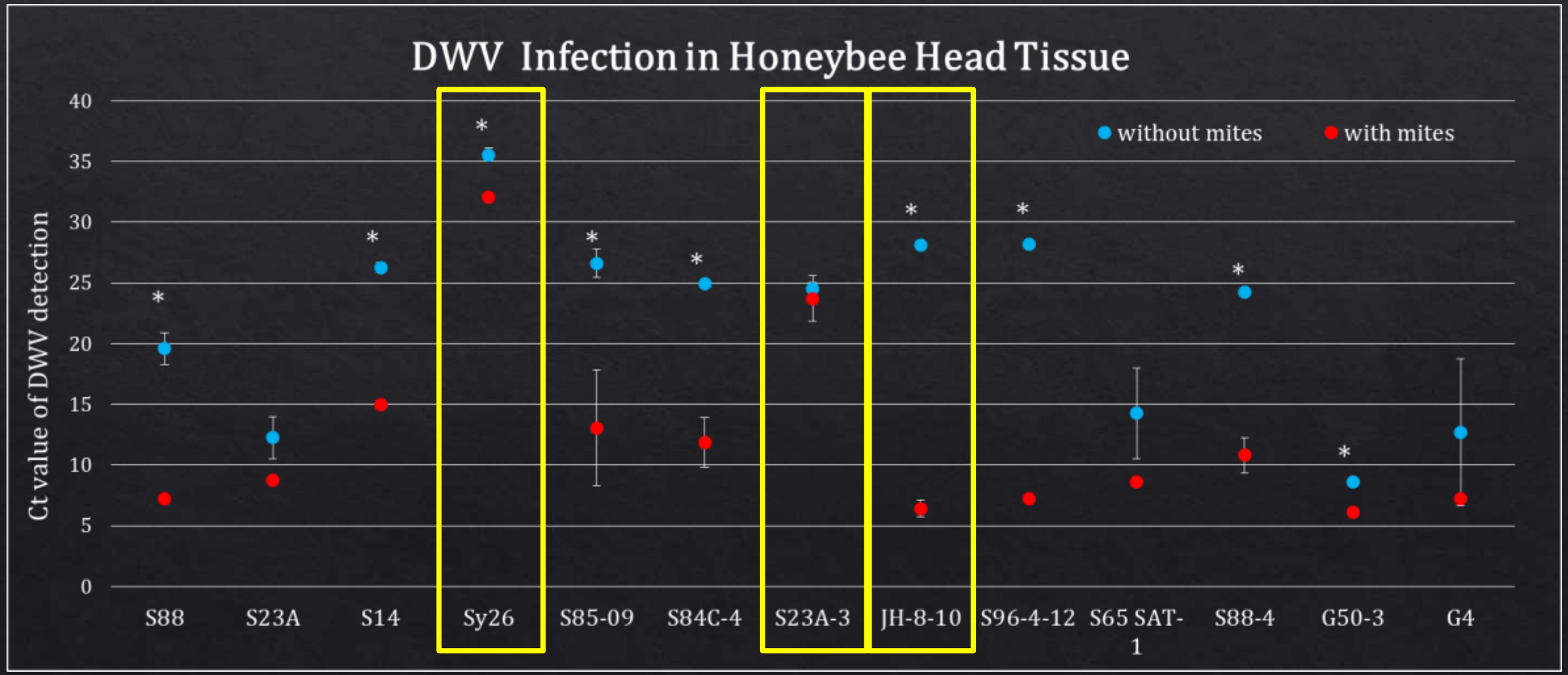
(DNA) ⟶ (RNA) ⟶ (Protein) ⟶ (Signal Transduction)



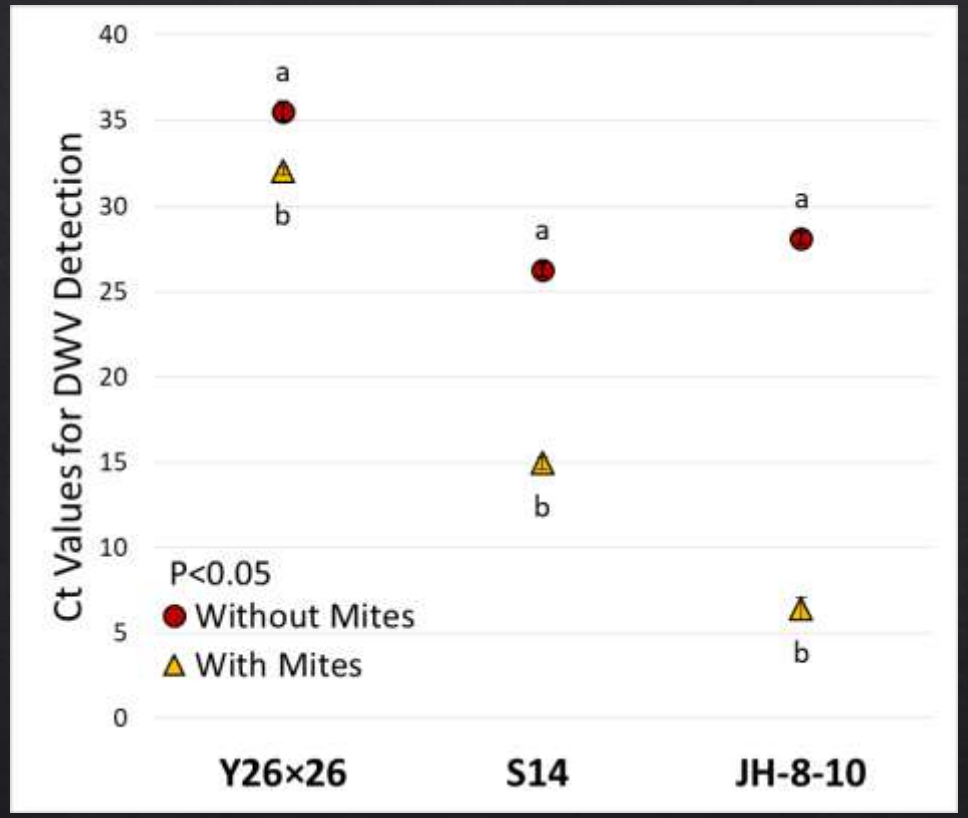
# Differentially Expressed Transcripts in G4 and S88 In Varroa Infected and Uninfected Pupa

| 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 |

# DWV Analysis of Saskatraz Phenotypes with and without Mites

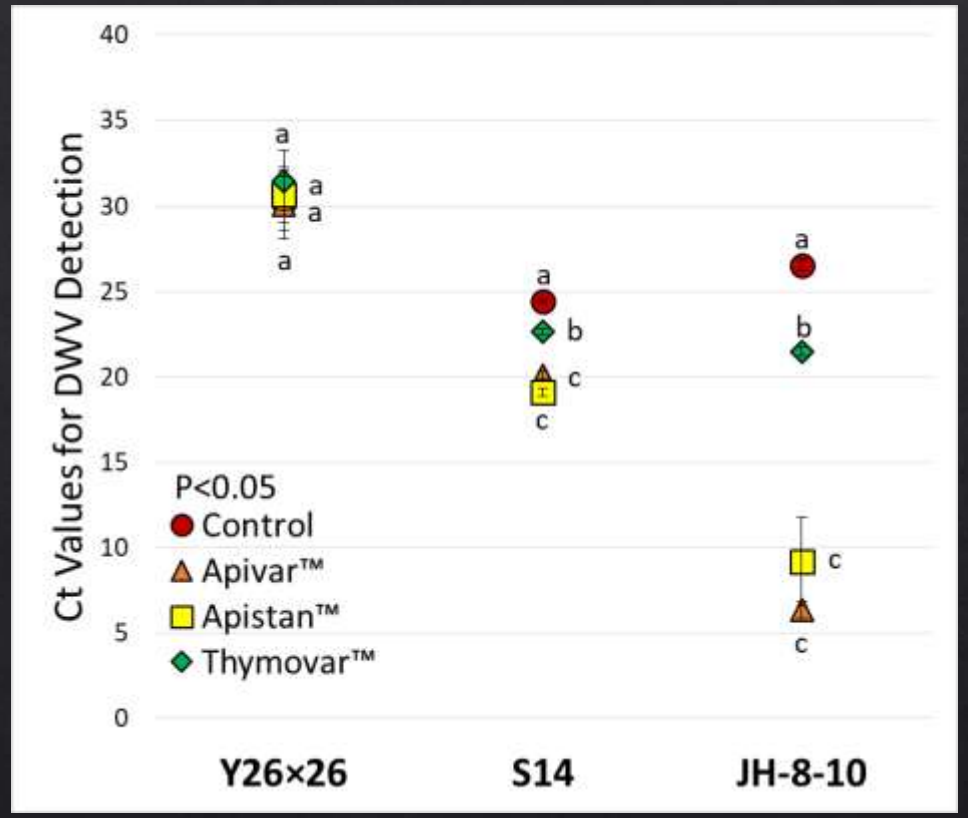


# DWV Levels in Response to Varroa Mite Infestation and Miticide Treatments




Tolerant Susceptible

DWV in Head Tissue w/ and w/o Varroa Mites

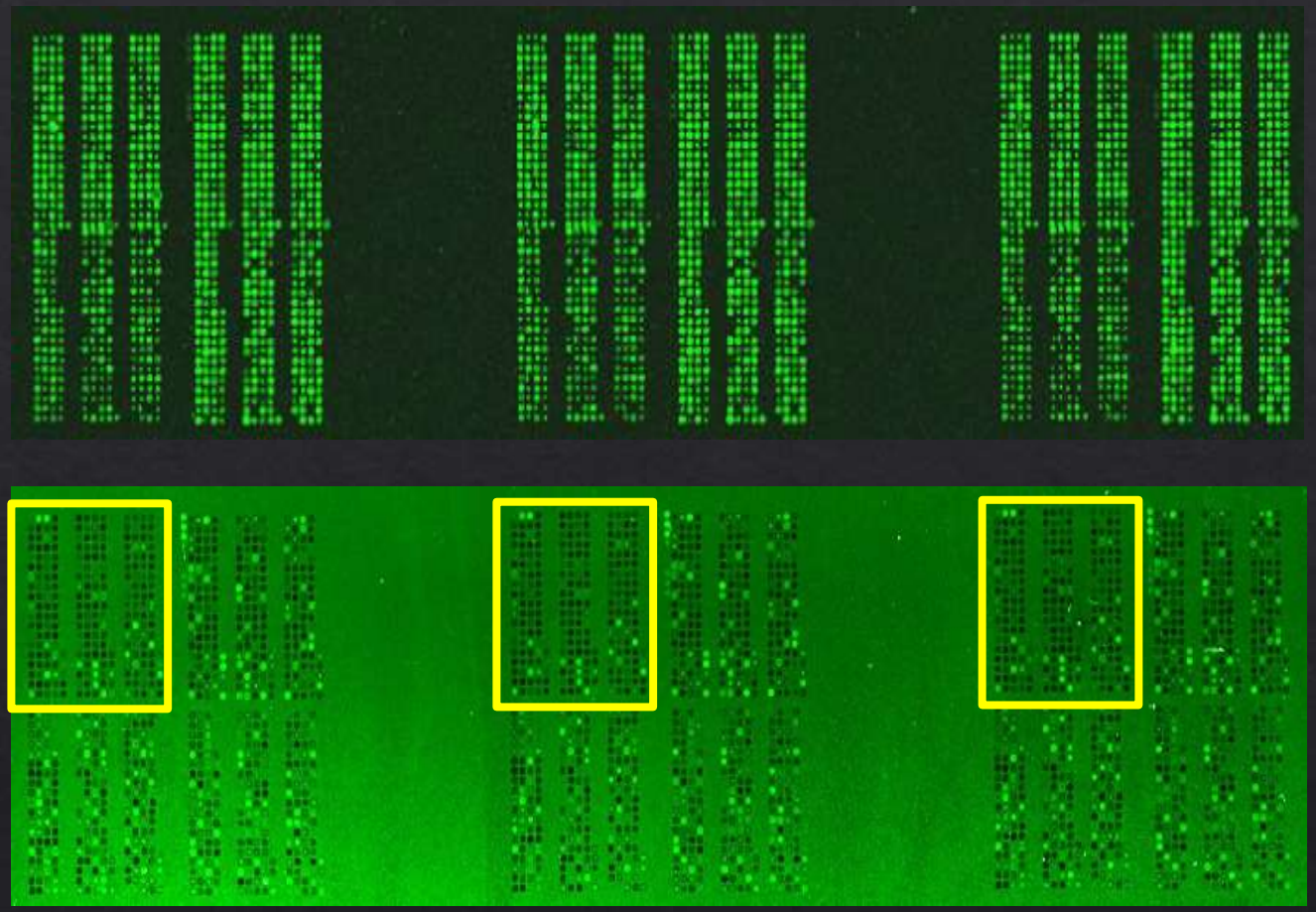



Tolerant Susceptible

DWV in Head Tissue in Miticide Experiment

**Quantitative measurements of DWV in two varroa tolerant (SY26 and S14) and one susceptible (JH-8-10) colony in response to varroa mite infestation and miticide treatments.** y axis: Ct values for DWV detection (mean± SEM, N=3); x axis: three colonies (SY26, S14 and JH-8-10). A. DWV in the head with and without varroa mite; B. DWV in the head with and without miticide treatments. The multi-treatment comparisons of Ct values used the LSD (least significant difference) method for difference analysis.

# Kinome Analysis of Colony Phenotypes

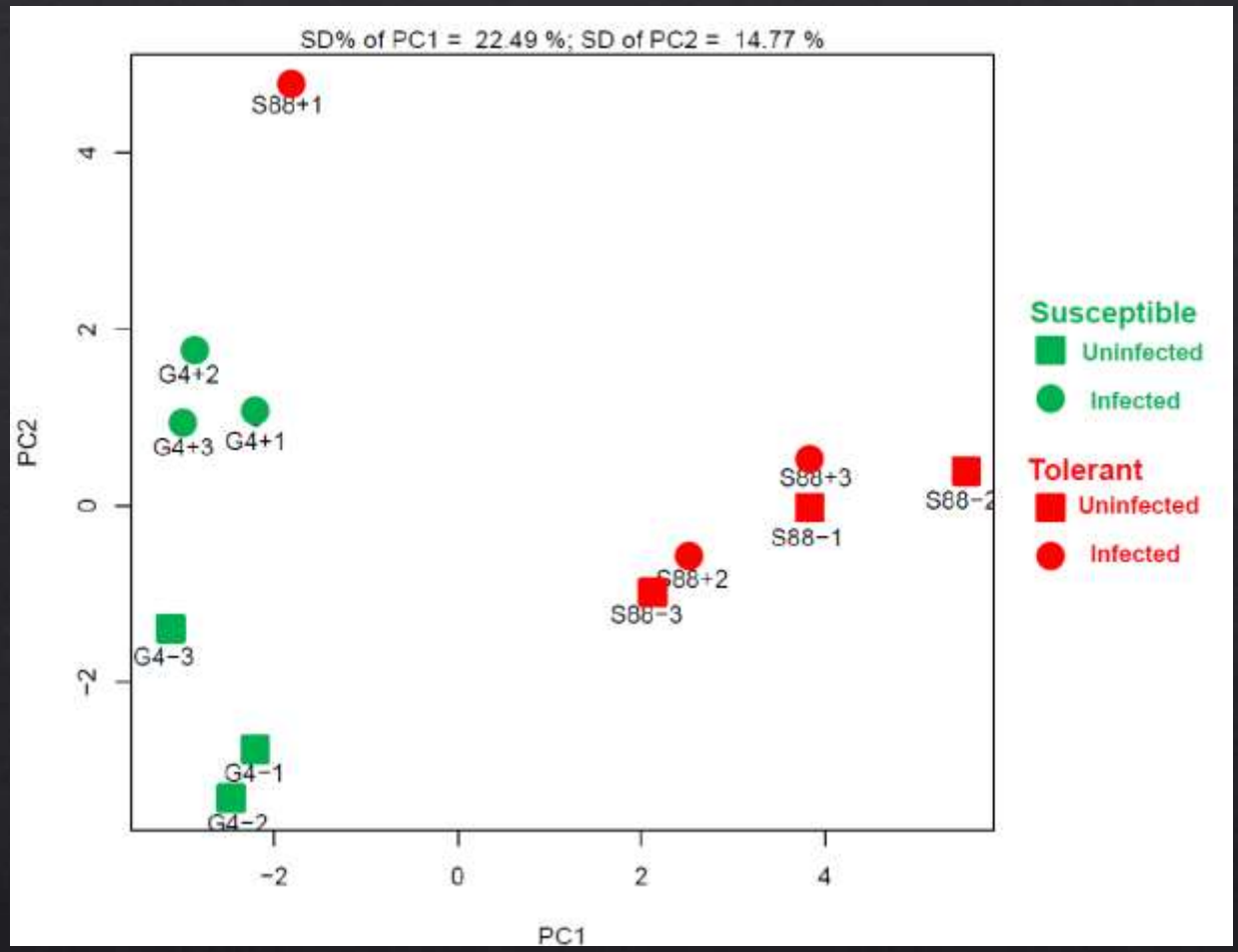


http://www.greendiary.com/hawaii-bees-infested-by-destructive-varroa-mites.html

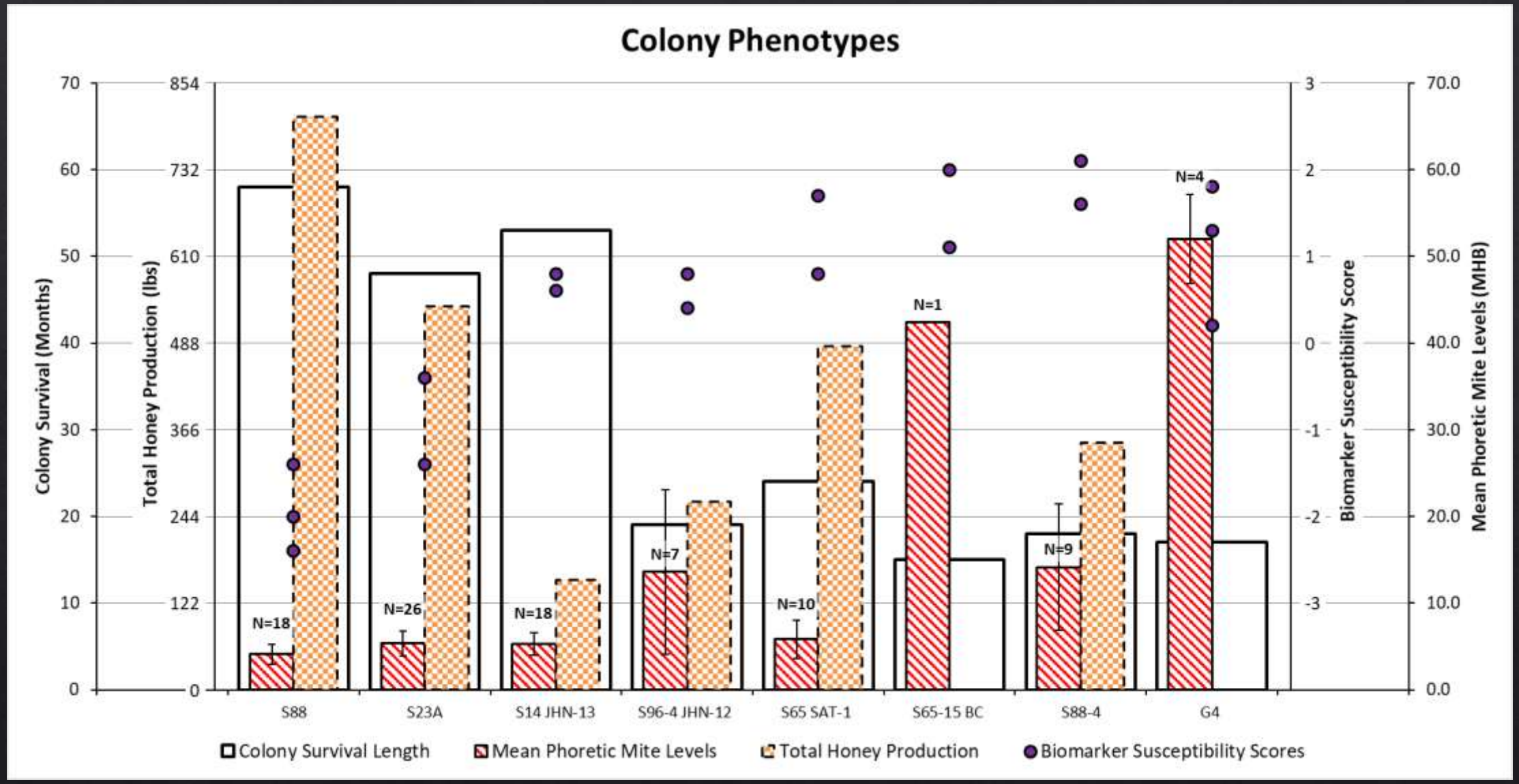
**Printing and Validation of the Bee Specific Peptide Array.** A) The arrays were printed by a commercial partner (JPT Technologies). For each array each spot is printed in triplicate within each block. Each block is then printed in triplicate for nine technical repeats of each peptide. This image, taken 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<br>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.




The survival time, mean phoretic mite infestation, total honey production, and biomarker susceptibility scores for 8 colony phenotypes are shown here. Error bars are shown as ±SE of the mean phoretic mite level where N is the number of samples tested to calculate the mean where S65-15 BC is represented only by a single sample. The purple dots represent the biomarker susceptibility scores calculated from the kinome array (n=299 peptides) analyses of dark-eyed pupae. Each dot represents a score calculated from one pupa.

# Conclusions:

• Possible to identify productive and mite tolerant colony phenotypes, and improve by established breeding procedures (back crossing, out crossing, re-current selection and progeny analyses).

• Colony phenotypes are difficult to stabilize because of considerable variability in progeny, from selected breeders. This may be due, in part, to multiple mating (sub-families), queen events (supersedure), high recombination rates, epistatis, and genotype – environment interactions (epigenetic effects). Evolutionary characteristic?

• New selection tools (biomarkers) for identifying phenotypes expressing genes involved in varroa tolerance, pathogen resistance immunity, and productivity should help to stabilize phenotypes and assist with breeding procedures.

• Kinome analysis showed varroa tolerant colonies had increased expression of Toll-like receptors which activate innate immune responses resulting in increased anti-microbial activity (proteolytic activity, lysozyme, phagocytosis, melanizing agent phenoloxidase, etc.)

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