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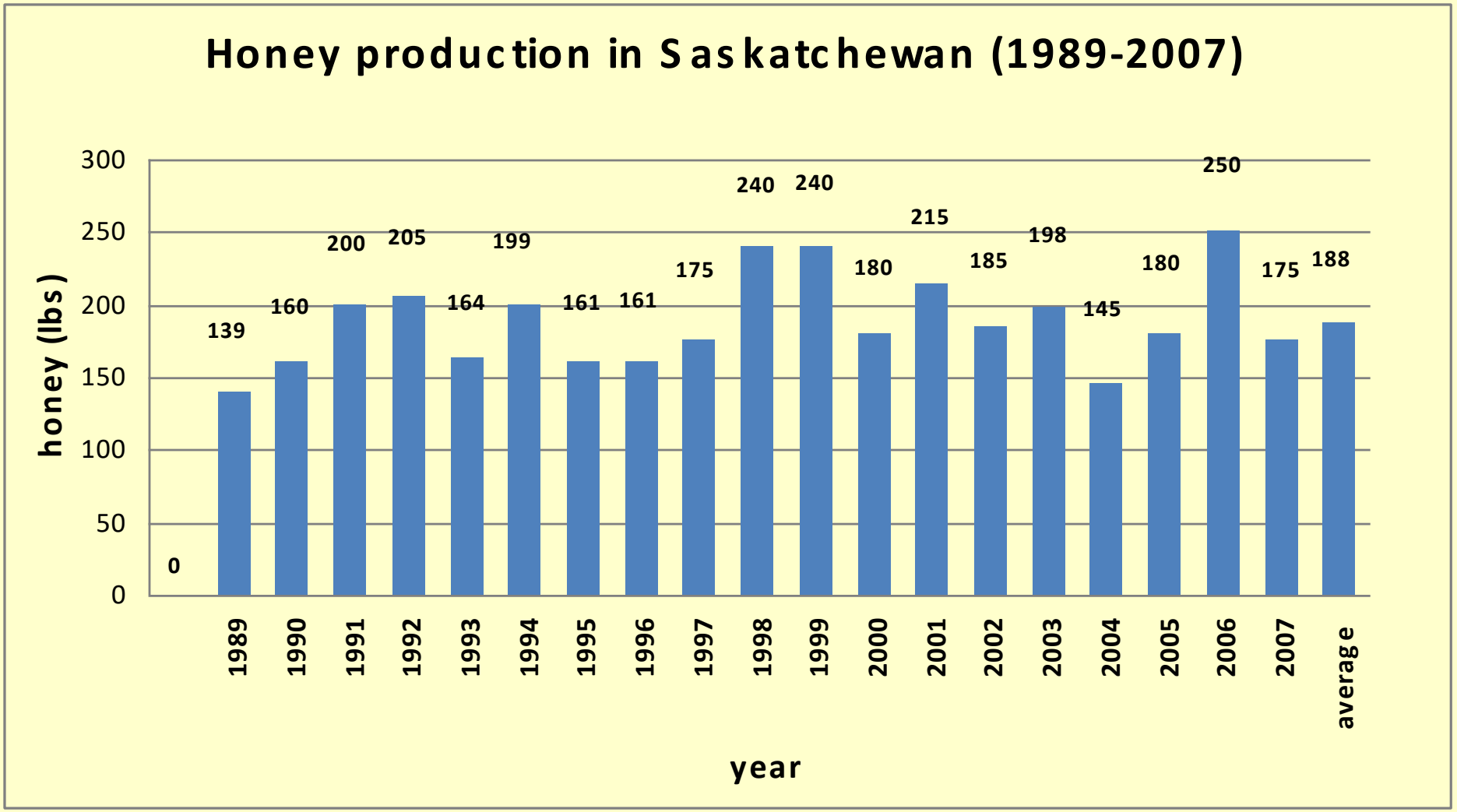
Honey production in Saskatchewan (1989-2007)
honey (lbs)
300
250
200
150
100
50
0
0
139
160
200
205
164
199
161
161
175
240
240
180
215
185
198
145
180
250
175
188
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
average
year

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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

Second World Symposium of Queen Bee Breeders, October 2008 - Mexico

Summer 2006

Second World Symposium of Queen Bee Breeders, October 2008 - Mexico

Dr. Abdullah Ibrahim

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Varroa Analysis

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Dr. Filipe Brizuela and Mohammad Mosterjan

Saskatraz yard site fall 2006 - fenced

Outline:

1. Brief history
2. Identification of superior phenotypes and genotypes. Selection of high performance colonies for “Saskatraz”
3. “Saskatraz” – Established 2004
   - selected colonies isolated for natural selection
   - monitor honey yield, mite populations
4. Molecular marker analysis to identify different populations and breeding lines – Microsatellites and SNPs

Brief History of Breeding Program and gene pool development (1992-2008)

- 1992 Closed population breeding program at Meadow Ridge: selections made for honey production and disease resistance annually from 1200-1600 hives
- 2001 purple-30-01, blue-40-01, green-01
- 2002 purple-30-02, blue-40-02, green-02, yellow-02
- 2003 yellow-03, purple Ont-03, orange-white-03
- 2004 red-04, yellow-blue-04, blue-04
- 2005 yellow-blue-05, yellow-green-05, Breeder queens – Charlie Harper

Brief History of Breeding Program (2001-2008), cont'd

- 2004 Drone semen from Dr. Ralph Buchler 2004 - two lines of pure Carnica (25 year program) selected for varroa tolerance and hygienic behaviour. Crossed to 2004 Russian (red-04,yellow-blue04,blue-04) and Canadian lines.
- 2005 Obtained semen from two additional lines G-08 and G-72. Made 35 crosses with virgin queens selected from the following lines: UM-163, UM-234, TSQ, SAT-30, yellow-green-05, SAT-28, BTP-30, yellow-blue-05, Car-04, and UM-147.

Brief History of Breeding Program (2001-2008), cont'd

- 2004-08 Canadian selections (donated by 25 Saskatchewan queen breeders representing selections from over 45,000 colonies).
- 2006 Saskatraz breeders selected in the fall of 2005 (SAT-14, 17, 23, 28, 30 and 34) were used to produce virgin queens for insemination with drone semen from New World Carniolian (S. Cobey) lines, and for closed population breeding at Saskatraz.

Brief History of Breeding Program (2001-2008), cont'd

- 2007 — Saskatraz was stocked with 28 selections from Saskatraz survivors, and new selections made from 1200 colonies in the spring of 2007. Some of the Saskatraz survivors were constructed in 2006 by closed population breeding of daughters from Saskatraz selections and AI33 (UM234XG72). Numerous control crosses were made by instrumental insemination by crossing high honey producers (SAT30, 17) and mite tolerant lines (SAT34, 28) in 2007.
- 2008 — 23 colonies from the 28, 2007 Saskatraz colonies survived into the honey flow.

Saskatraz

- An isolated apiary stocked with colonies pre-selected for desirable “colony” traits (honey production, temperament, over-wintering ability, longevity, low temperature flight, pollen storage, burr comb). Selections obtained from a large gene pool.
- All colonies placed into Saskatraz in 2004 were infected with Varroa and tracheal mites.
- Monitor
  - Honey production
  - Changes in tracheal and varroa mite populations
  - Hygienic behaviour and varroa sensitive-hygiene (VSH)
  - Varroa mite populations, their distribution (partitioning) between worker and drone brood as well as damaged (grooming behaviour) and immature mites.

Saskatraz, cont'd.

- Management of Saskatraz apiary follows standard practices except no chemical miticides or excluders are used.

  - Fall Colonies are fed liquid sucrose (Sept., Oct.) with Fumidil B and Oxytetracylcline.
  - Winter Colonies are wintered outdoors in 4 packs with standard insulated wraps applied in mid-October. No pollen substitutes are fed.
  - Spring Colonies are fed 1 pail (2.5 gal) of liquid sucrose or corn syrup in the spring. No pollen substitutes are used. Colonies are opened between the 7th and 20th of May for inspection and sampling.
  - Summer Honey is collected 3 to 4 times between July 10th and September 10th each year.

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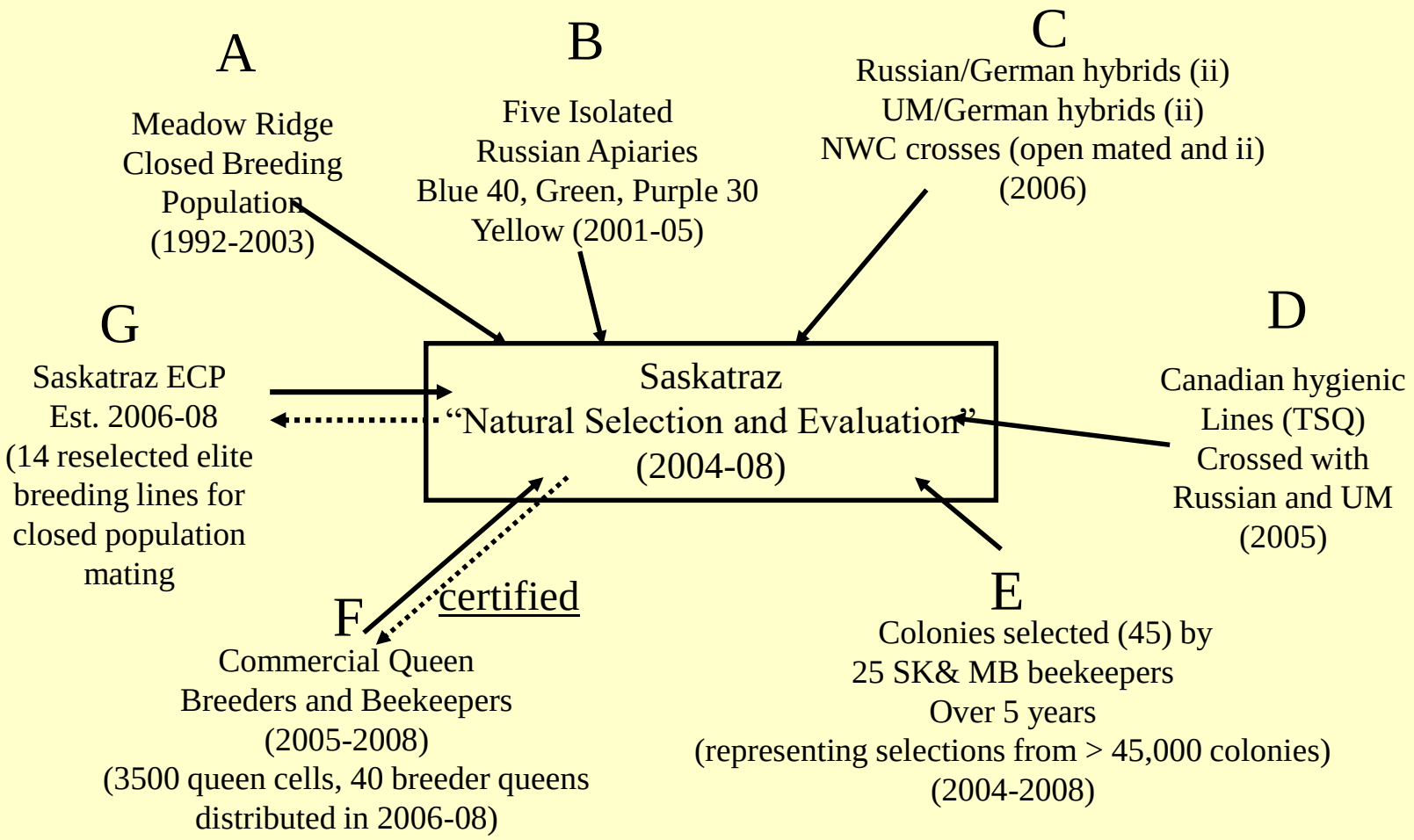
Saskatraz Breeding Program Logistics



Letters A to G represent isolated apiaries and the year of establishment at Meadow Ridge. Solid arrows indicate gene flow into Saskatraz, dashed arrows gene flow out of Saskatraz. (ii) denotes instrumental insemination.

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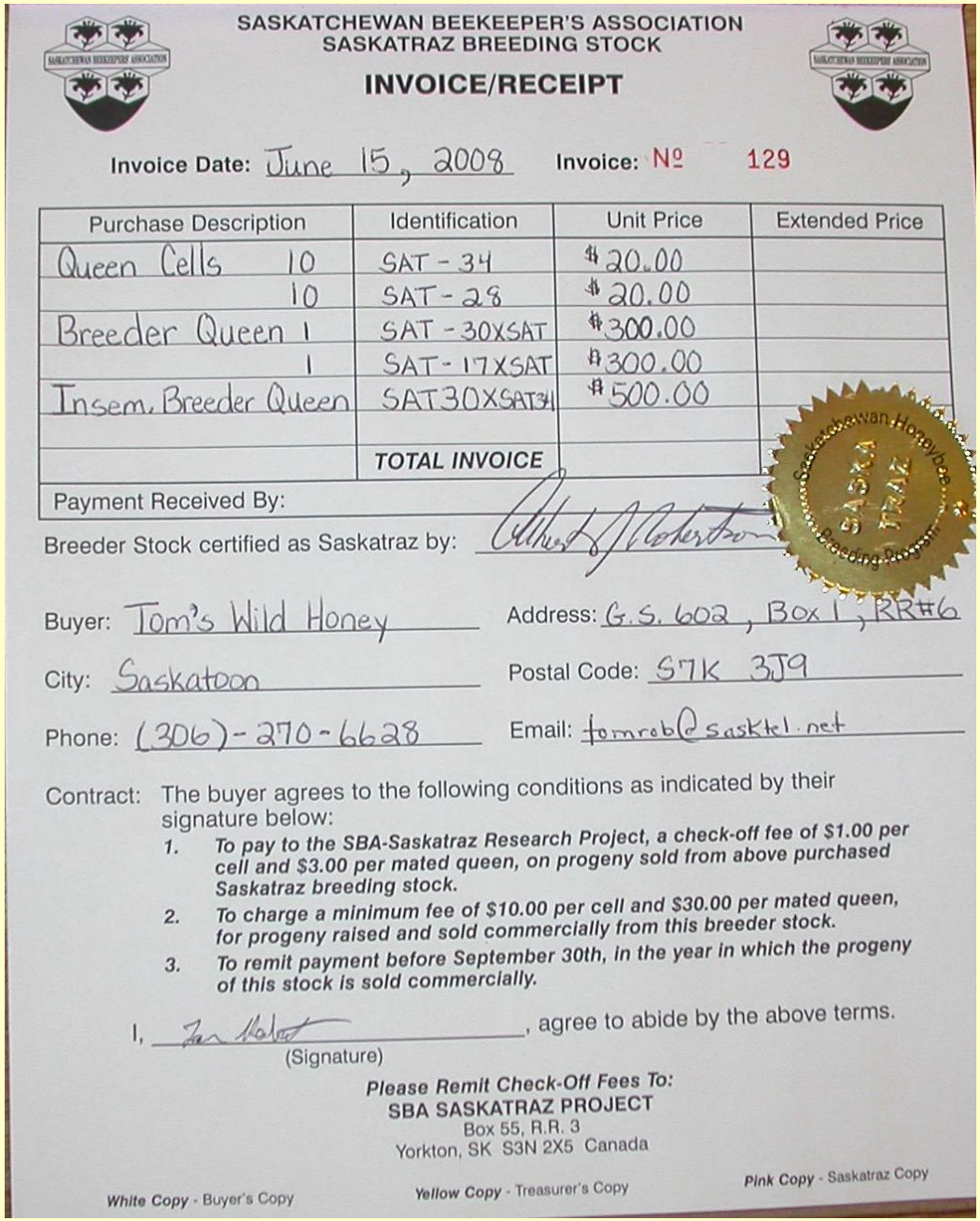

SASKATCHEWAN BEEKEEPER'S ASSOCIATION
SASKATRAZ BREEDING STOCK

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	SAT30XSAT31	$500.00	
	TOTAL INVOICE		

Payment Received By:

Breeder Stock certified as Saskatraz by:

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

The Performance of A Colony of Honey Bees Depends On:

1. The genotype of the queen and the genotype of the drones she has mated with.
2. The colony environment and management of the colony (genotype-environment interaction).
   - Logic behind Saskatraz ECP colony selection.

Identifying Genetic Diversity: Molecular Marker Analysis

1. Is it possible to identify different populations (genetic diversity) of honey bees by microsatellite marker analysis? – YES (Russian, Canadian, German, Africanized and Asian)
2. To eventually use molecular markers for marker assisted selection of important traits. Eliminate many years labour and intensive field work. Valuable efficient tool. Phenotypes and genotypes are being identified to pursue this goal, using microsatellites and single nucleotide polymorphisms (SNPS).

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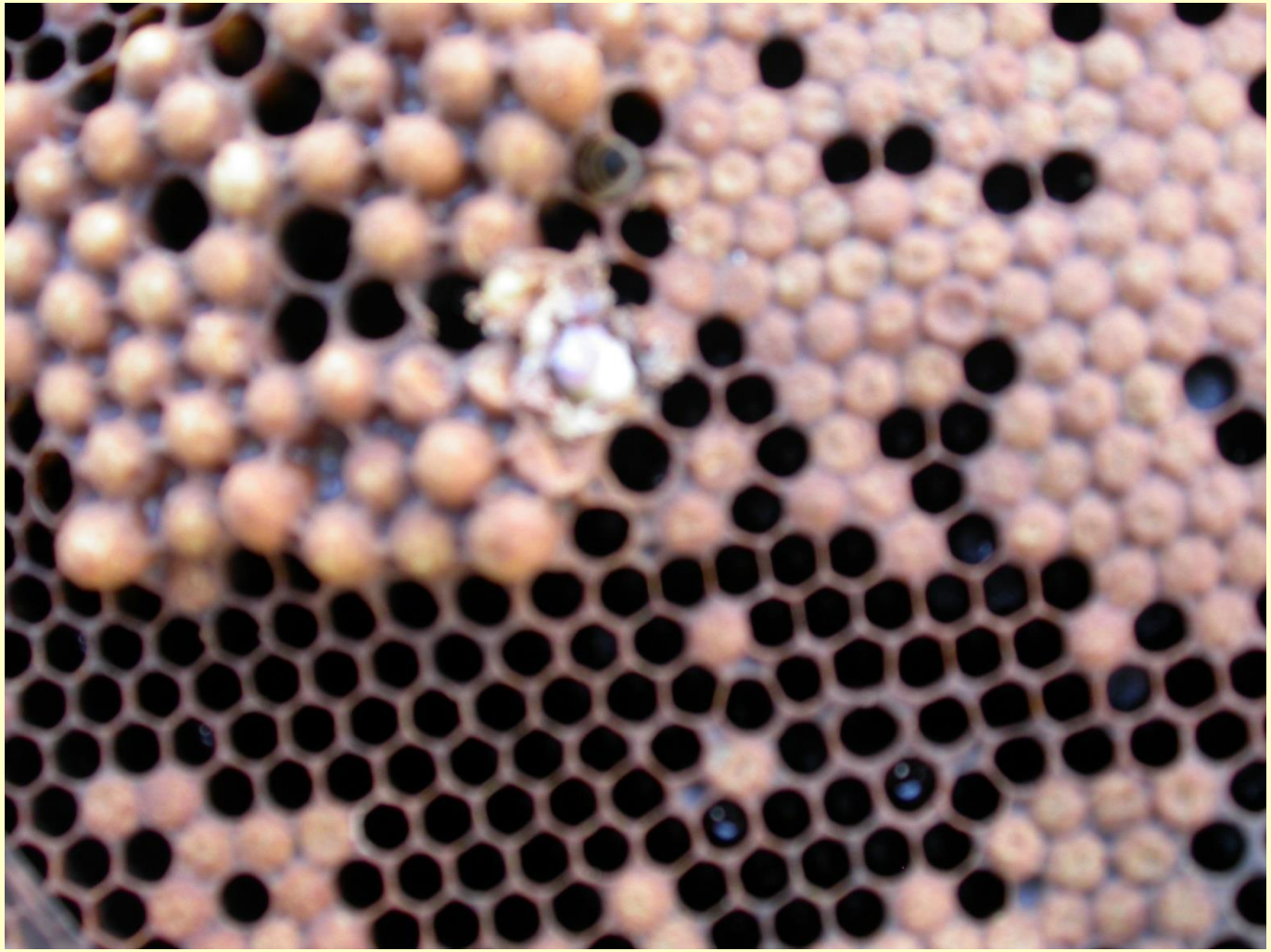
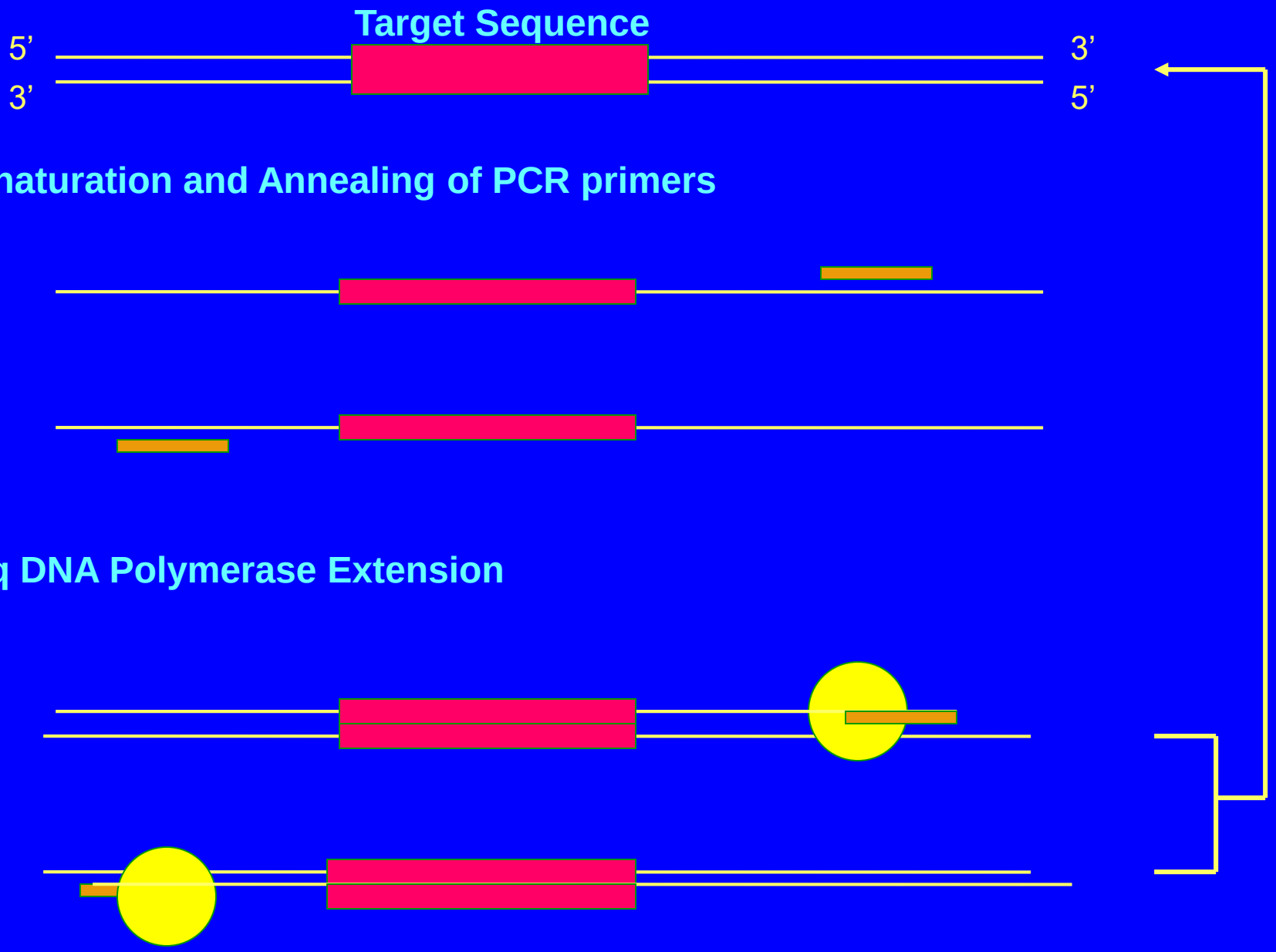


Drone Brood



*Solignac, M. et al., Genetics (2004) 253:541

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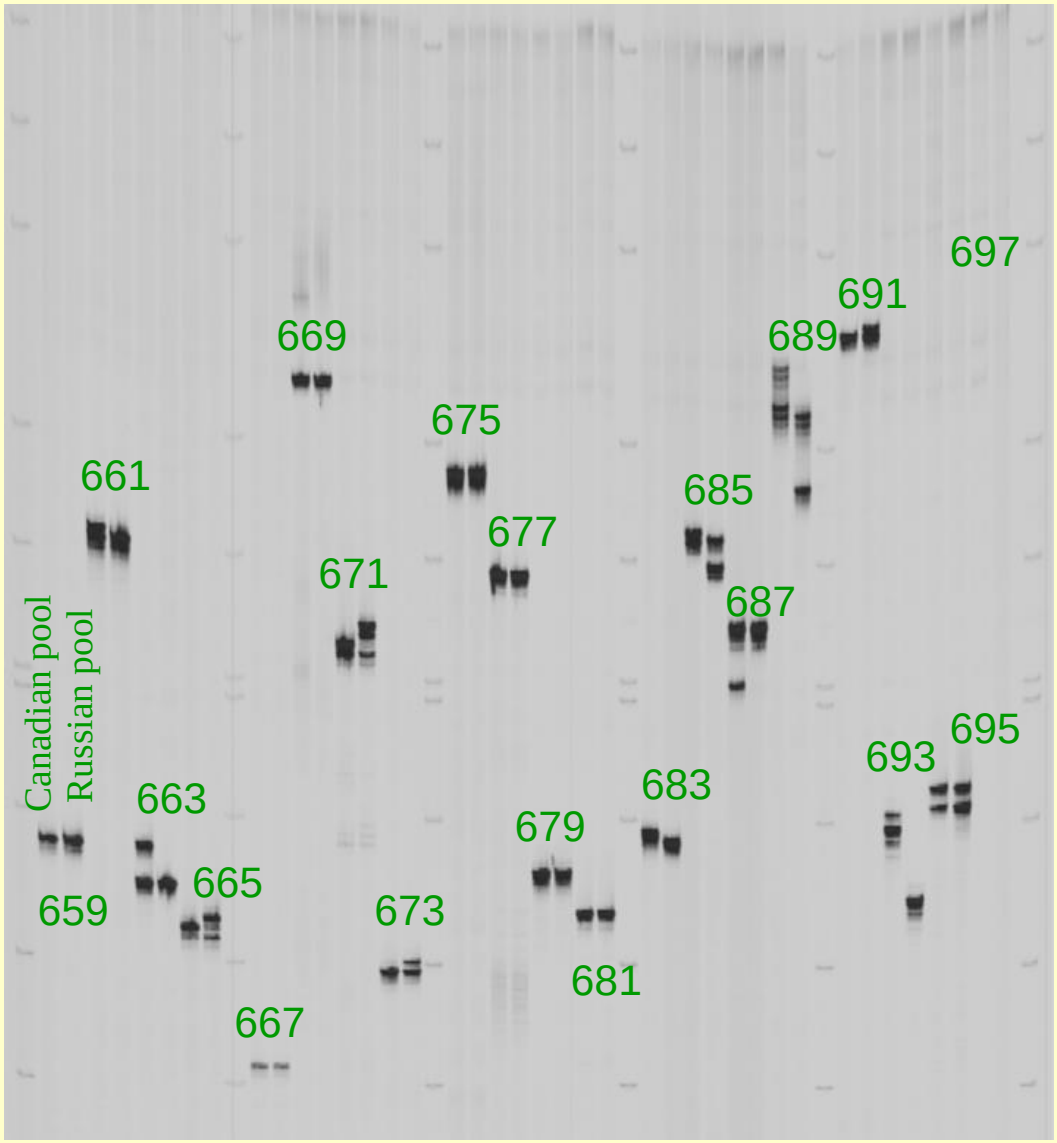
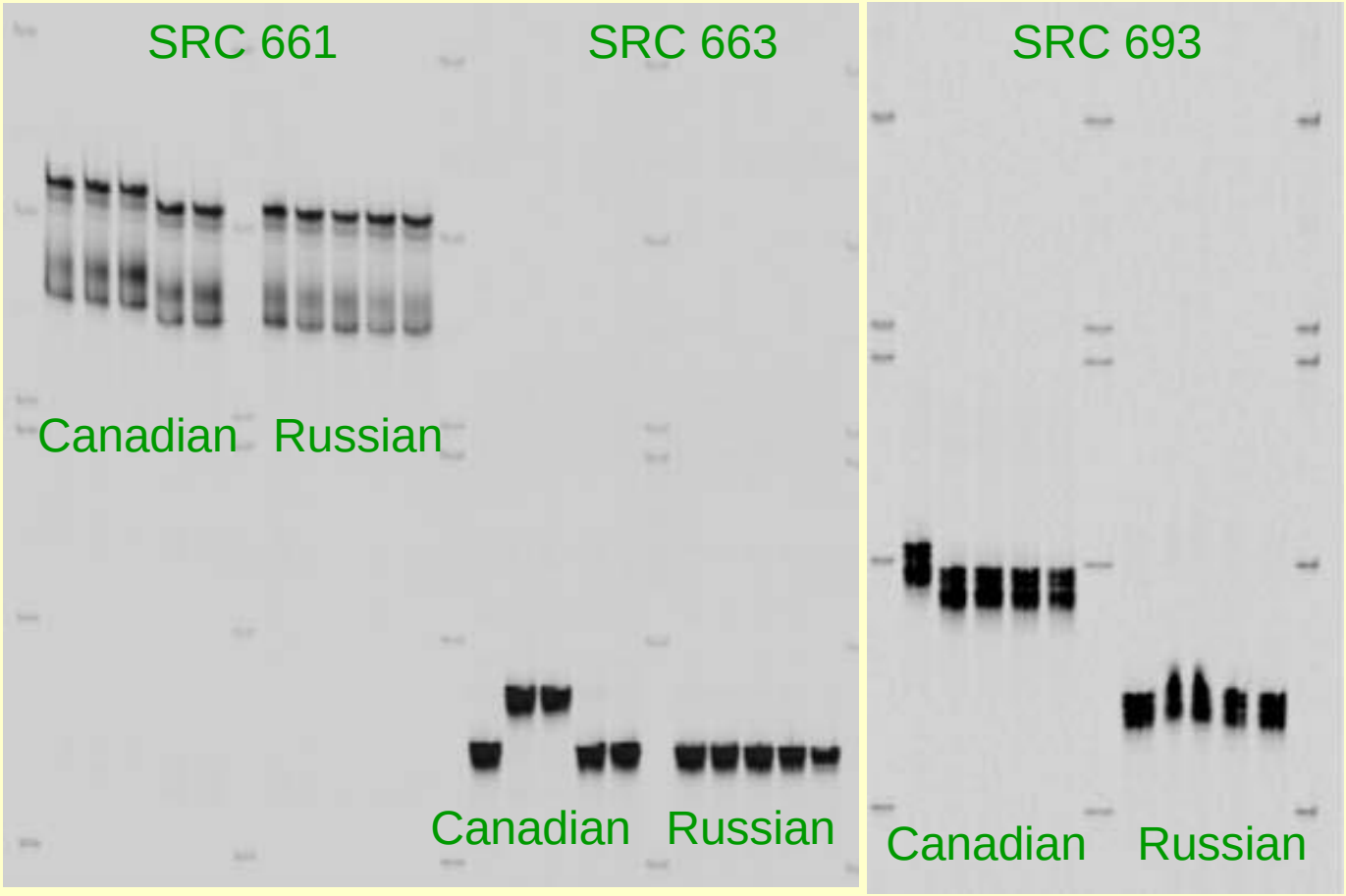


Initial 20 marker screen, 1/10 dil of original PCR product



Optimized Markers

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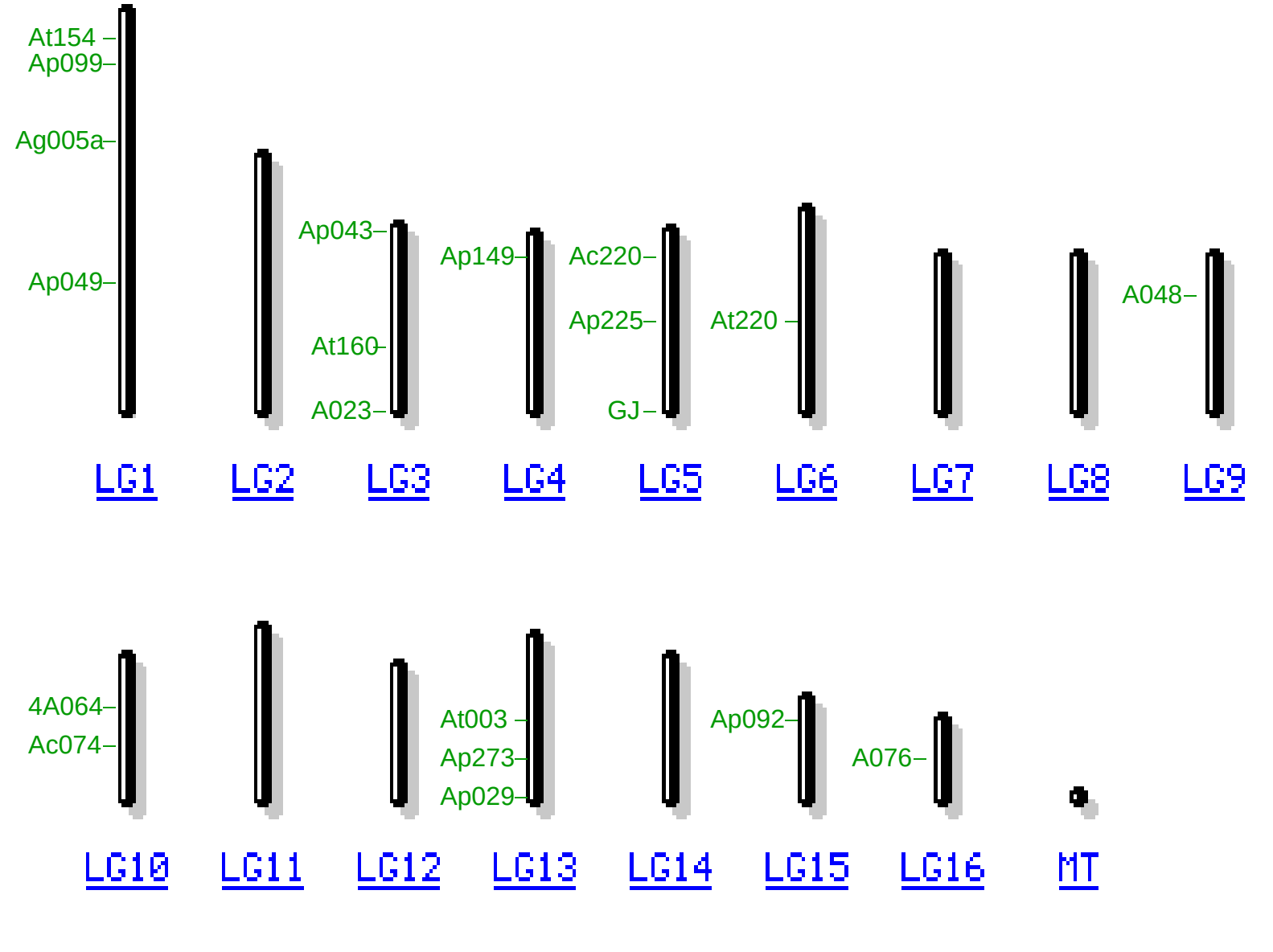

Apis mellifera (honey bee) genome – NCBI Website

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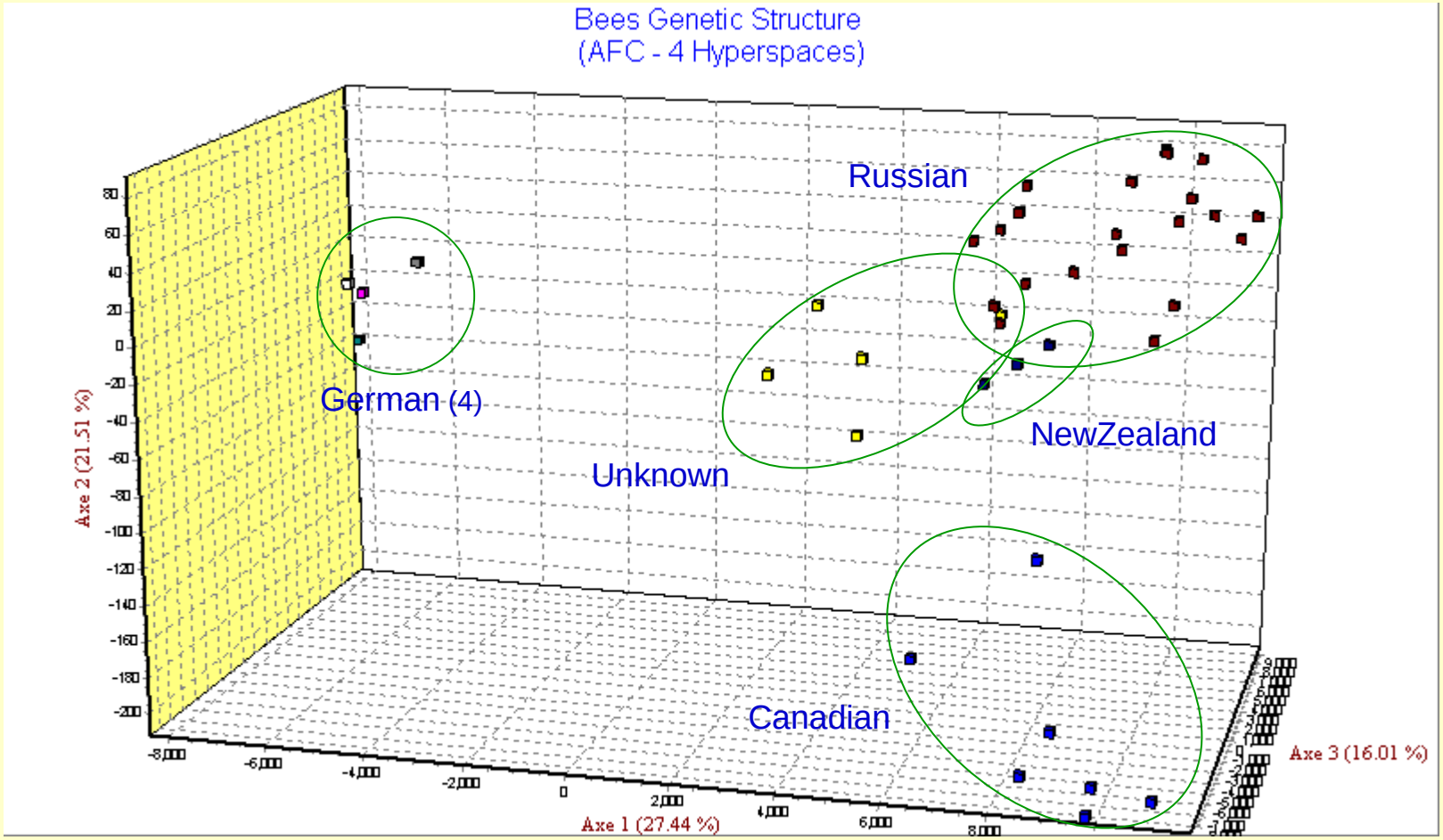

A three dimensional plot showing the grouping of 5 different honeybee populations using 20 informative microsatellite markers.

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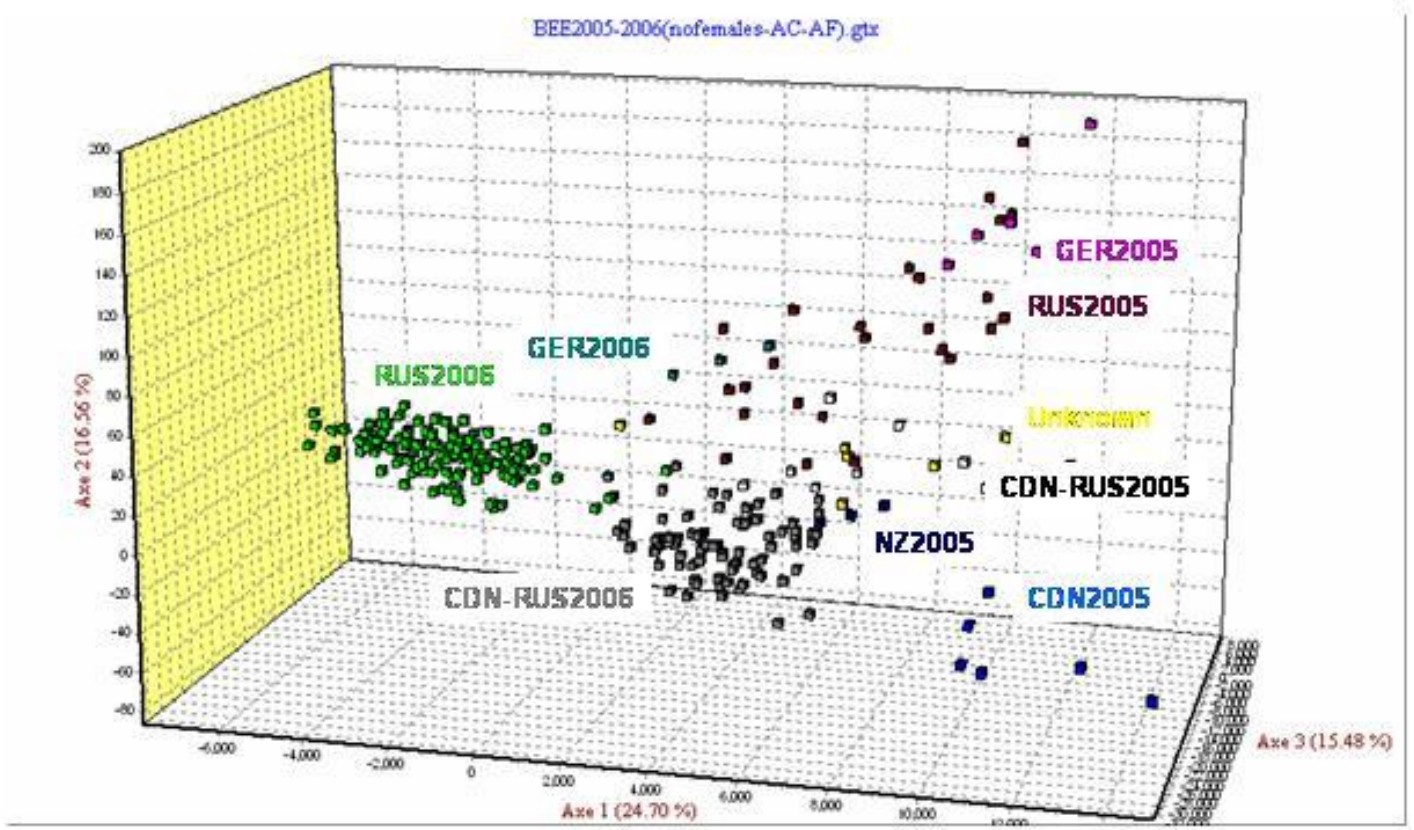

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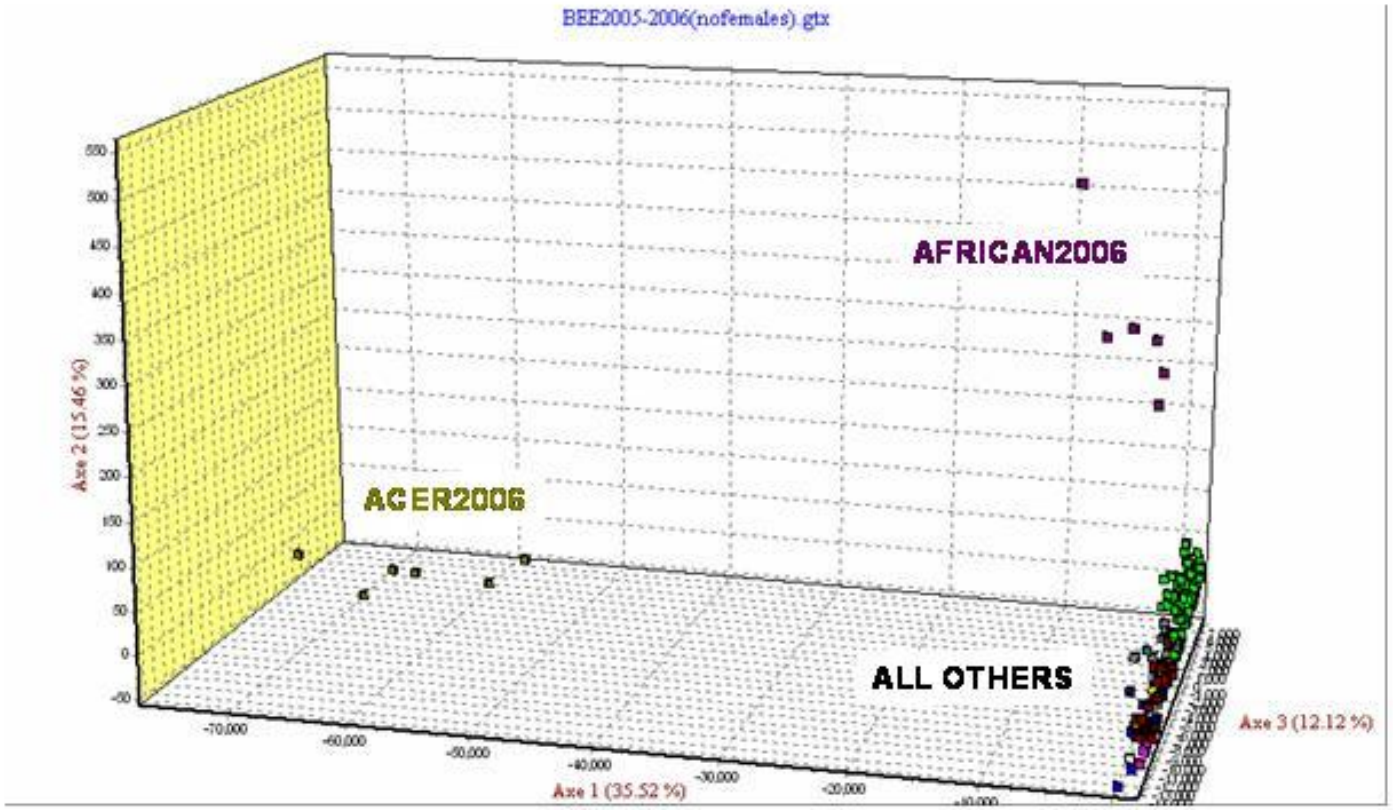

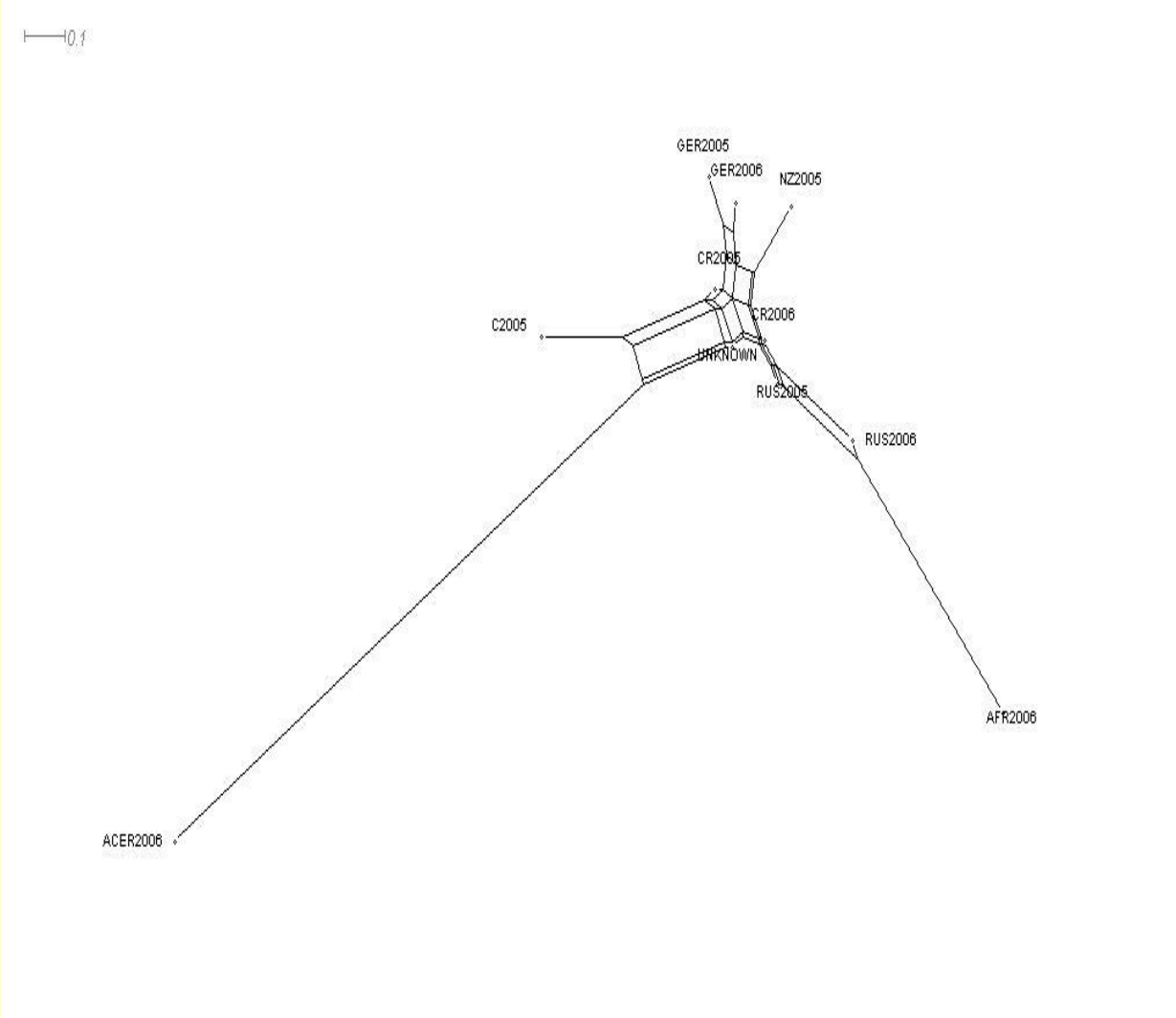

Genetic Distance: Nei's Standard Genetic Distance
(Corrected for small samples)

Characteristics of diseased hives

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2G Car-02 selected colony

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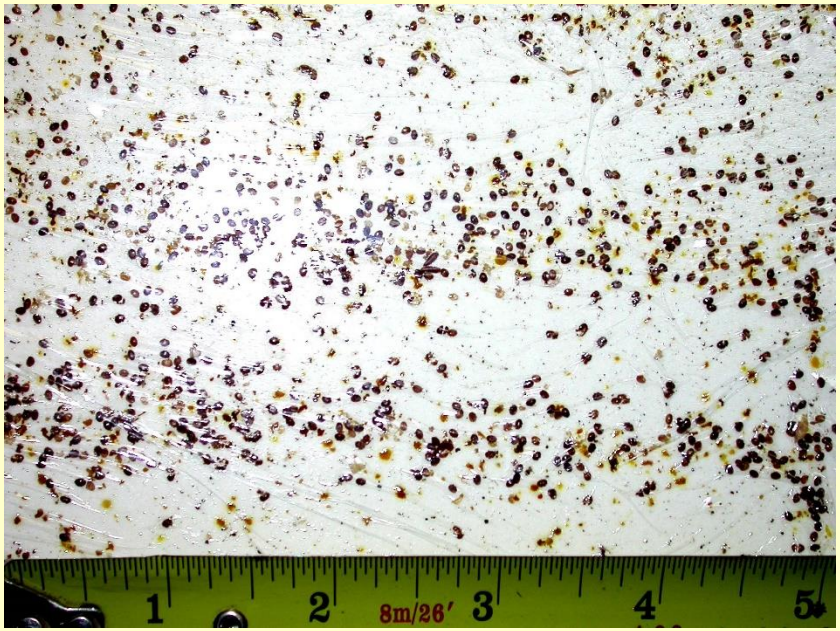
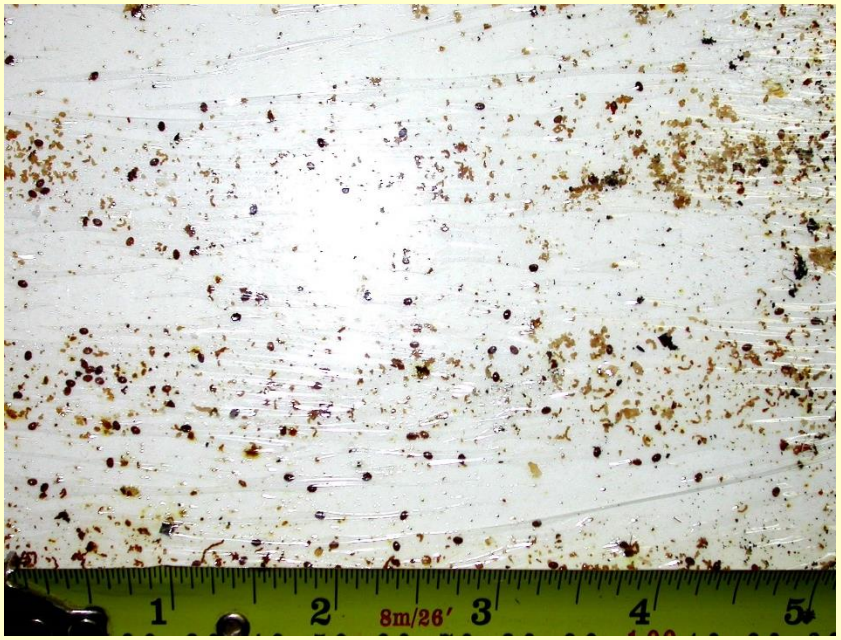


Control Hive



Russian-Canadian Hybrid-02, Purple-1

48 h Varroa Mite Drop With Apistan Treatment
Drones sampled for DNA analysis.

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Progeny of Russian-04/German G-3 hybrids

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Brood Pattern of Russian/German hybrids

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Selections done during honey flow
(Most important selection criteria)

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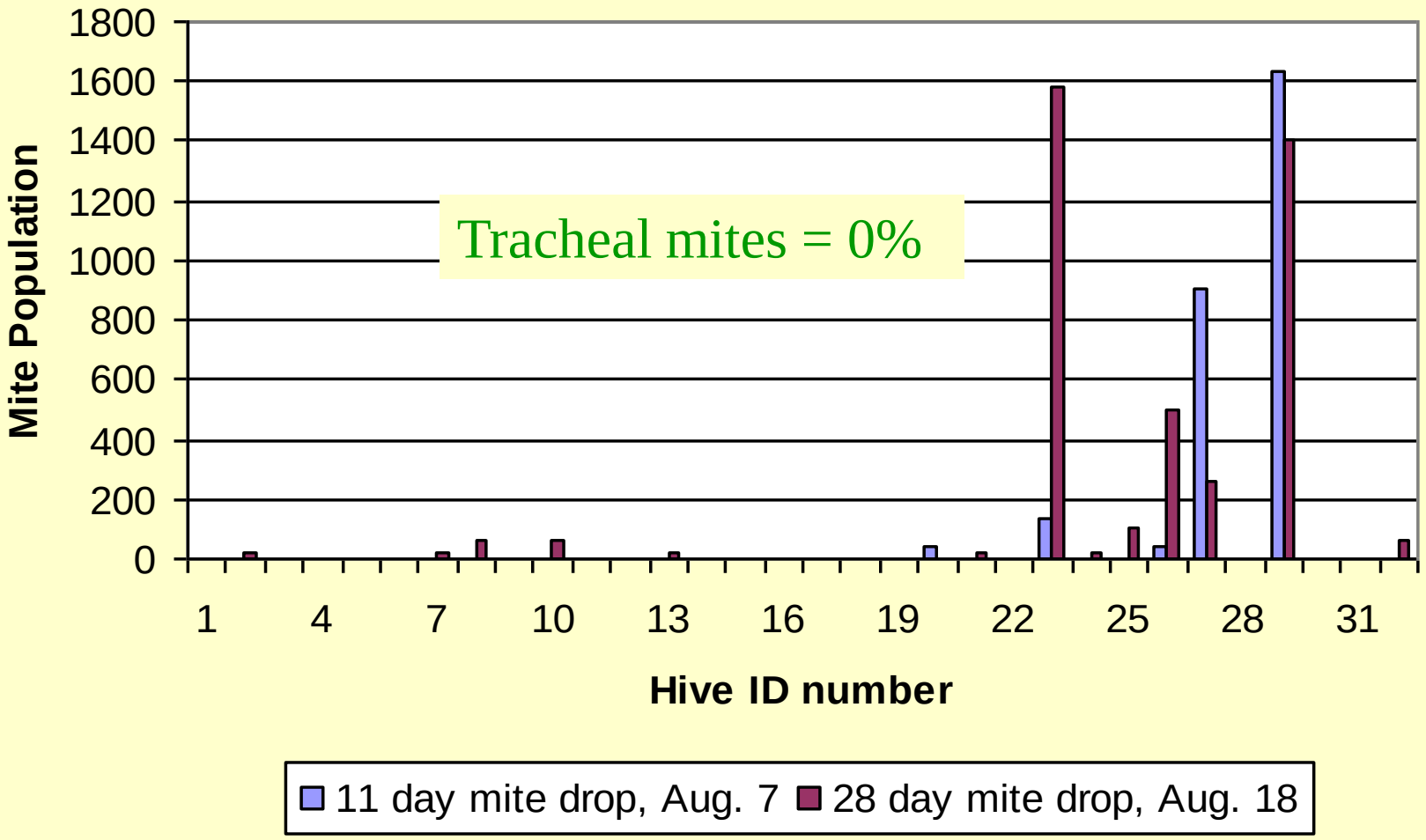
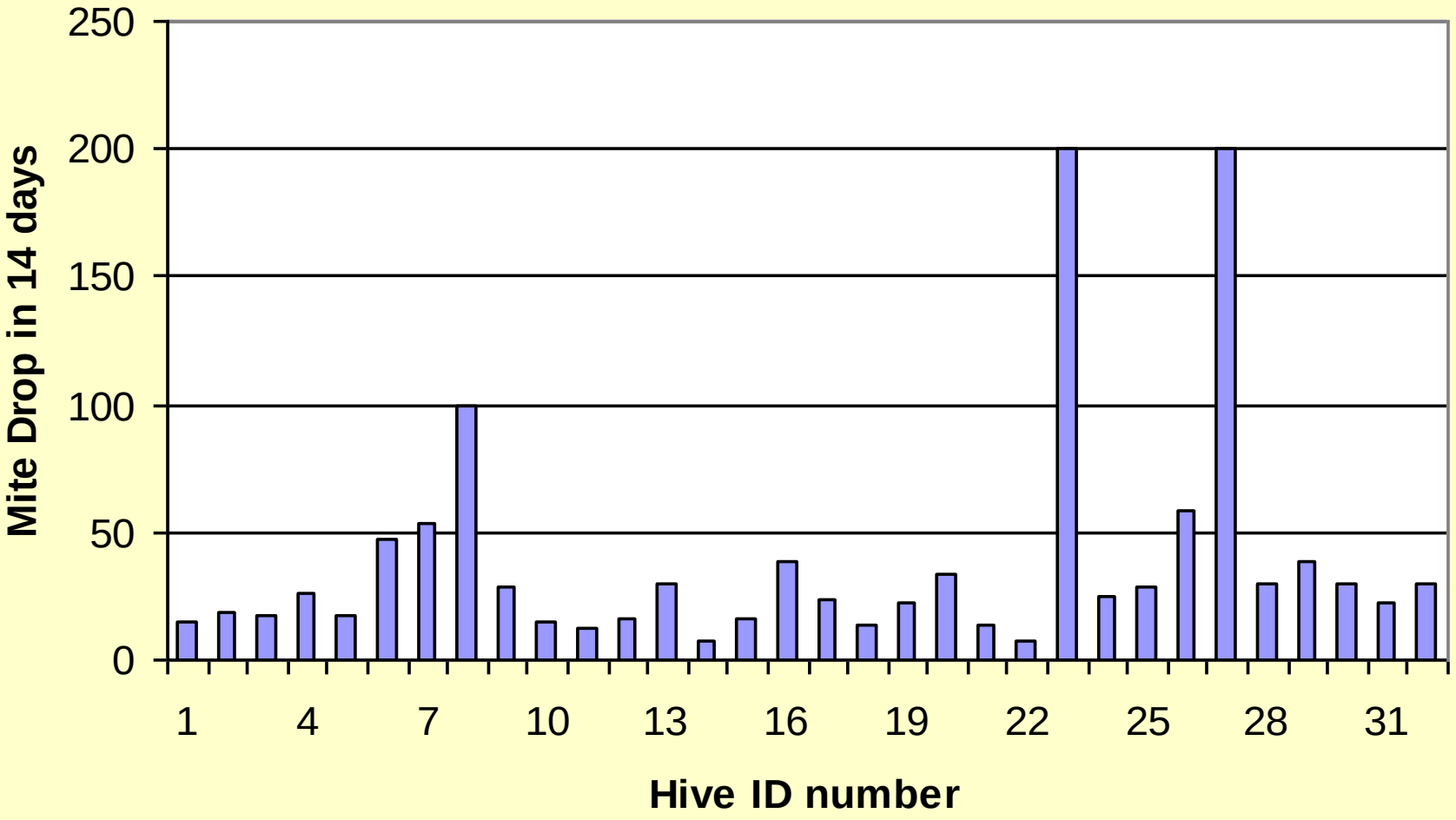
Saskatraz – 2004

Natural drop – Varroa population analysis showing Apinovar boards

Saskatraz Varroa Analysis - Natural Drop (2004)



Saskatraz Varroa Analysis - Apistan Treatment (2004)



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Tracheal mite infested colony

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Introducing tracheal mites

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Saskatraz – May, 2005 First Selection – SAT-30

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Saskatraz – May, 2005 Second Selection – SAT-23

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Saskatraz – July, 2005 SAT-17

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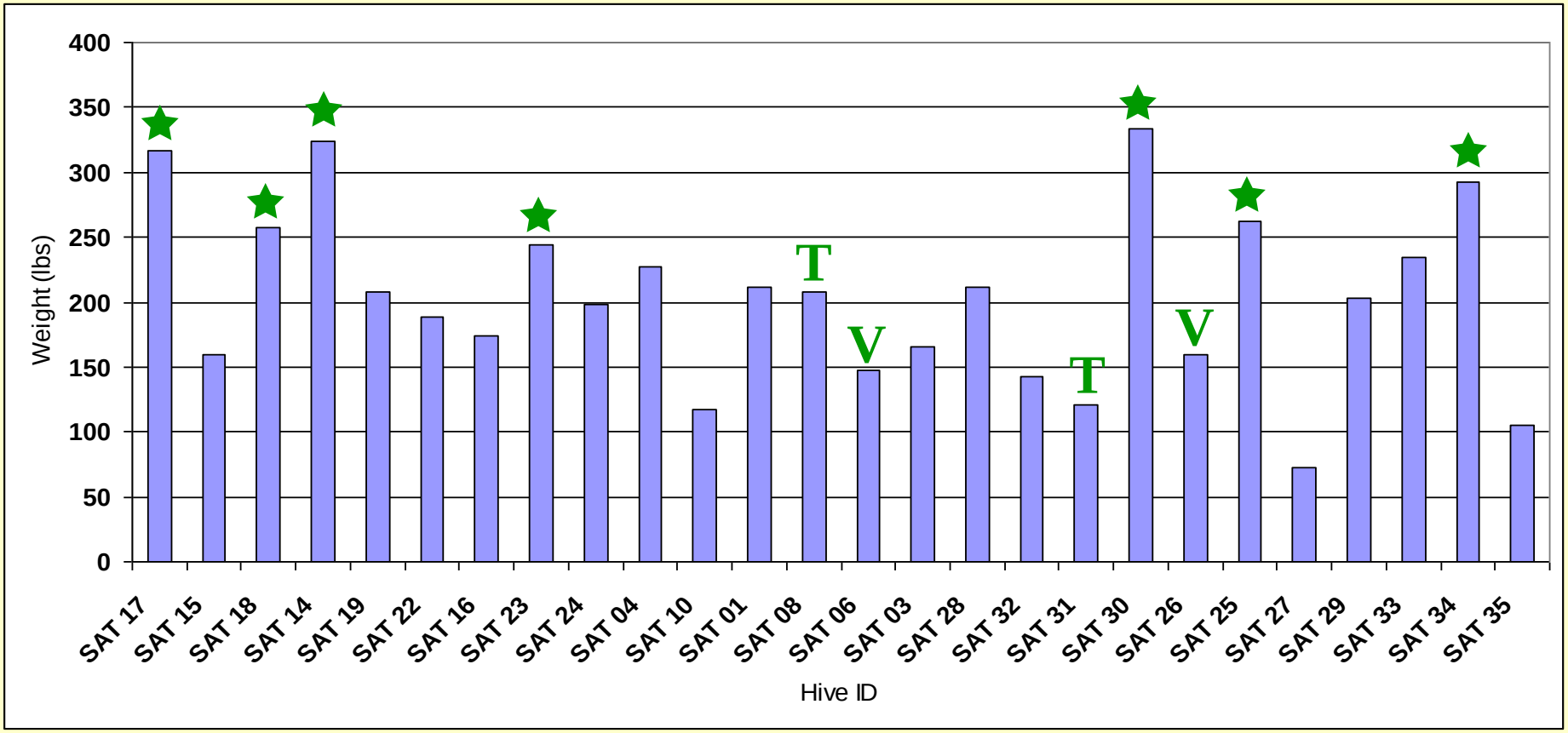
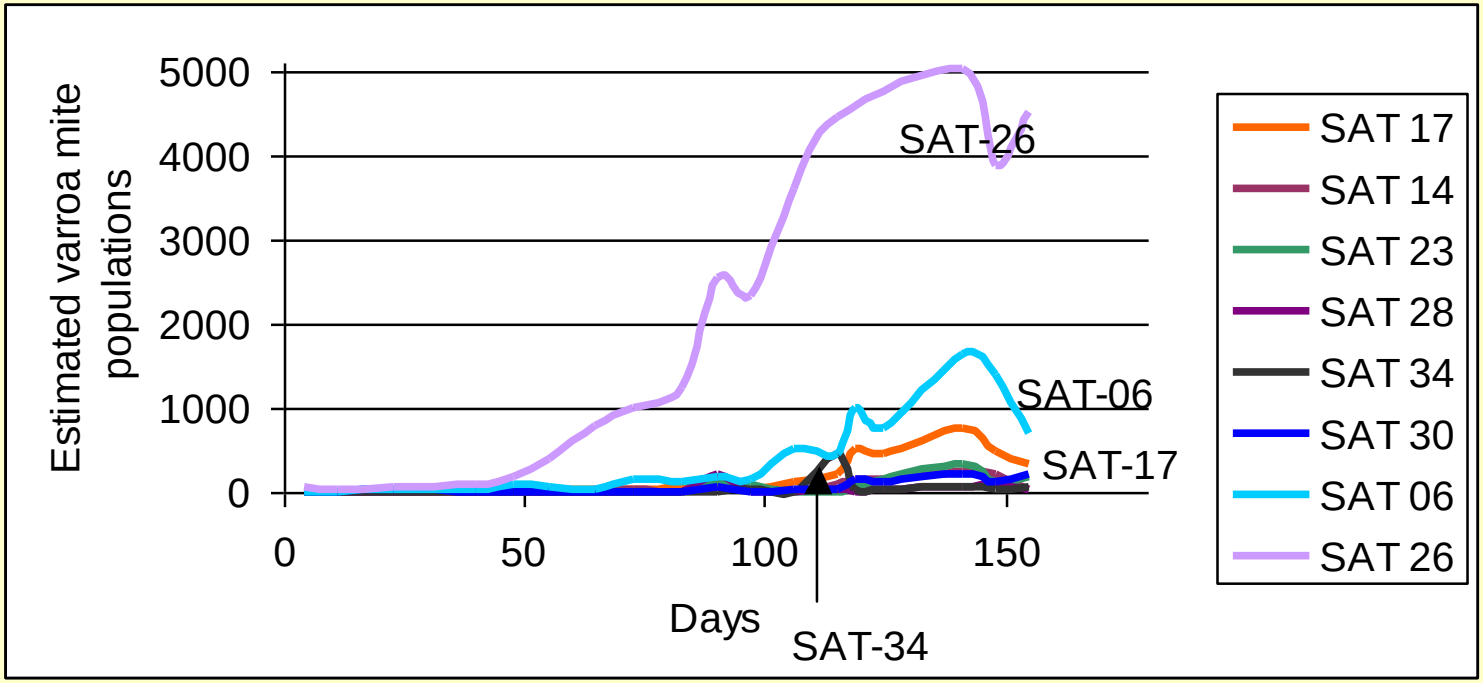
Saskatraz – October, 2005 - Sampling

Saskatraz – Honey Production: 2005



Yard Average: 203 lbs per hive

May to October, 2005 Change in Varroa mite populations



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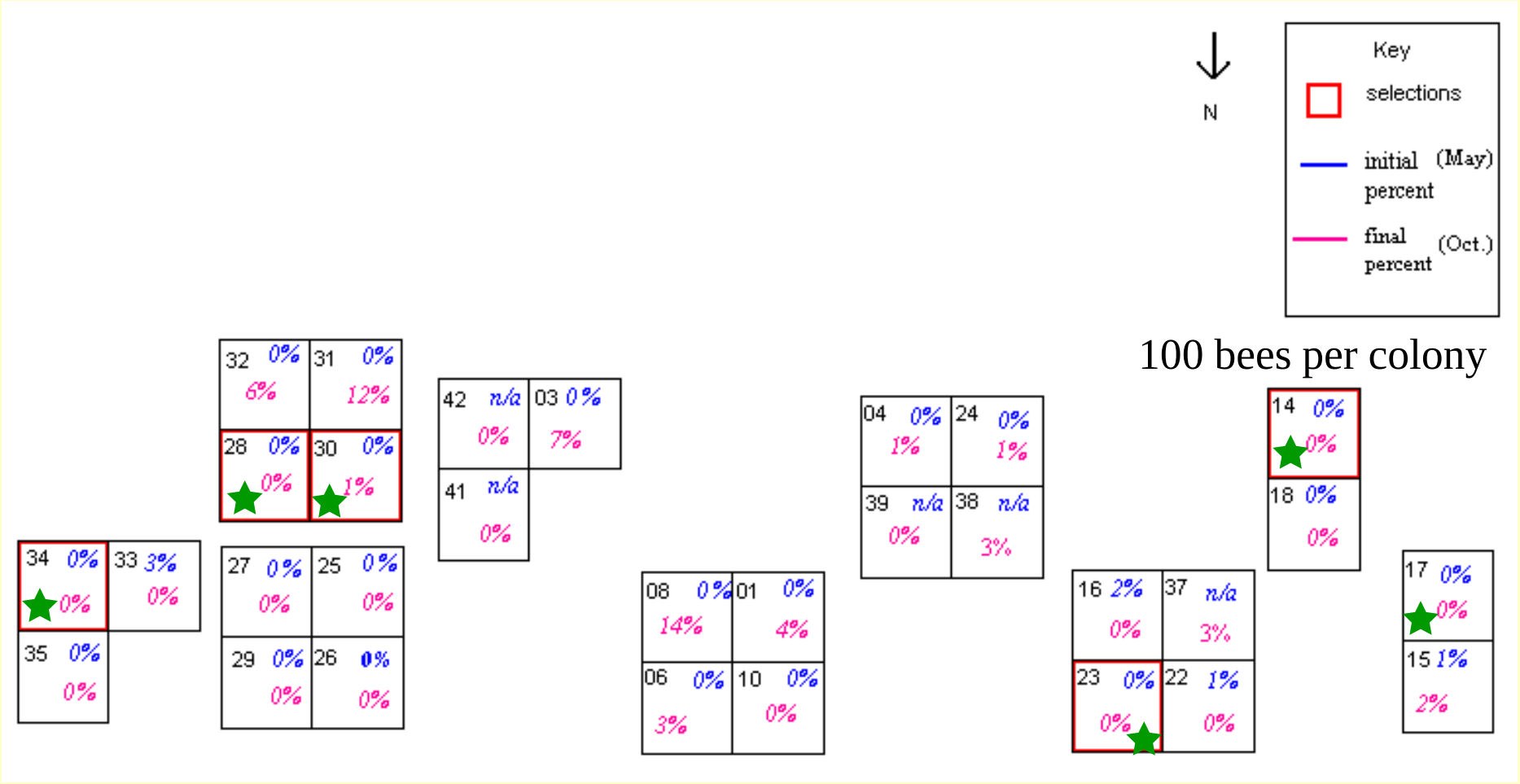
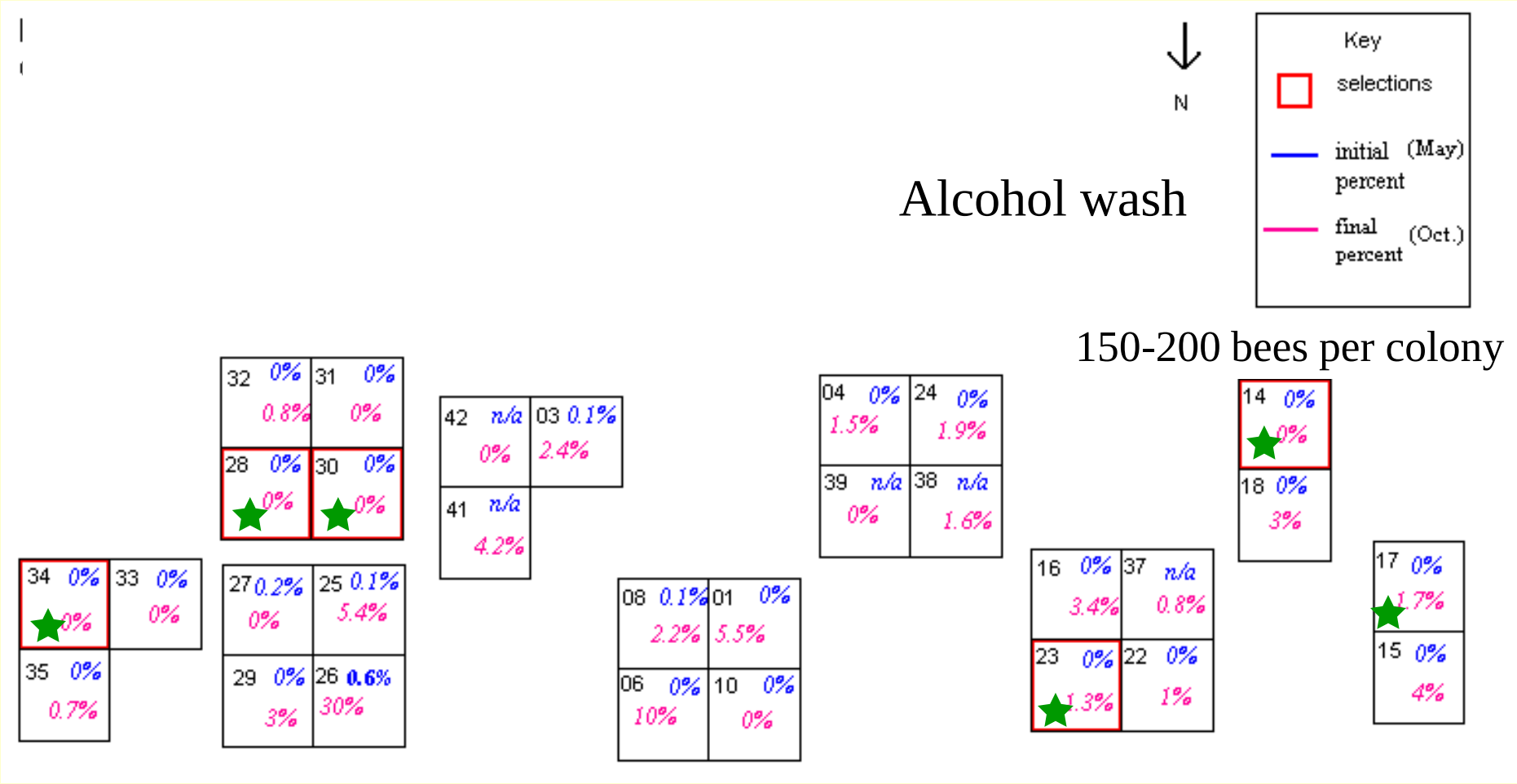


Saskatraz Tracheal mite levels and hive locations (2005)



Saskatraz Varroa mite levels and hive locations (2005)

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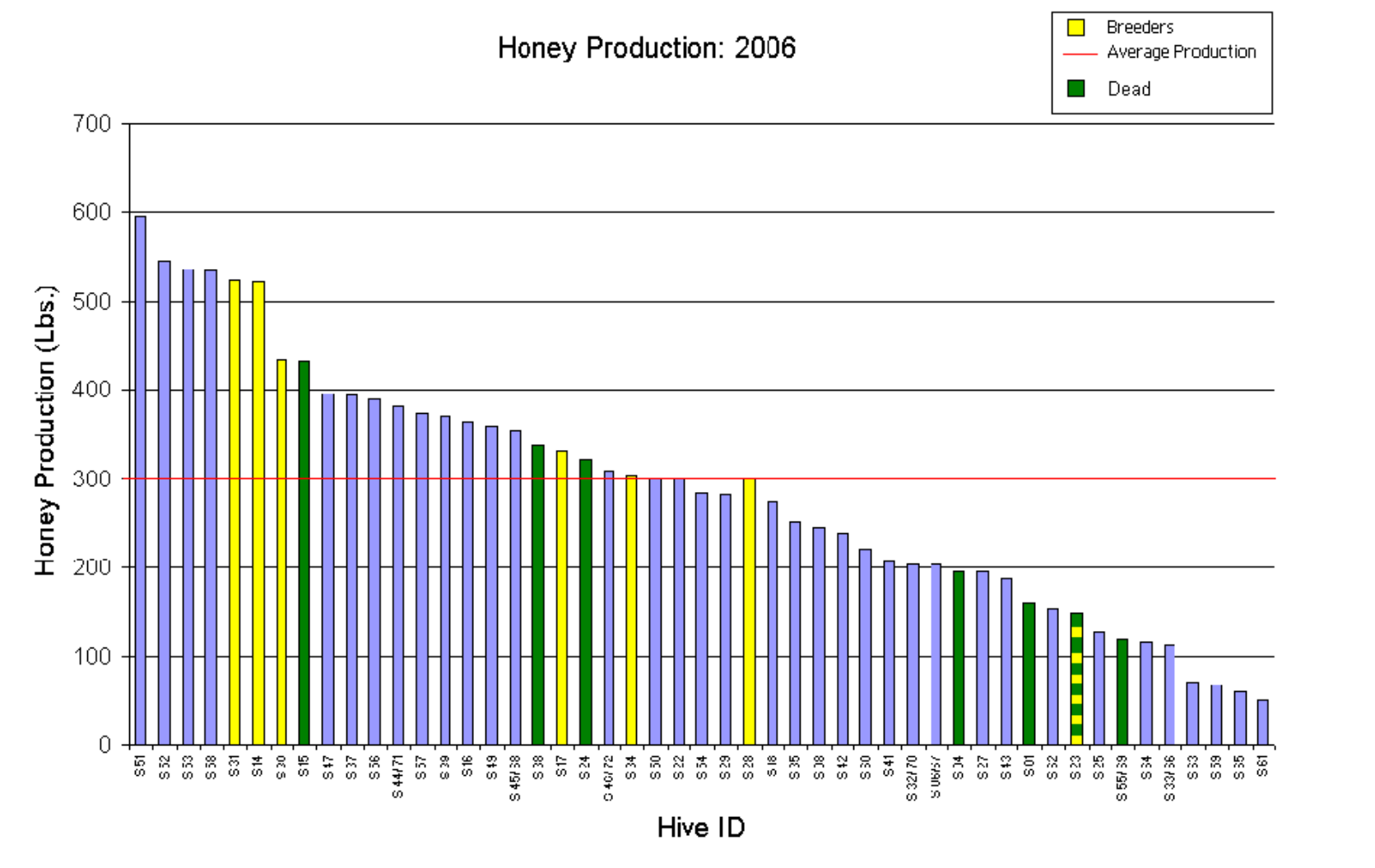

Honey production in 2006 shown for all Saskatraz colonies (48), including new additions made in 2006.

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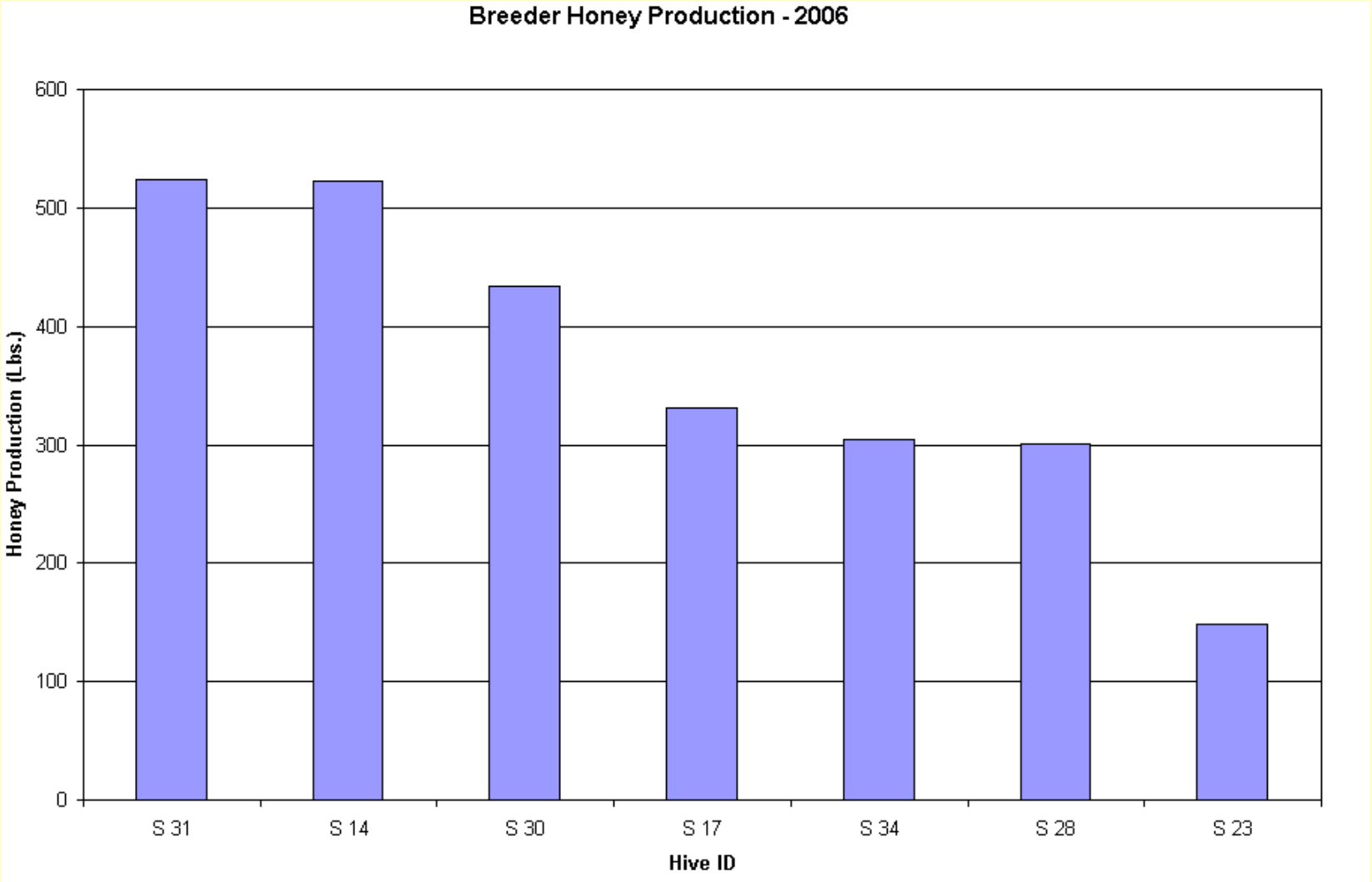
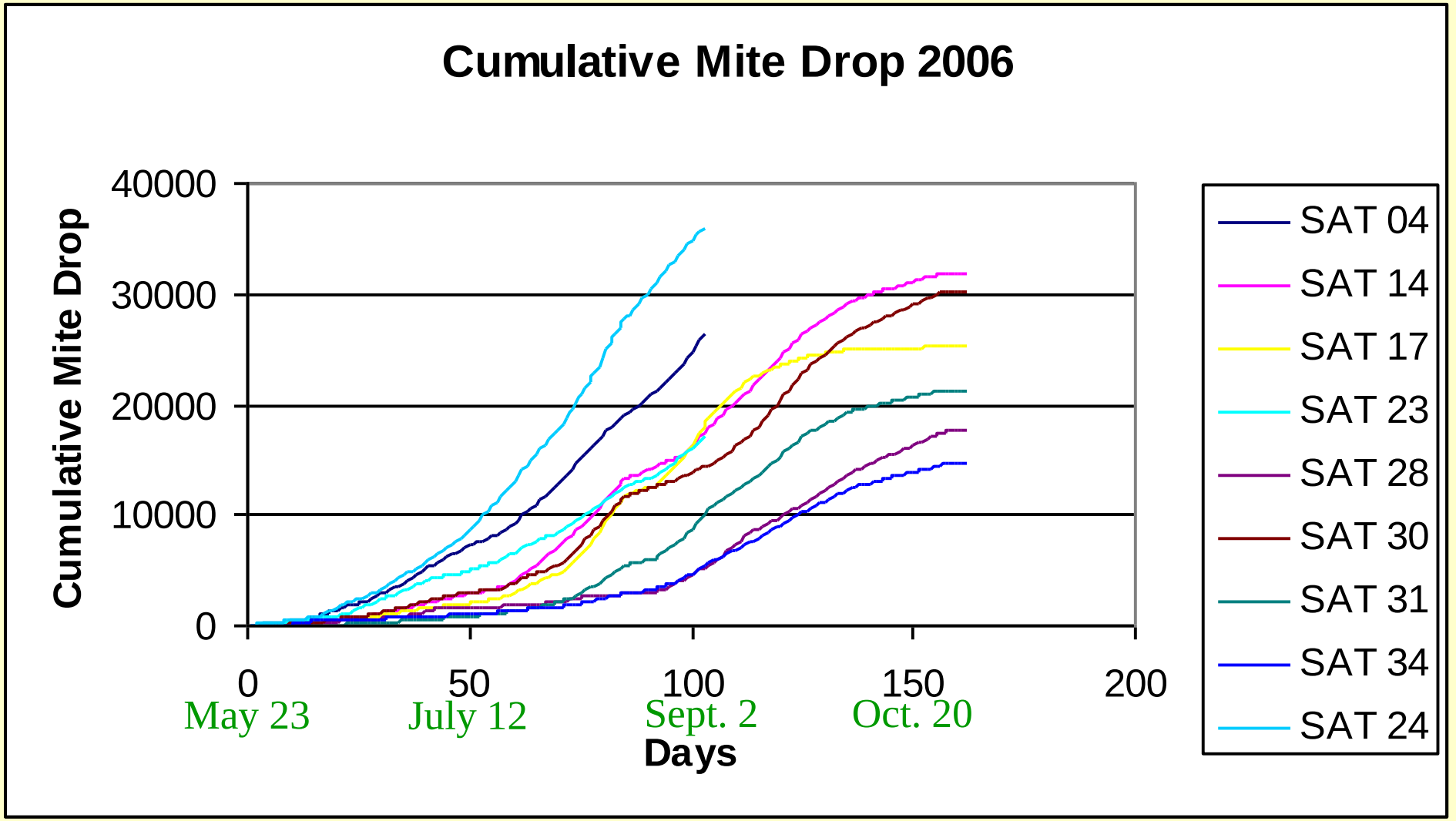


Total honey production in 2006 shown for 2005 selections and SAT-31 selected in 2006.


Cumulative Mite Drop 2006
Cumulative Mite Drop
40000
30000
20000
10000
0
0
50
100
150
200
May 23
July 12
Sept. 2
Oct. 20
Days
SAT 04
SAT 14
SAT 17
SAT 23
SAT 28
SAT 30
SAT 31
SAT 34
SAT 24

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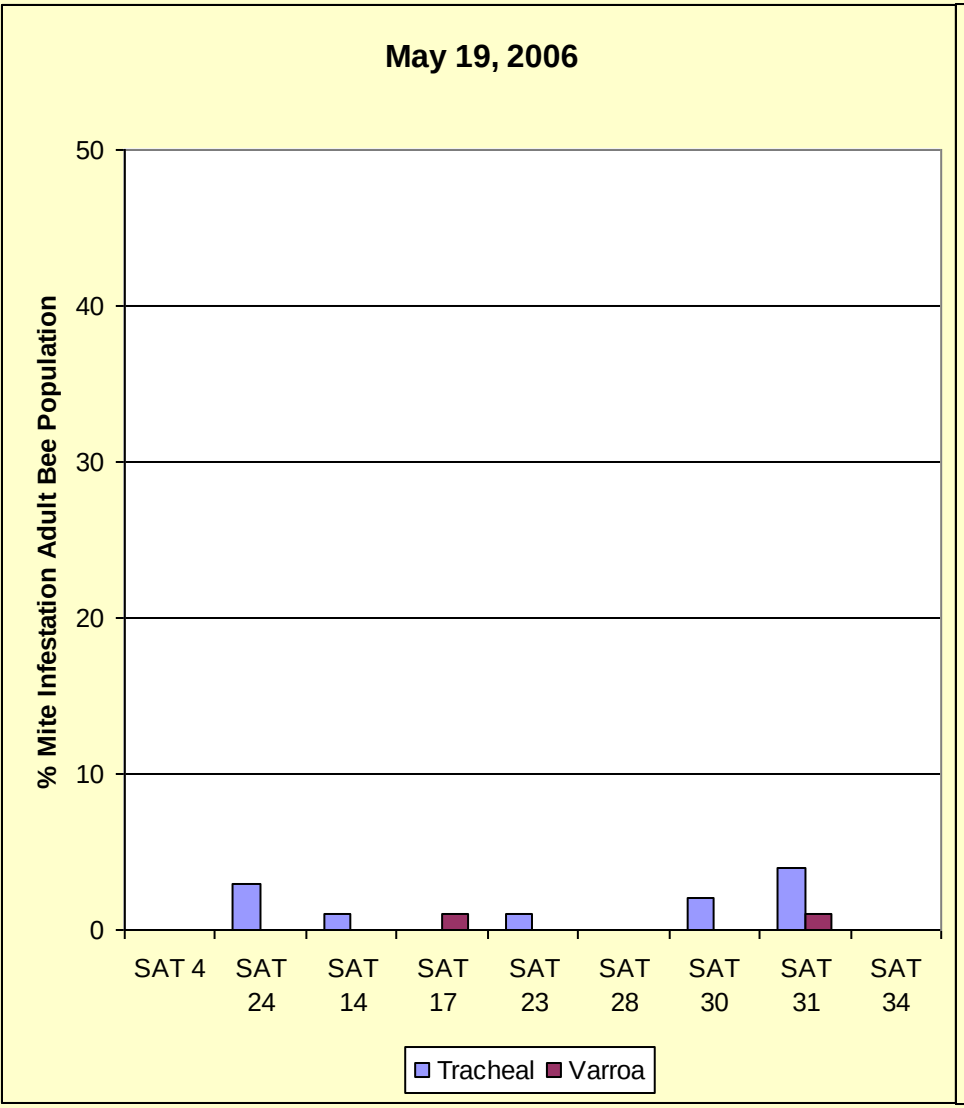

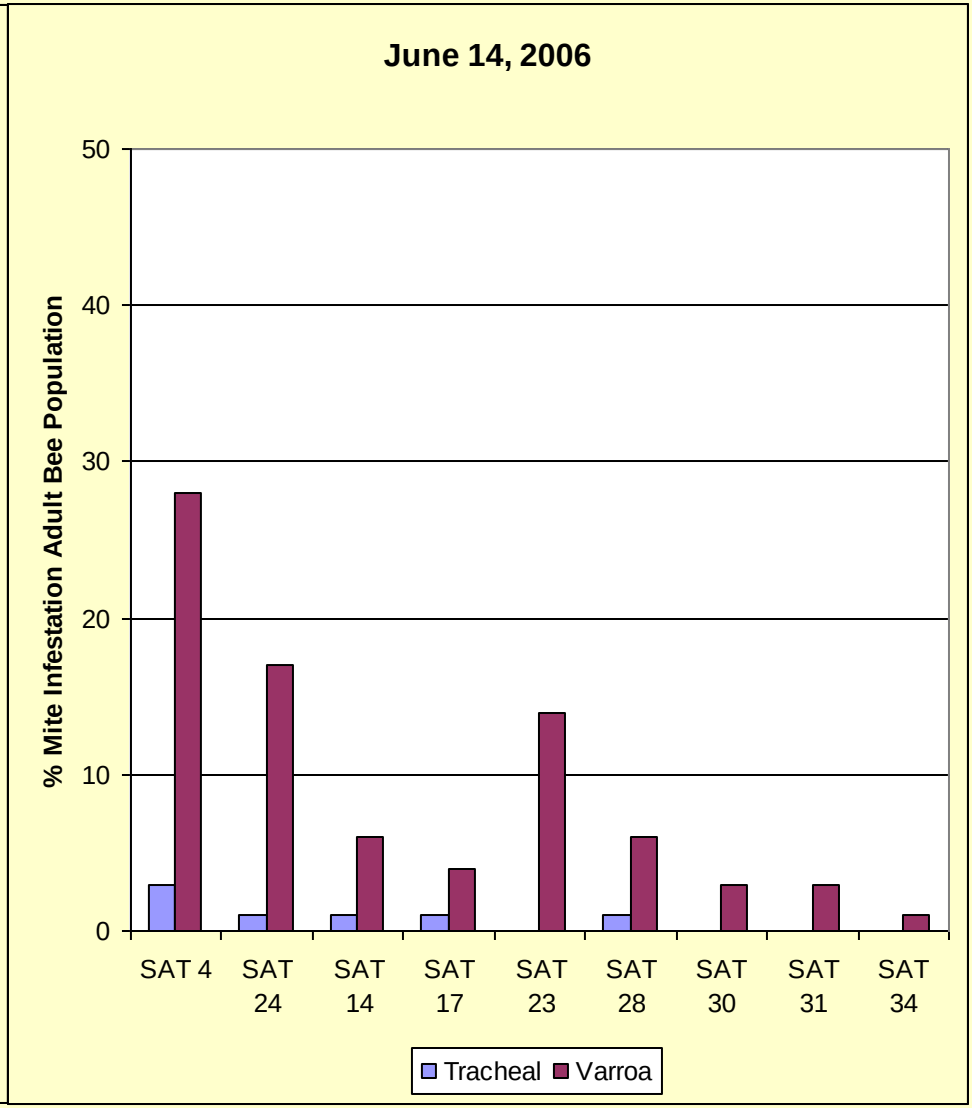

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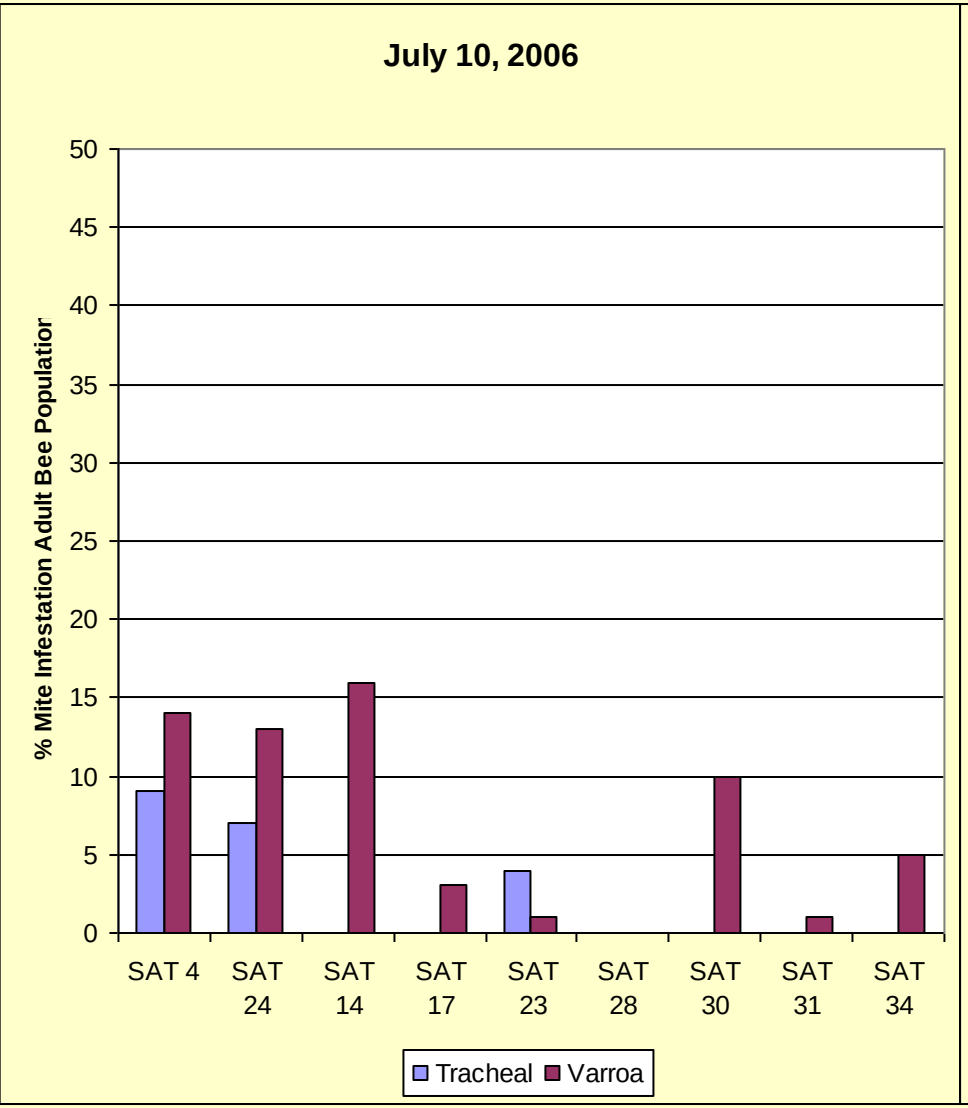

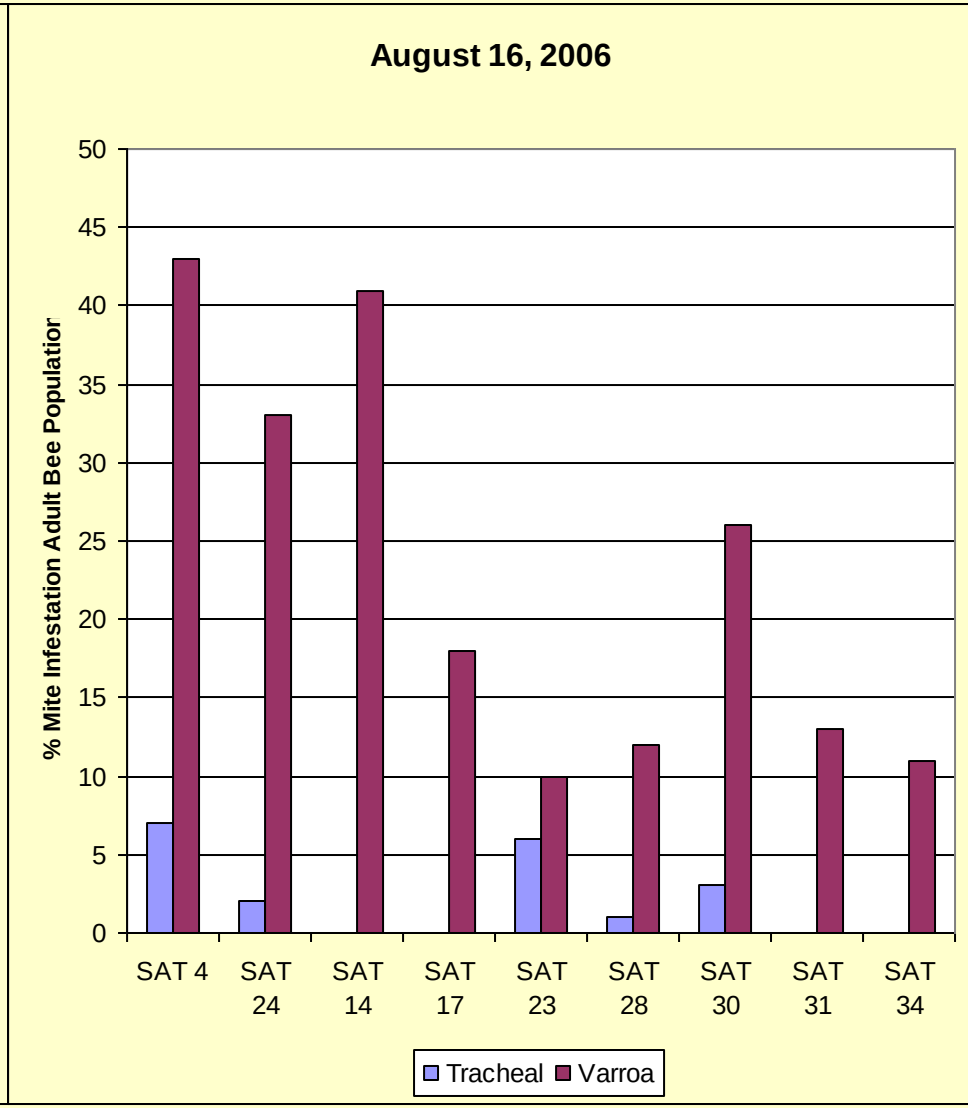

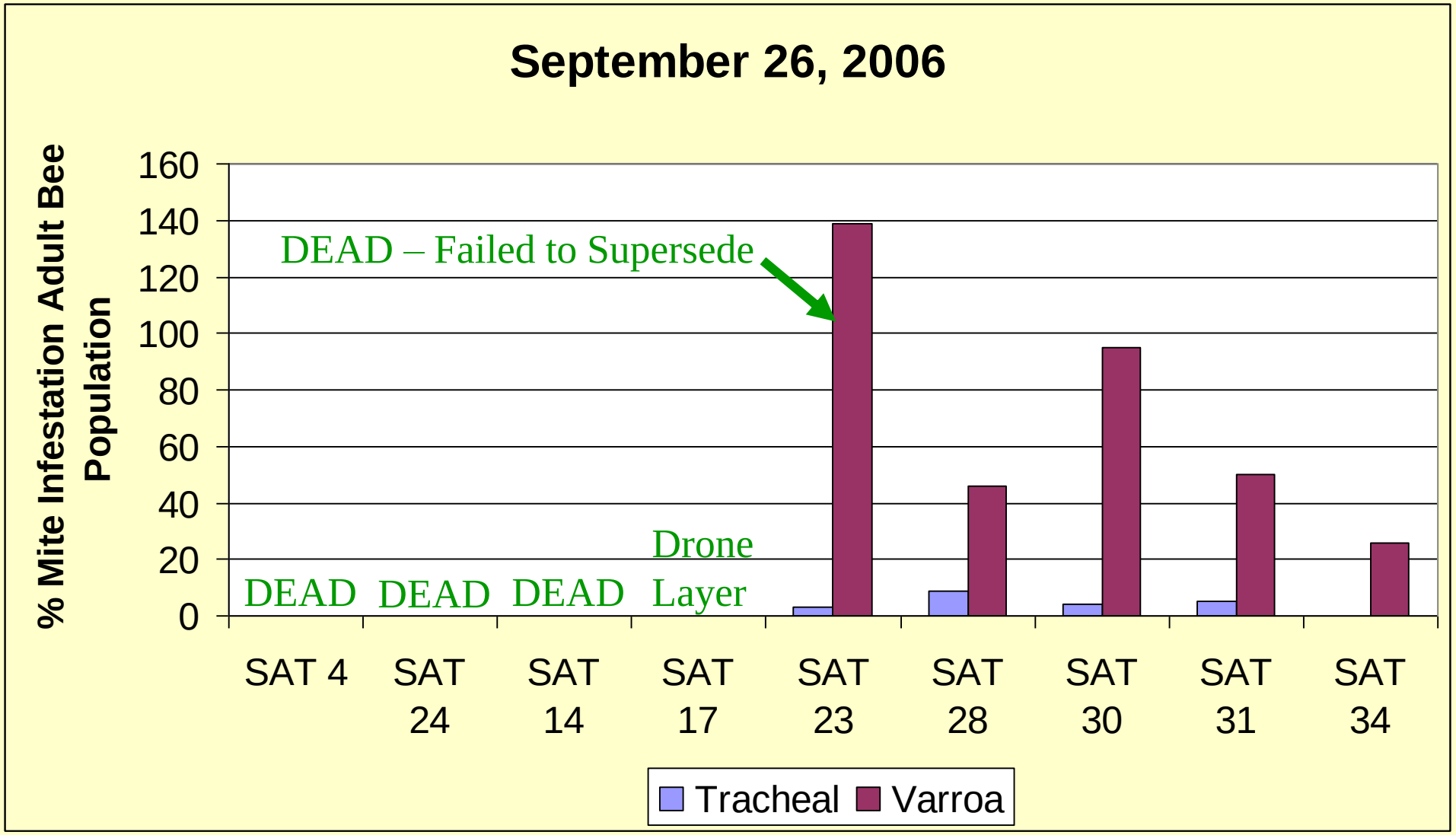
September 26, 2006
% Mite Infestation Adult Bee Population
160
140
120
100
80
60
40
20
0
DEAD – Failed to Supersede
Drone Layer
DEAD
DEAD
DEAD
SAT 4
SAT 24
SAT 14
SAT 17
SAT 23
SAT 28
SAT 30
SAT 31
SAT 34
Tracheal
Varroa

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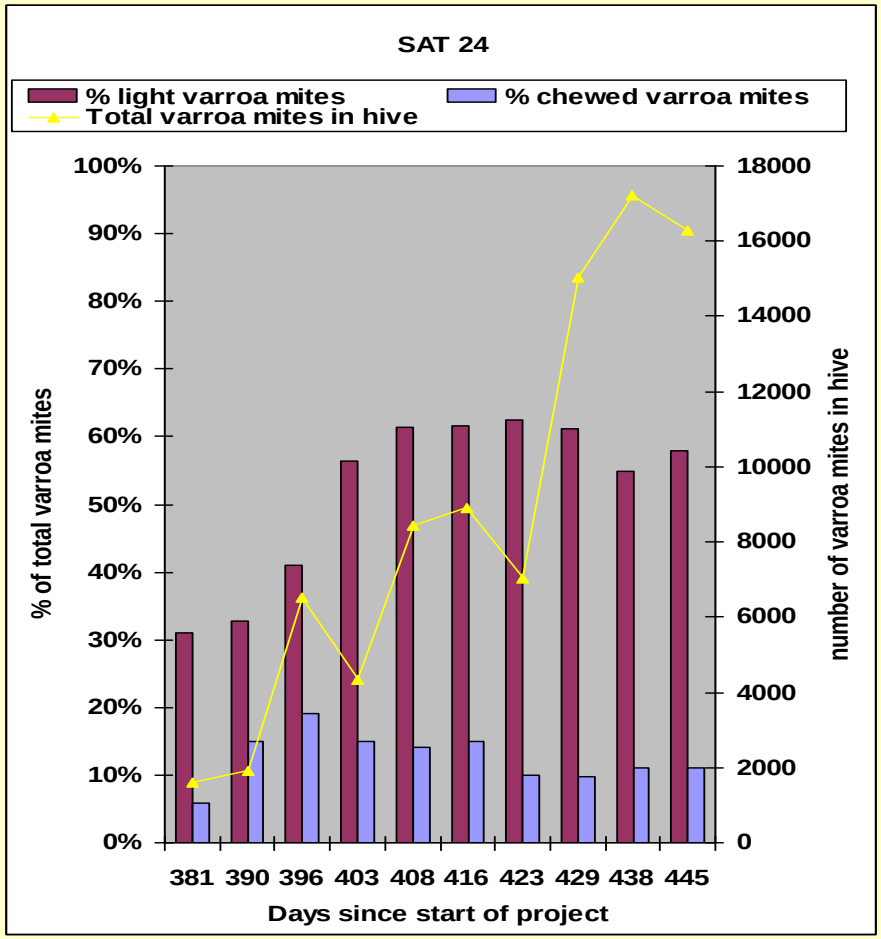
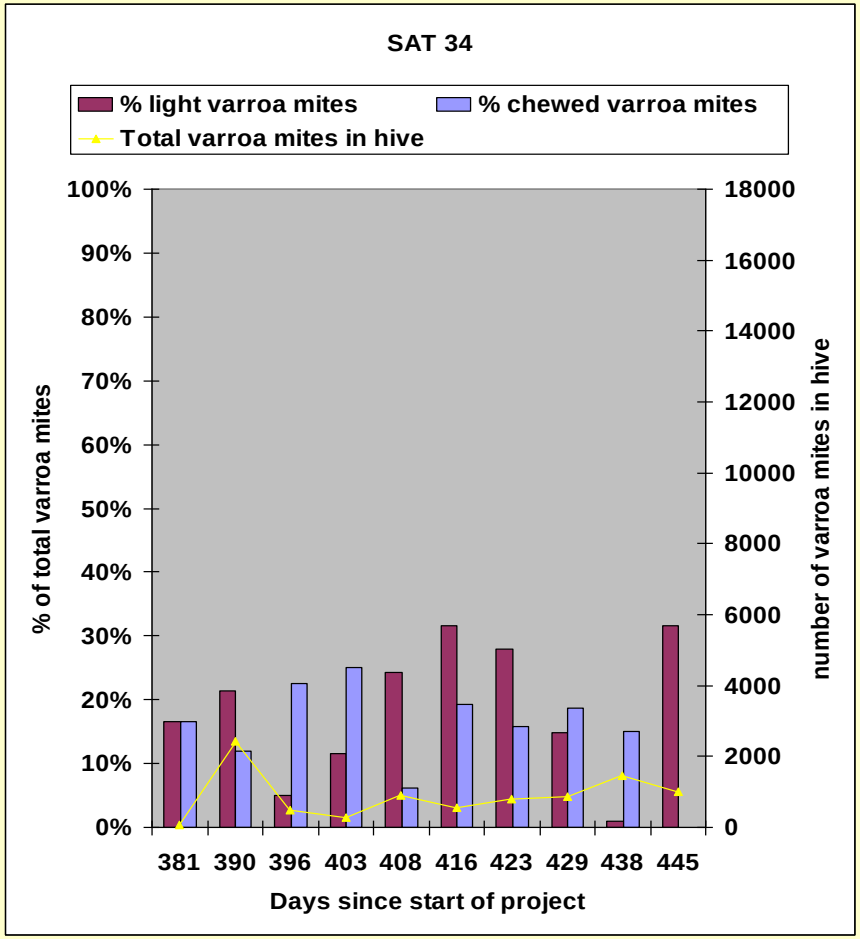
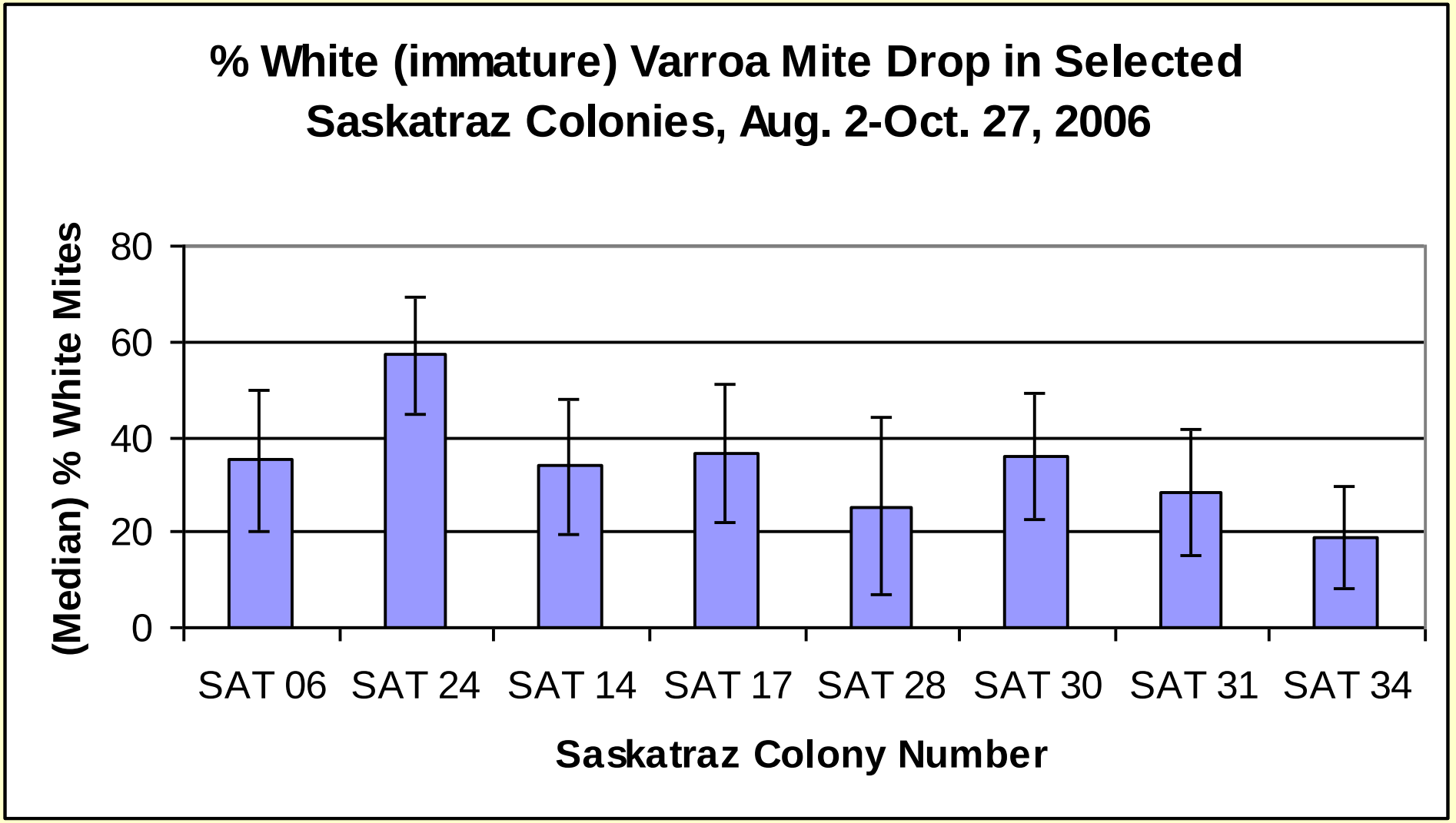




During natural drop Varroa mite population analyses 100 mites from each sticky board were sampled and microscopically assessed for light mites (immature) and mites damaged by chewing. SAT-24 (non selected), SAT-34 (selected).


% White (immature) Varroa Mite Drop in Selected Saskatraz Colonies, Aug. 2-Oct. 27, 2006
(Median) % White Mites
80
60
40
20
0
SAT 06
SAT 24
SAT 14
SAT 17
SAT 28
SAT 30
SAT 31
SAT 34
Saskatraz Colony Number

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Intact Varroa Mite

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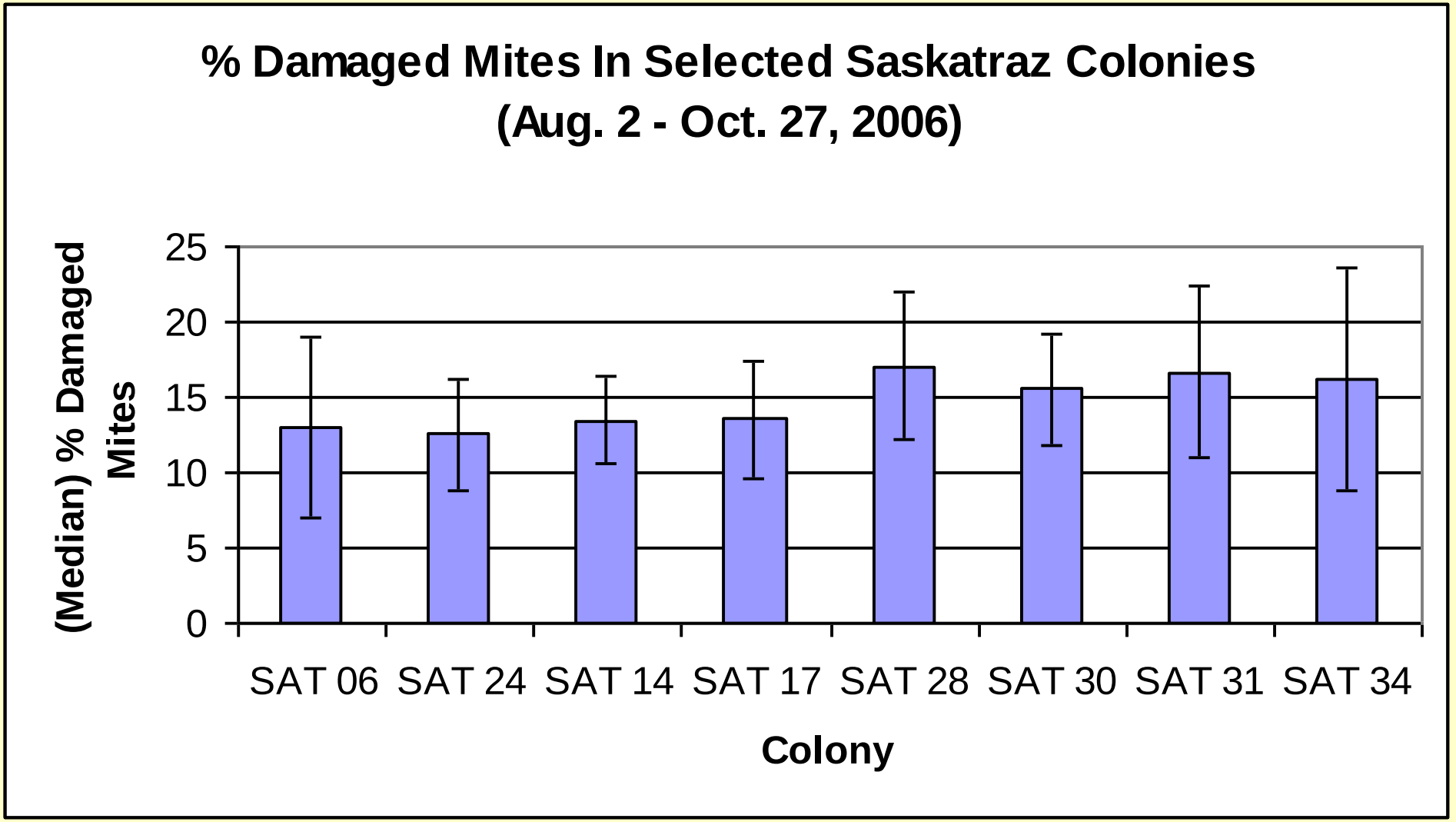
Mutilated Varroa Mite


% Damaged Mites In Selected Saskatraz Colonies
(Aug. 2 - Oct. 27, 2006)
(Median) % Damaged Mites
25
20
15
10
5
0
SAT 06
SAT 24
SAT 14
SAT 17
SAT 28
SAT 30
SAT 31
SAT 34
Colony

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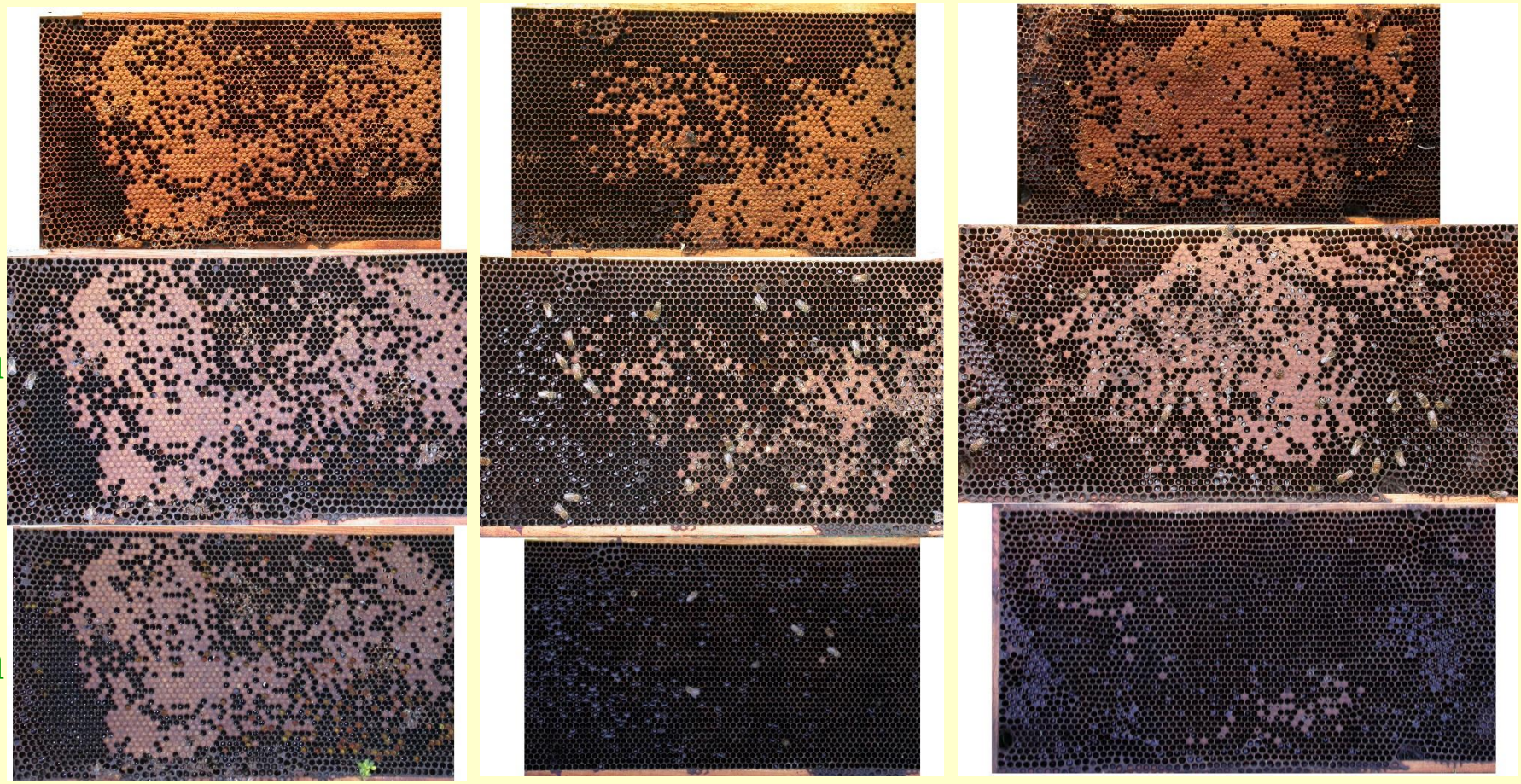

SAT-24 SAT-28 SAT-34

Hygienic behaviour of Saskatraz selections after exposure of whole frames of dead pupae. Both SAT-28 and SAT-34 showed significant uncapping after 24 h and extensive removal of dead pupae after 48 h exposure.

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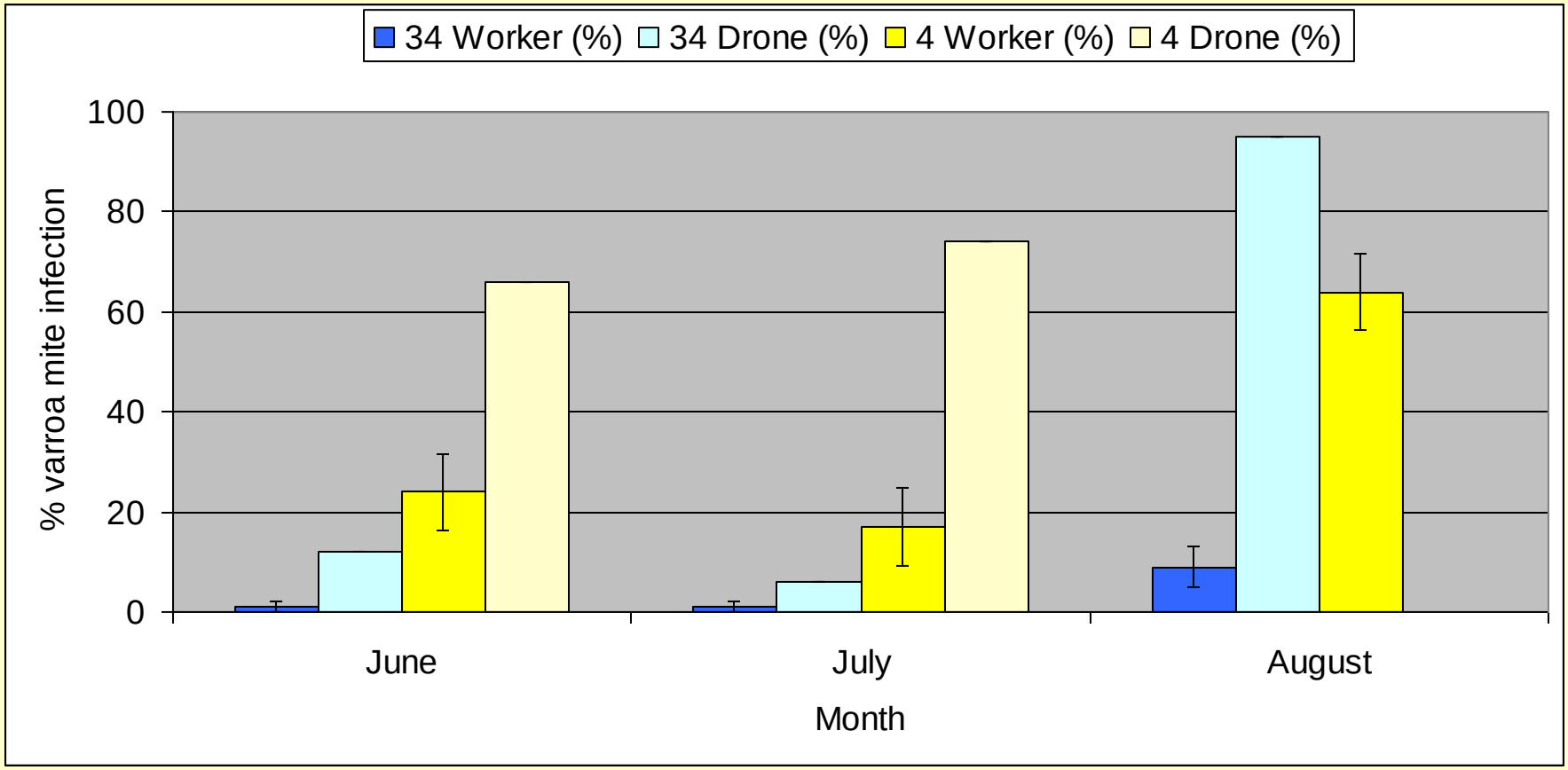

Drone and worker brood was randomly sampled between June and Aug. to determine if differences exist between colonies in percent varroa mite infestations between worker and drone brood. SAT-34 showed low levels of infestation in worker brood, but high levels in drone brood by Aug. A non-selected colony showed a general increase in worker brood infestation by Aug.

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September 2006

These bees look good, but they are dying. Varroa mites will Kill these bees and themselves in less than 3 months.

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Where have all the bees gone?

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Frames full of honey and pollen, no evidence of robbing.

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Hives full of honey and pollen, but no bees.
No sign of Winter dysentery, queen failure, supersedure or starvation
In most cases. No bees robbing.

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Clean bottom boards with no accumulation of dead bees in front of hives.

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Saskatraz Survivor

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Saskatraz Survivors
May 2007

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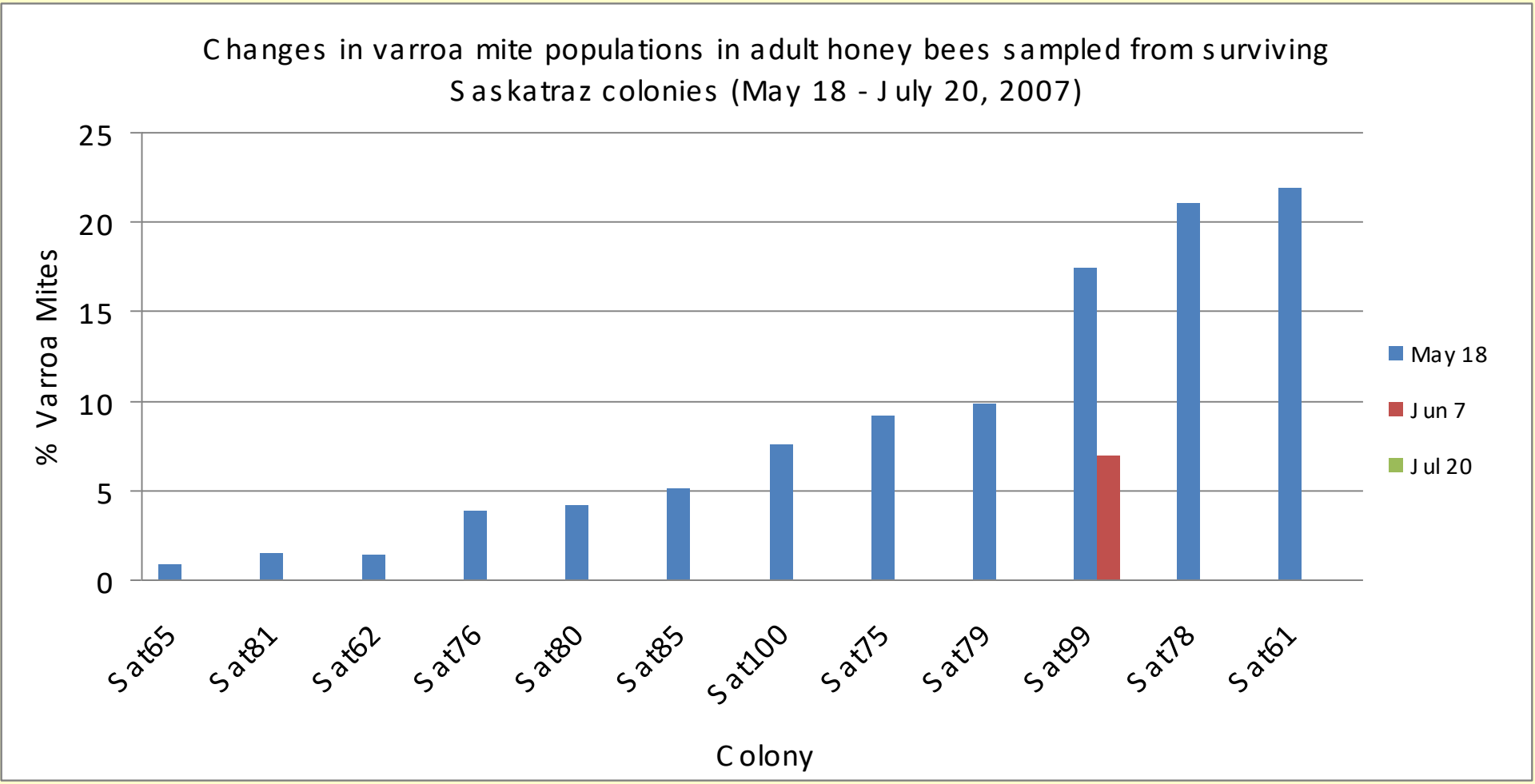

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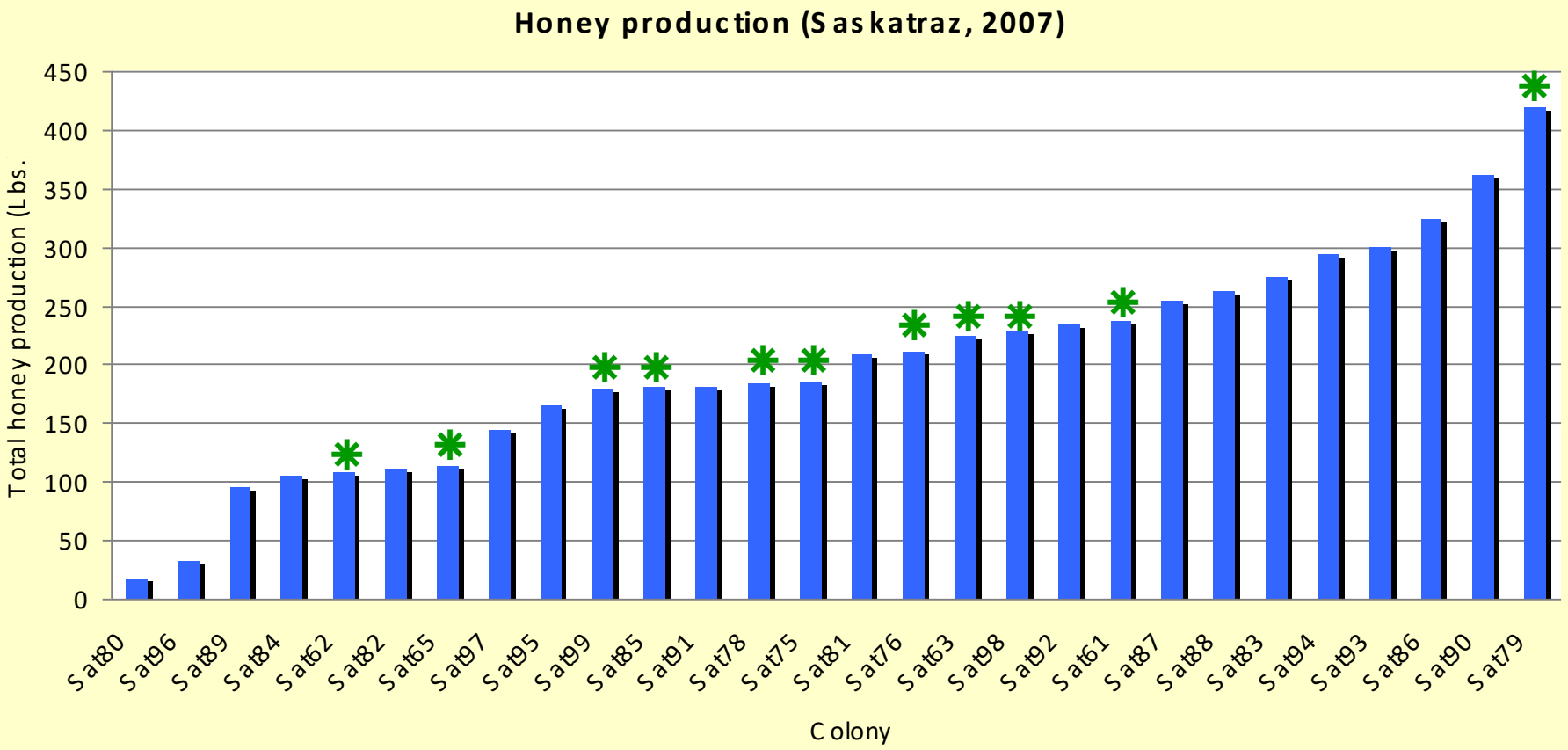

Honey production per hive was determined by weighing all supers of honey produced by each colony. Honey was harvested at two time periods between July 15 and Sept. 10, 2007. Bars with stars denote Saskatraz survivor colonies, yard ave. 200 lbs. per hive

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Saskatraz – May 2008

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SAT-65, 86, 87, 88 – added 2 supers by end of May – No spring feed.

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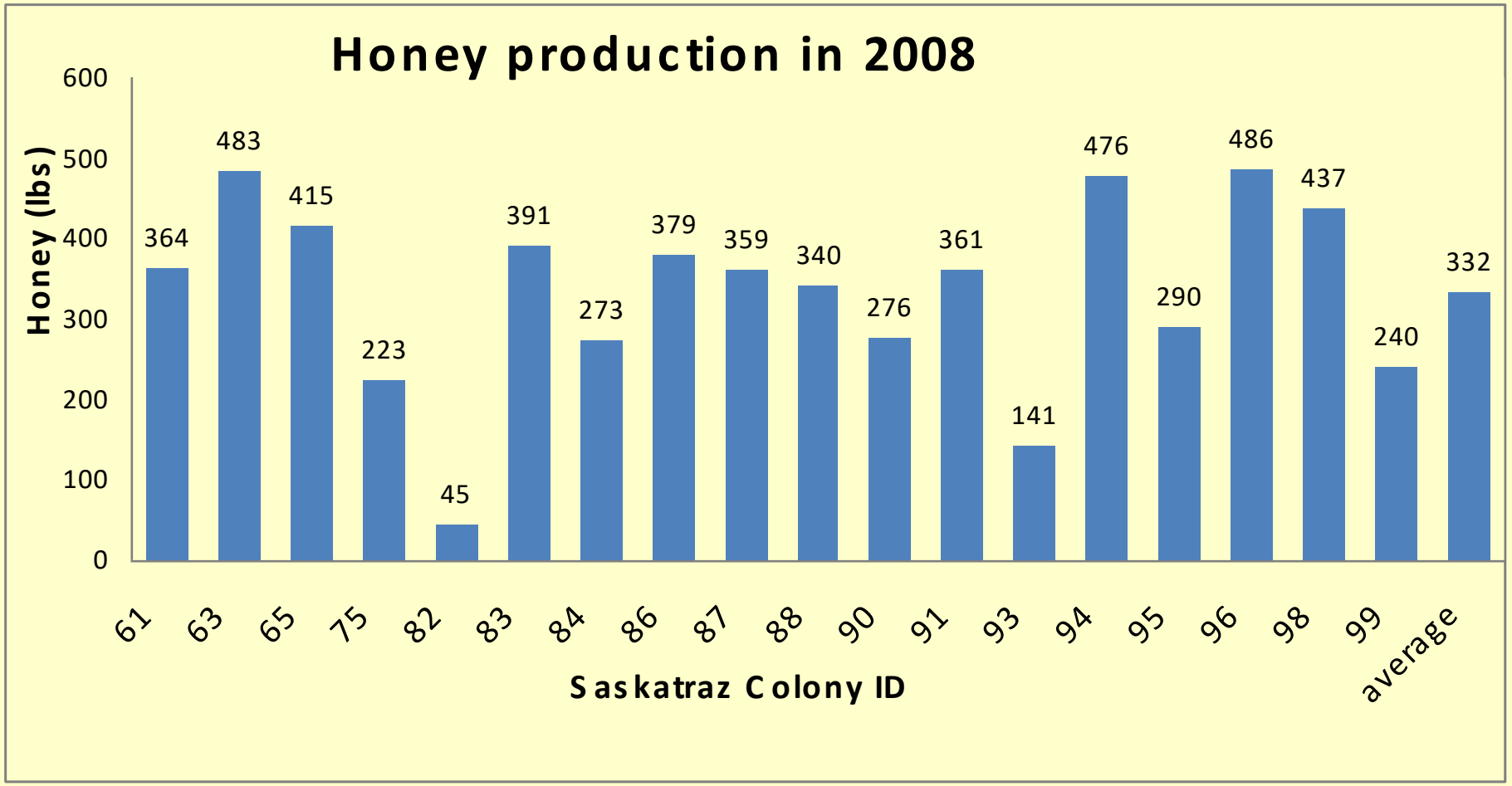

Eleven Colonies produced over 332 lbs. SAT-63, 65, 94, 96, and 98 produced over 400 lbs.

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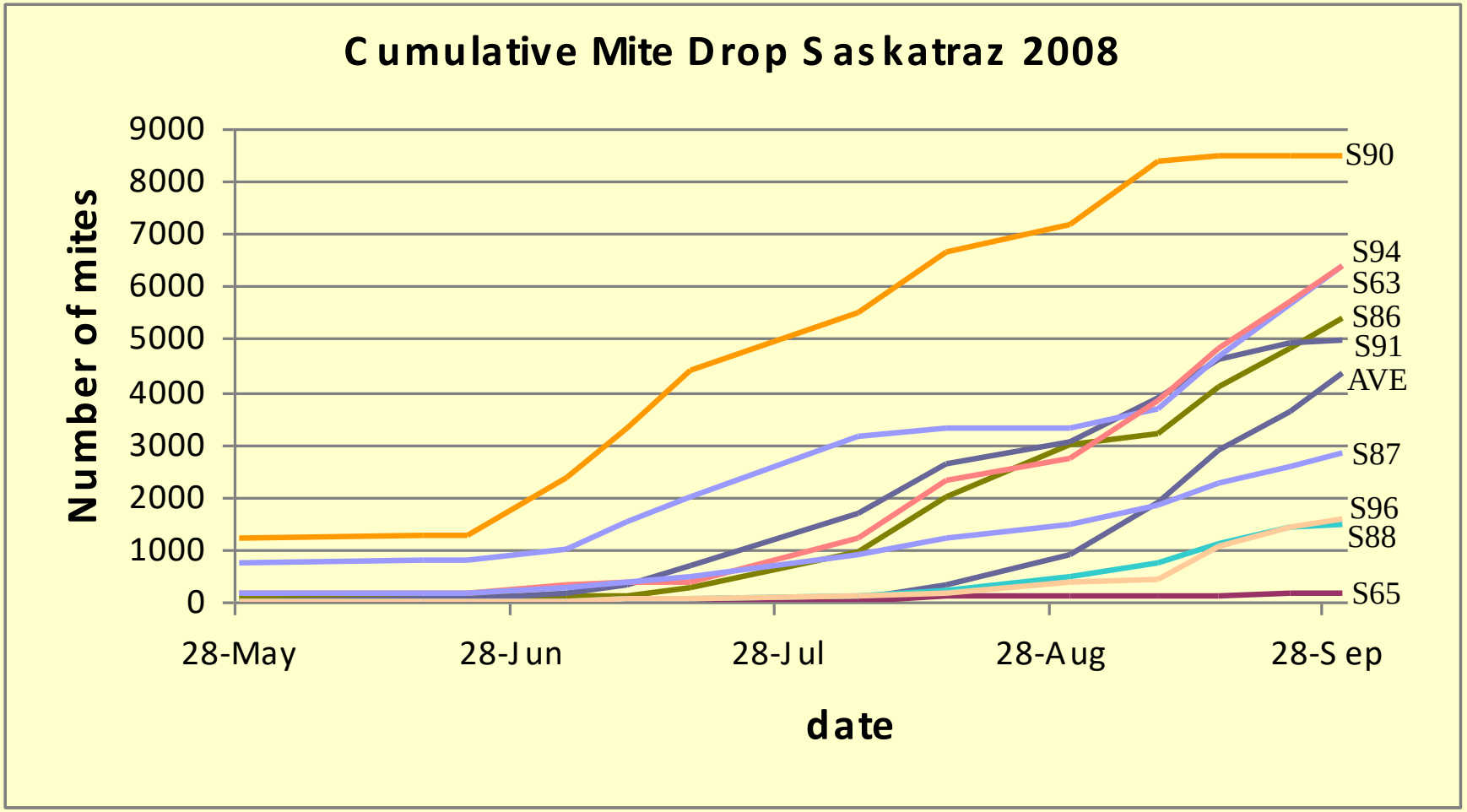

Mites were counted weekly on sticky boards (natural drop)

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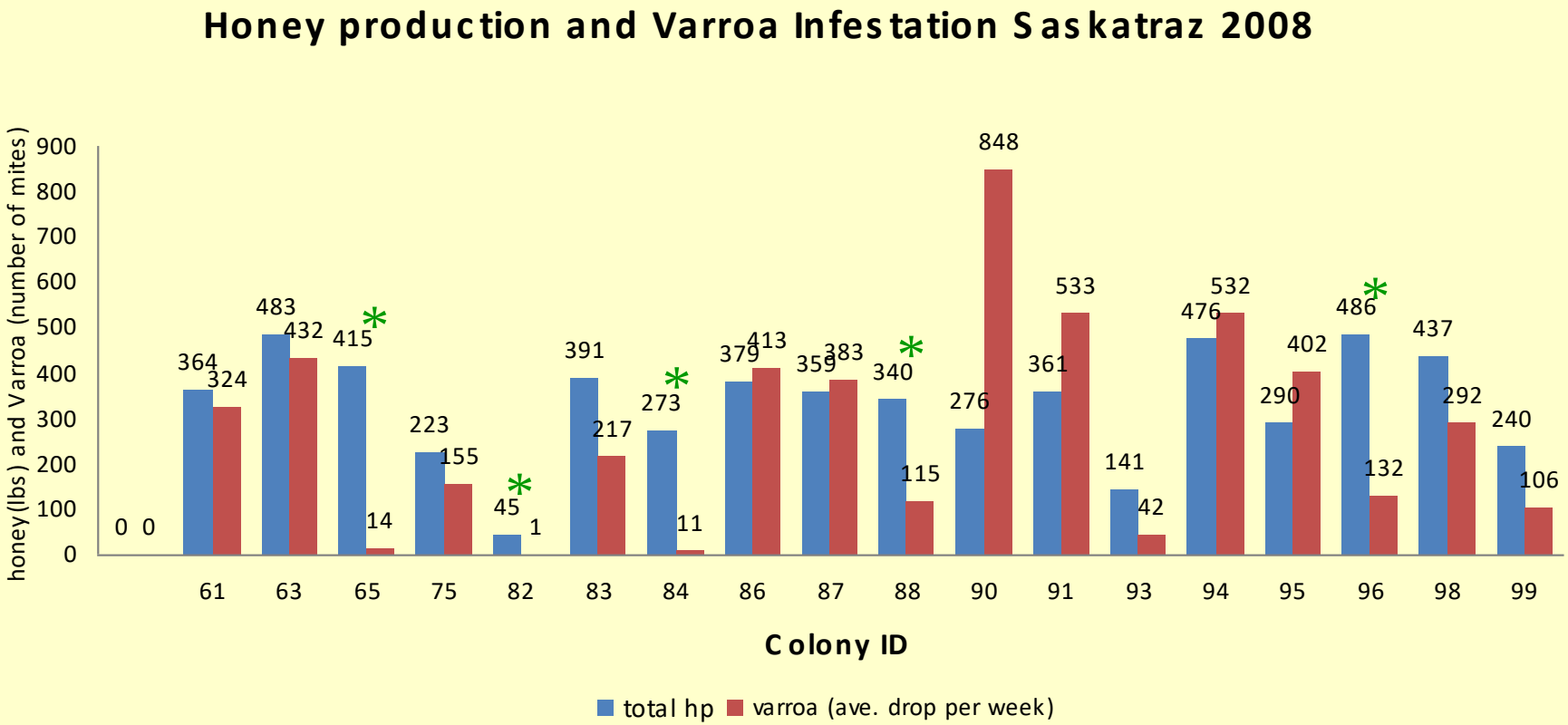

SAT-65, 84, 88, 96 and 98 produced economical honey yields and showed the best varroa mite population growth suppression. Some colonies showed no detectable varroa, but no significant honey production (SAT-82)

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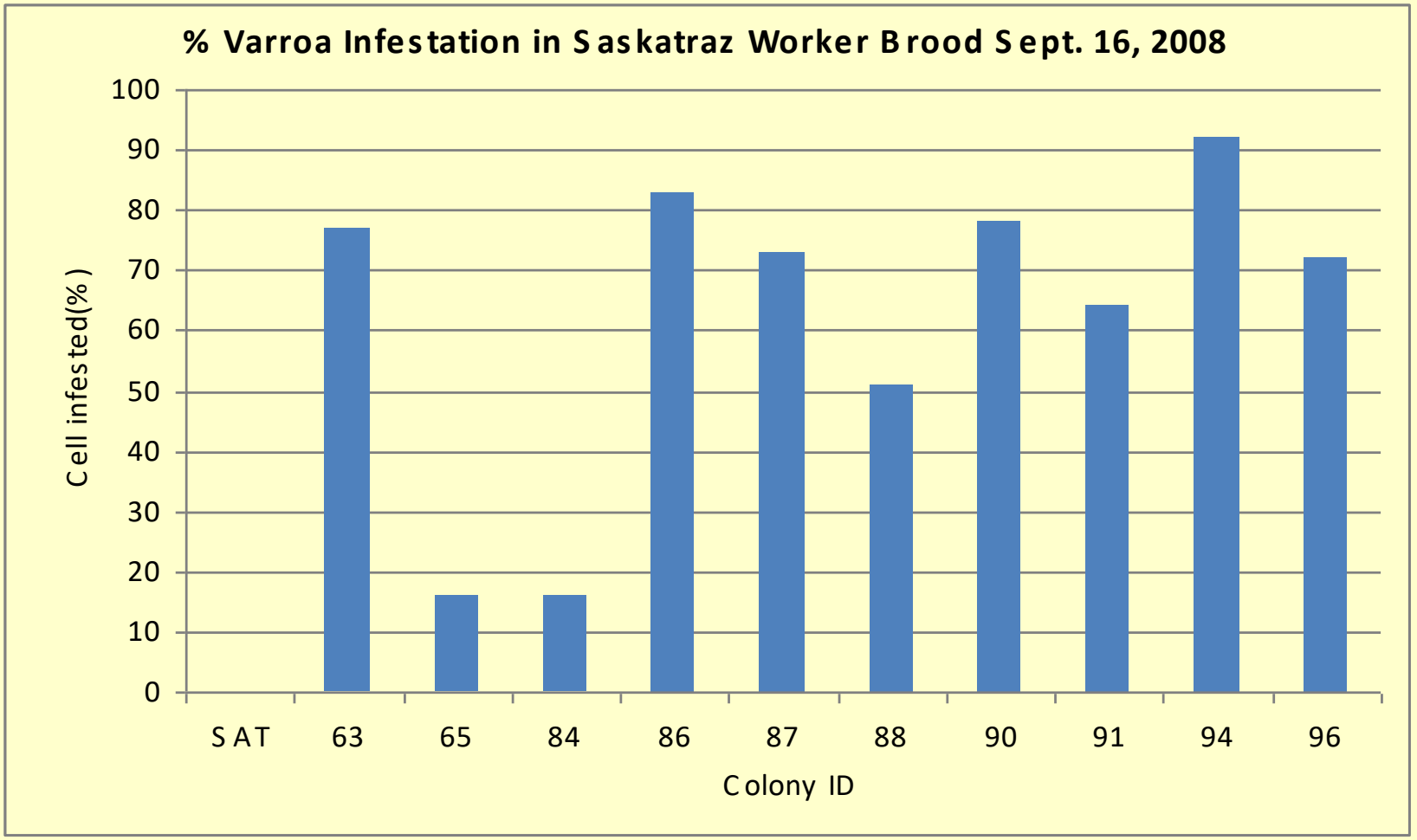

Only selected Saskatraz colonies are shown. Saskatraz-65 and 84 showed the lowest percent varroa infestation in worker brood.

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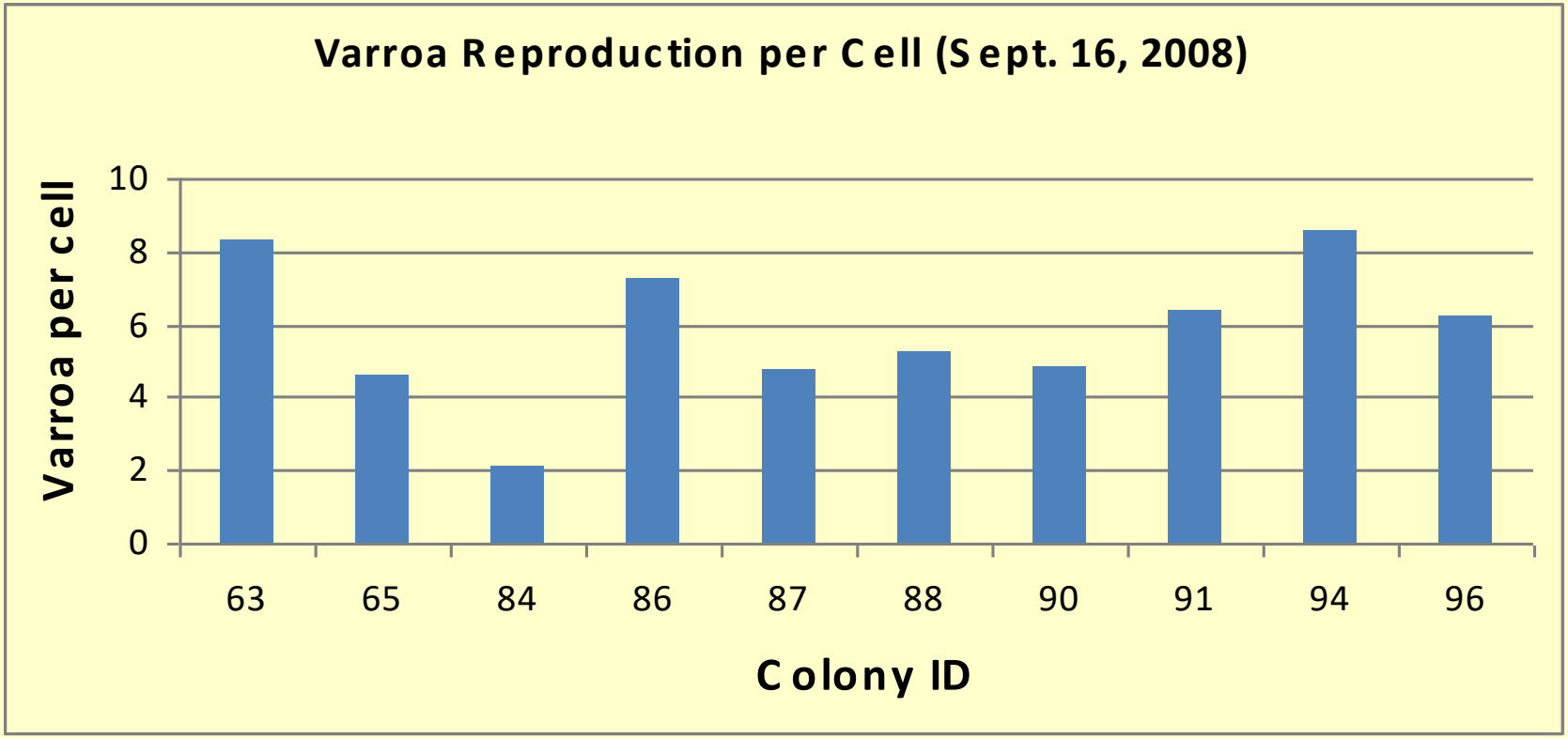

Saskatraz 84 and 65 showed the lowest number of varroa per cell. Comb were randomly sampled from sealed brood of all colonies.

Summary and Future Directions

- Selections made between 2004-06:
  - SAT-14, 17, 23, 28, 30, 31, and 34
- Selections showing best honey production:
  - SAT-14, 17 and 30
- Selections showing best varroa mite suppression: SAT-28, 31, 34
- All selections showed low to non-detectable tracheal mite infestations .
- SAT-34 and 28 showed excellent hygienic behaviour, but intermediate honey production. Also, maintained lower levels of varroa mites in worker brood, and showed more aggressive behaviour towards varroa mites (more damaged mites).
- Progeny of 2004-06 selections undergoing second year of evaluation under commercial conditions at Meadow Ridge.

Summary and Future Directions, cont'd:

- Re-selected outcrosses established in closed population (Saskatraz ECP) for mating with new Saskatraz selections.
- Specific crosses continue to be made by instrumental insemination between selections made for honey production and varroa tolerance.
- Selections made between 2007-08:
  - SAT-63, 65, 84, 87, 88, 94, 96 and 98
    - Best for honey production and varroa suppression: SAT 65 and 96
    - Daughters of the 2007-08 selections were crossed at Saskatraz ECP in the summer of 2008. Progeny analyses of these crosses will begin in 2009.
    - Two of the best selections; SAT 31 and 65 were the results of previous selections superseding and mating at the Saskatraz yard site.

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- All selections and varroa sensitive lines are being subjected to microsatallite analysis with 20 informative markers.
- The "Saskatraz Project" has become a valuable model system to study colony collapse disorder (CCD).
  - Diverse genetic pool
  - Minimal exposure to environmental pesticides
  - No chemical miticide treatments
  - Permanent yard site
  - Isolated area with diverse sources of pollen and propolis from many wild species of plants (good nutrition from natural sources)
  - Colonies fed sucrose not corn syrup
  - Colonies were thoroughly monitored for varroa and tracheal mite growth since 2004
  - Bees and mites were sampled from all colonies for molecular analysis from 2006 to 2008.

Severe mite infestation at Saskatraz, produced symptoms similar to those reported for CCD

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Post-mortem analyses. Dead pupae loaded with varroa mites

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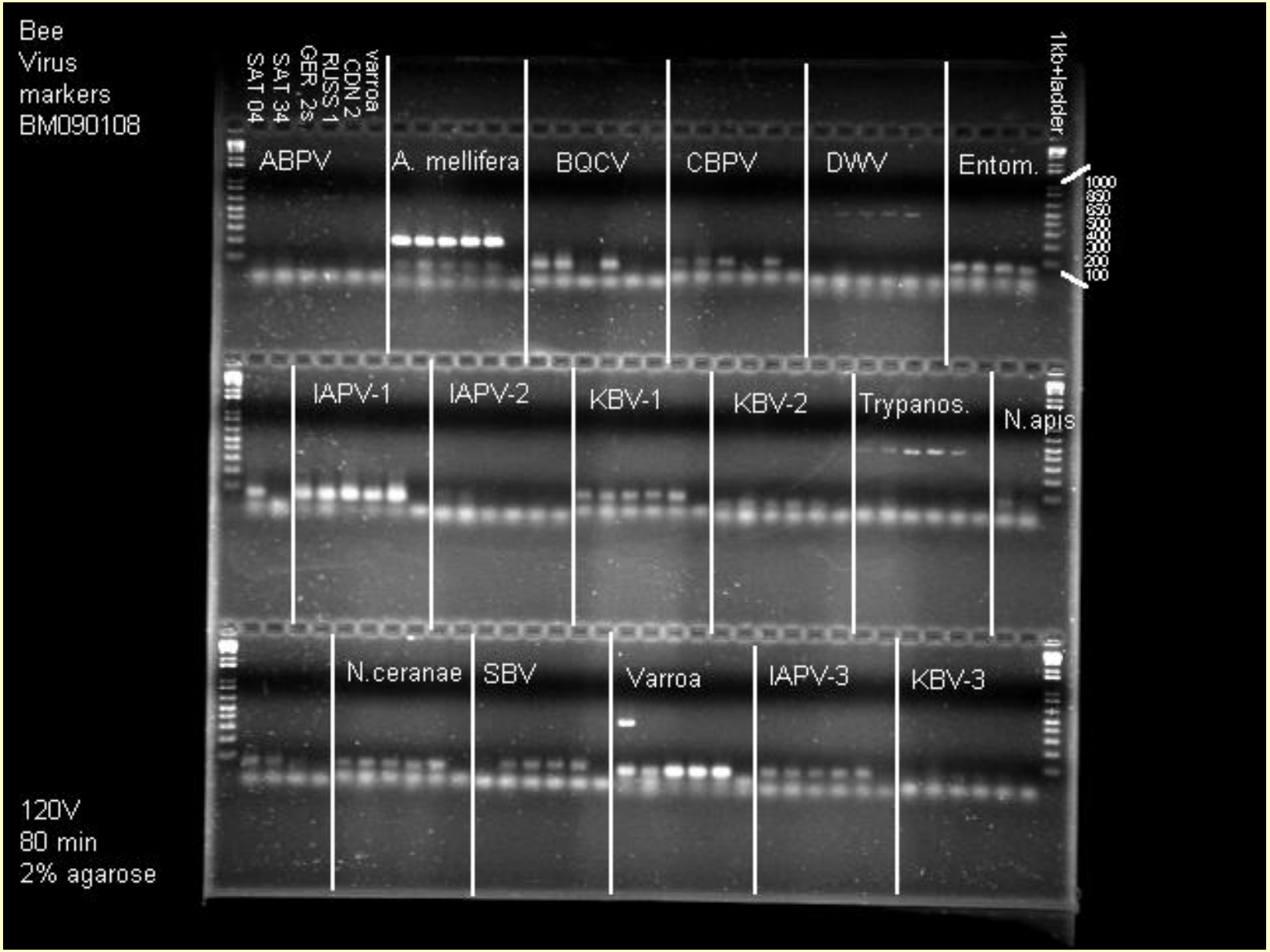

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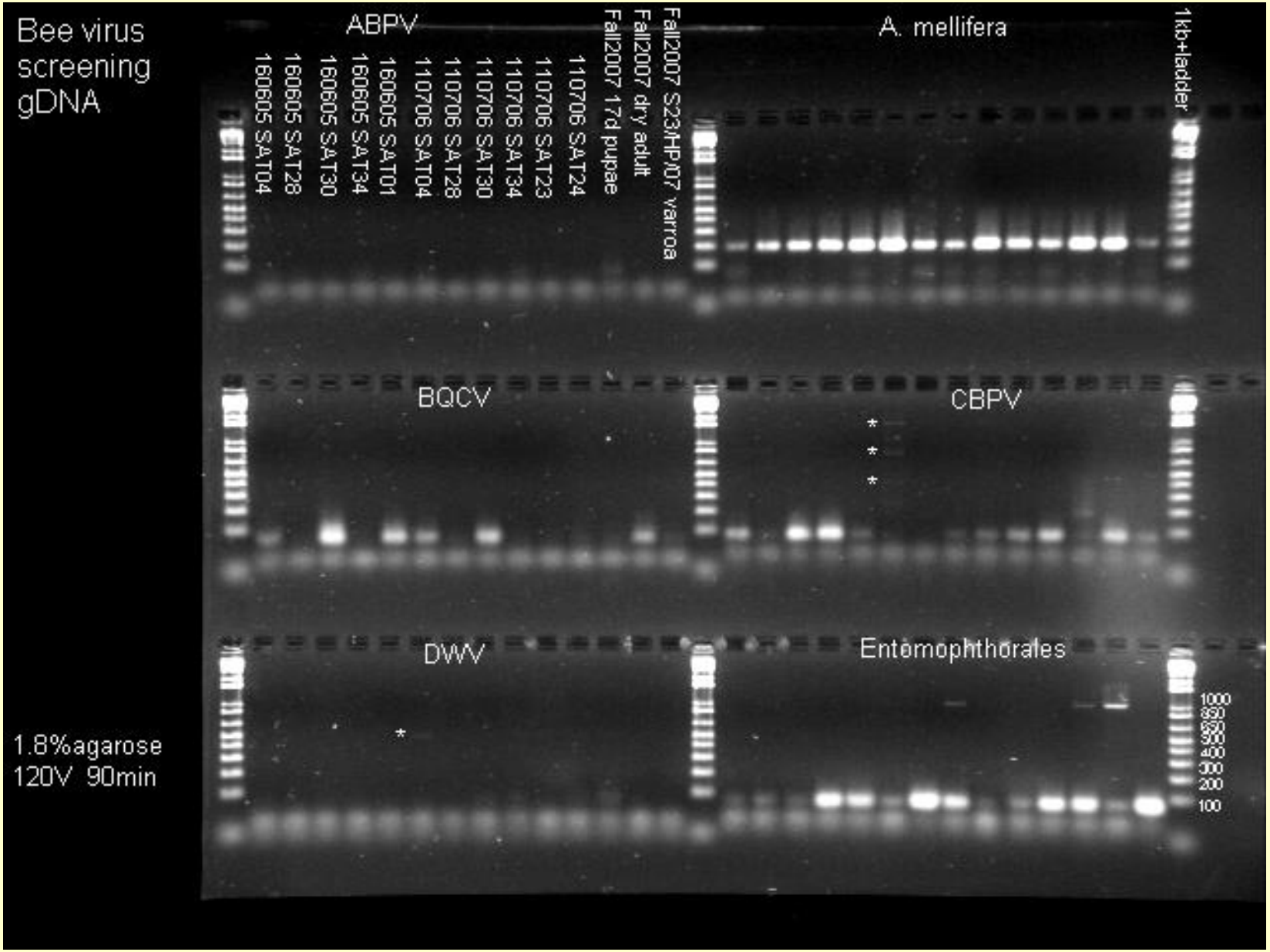

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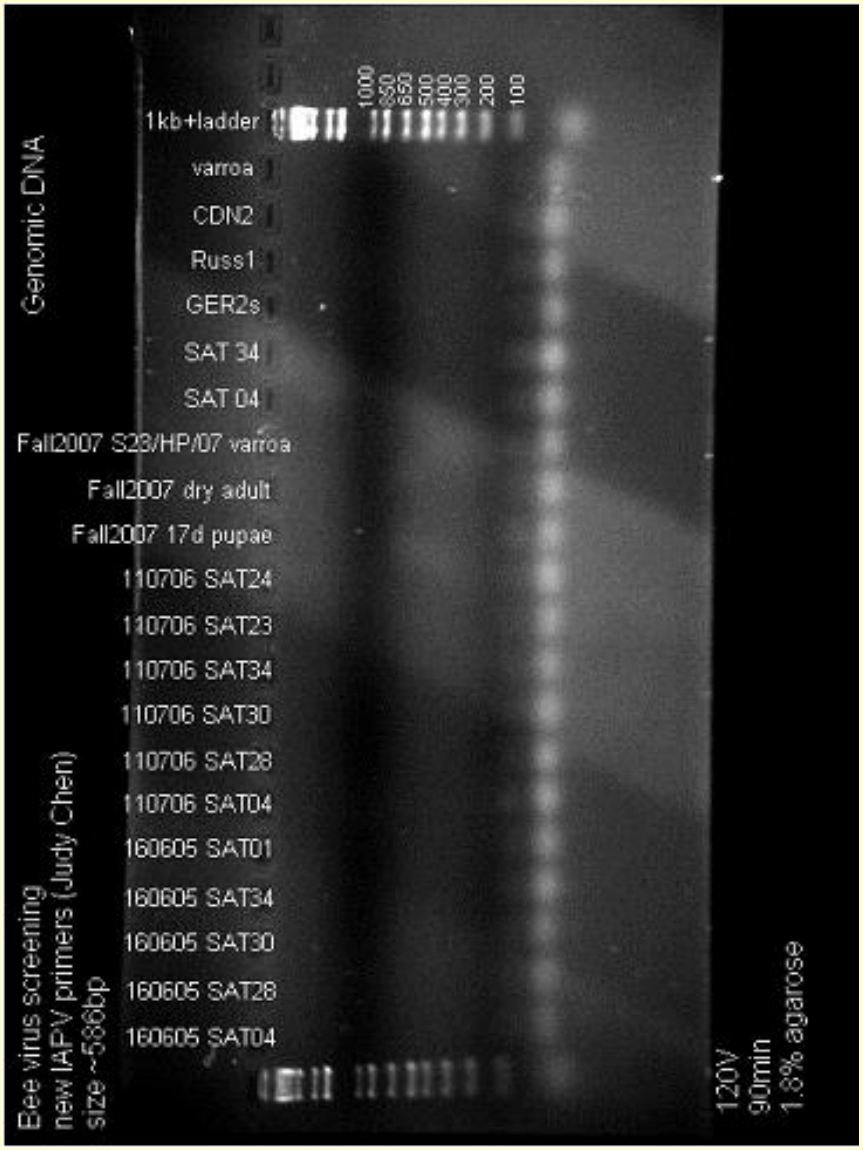

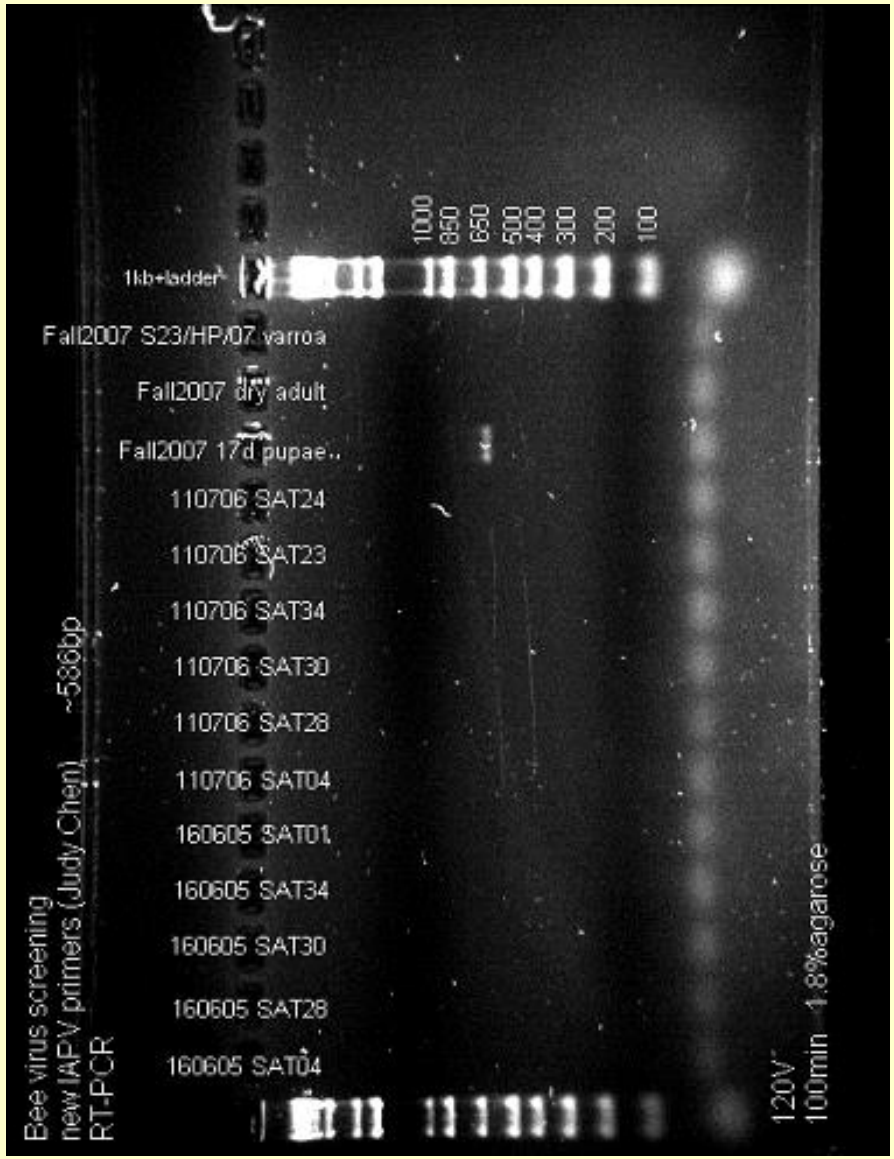

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Saskatraz-90: Average number of mites per cell = 6.3 (in infested cells)

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SAT-94: Healthy bee, pre-emergent pupae – no varroa infestation

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SAT-94: Pre-emergent pupae - varroa infested, Ave. 8.5 mites/cell

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SAT-94: Healthy pre-emergent pupae

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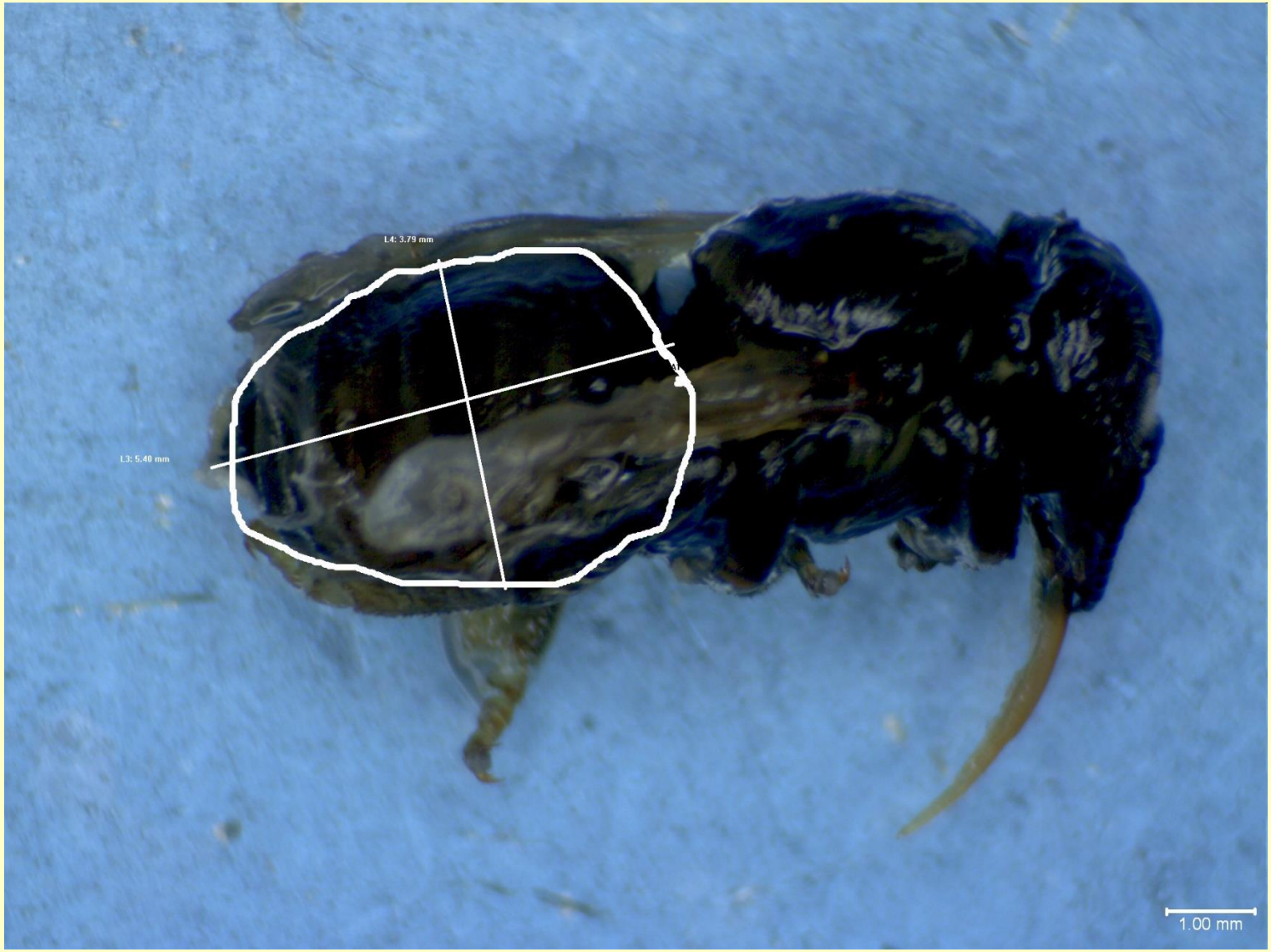

SAT-94: Pre-emergent pupae - varroa infested, deformed abdomen and wings

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Conclusion

- Currently testing hypothesis that severe varroa mite infestation is causing CCD by inducing the expression of pathogenic viruses such as IAPV and DWV.
- Extensive RT-PCR analysis is being performed on Saskatraz samples collected from healthy and collapsed colonies.

Questions?

Acknowledgements

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