Teabe- ja õppepäeva korraldamist toetab Euroopa Liit Eesti riikliku mesindusprogrammi 2010 – 2013 raames

Breeding for resistance against diseases

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American foulbrood

- Park 1937: genetic differences. Transferred pieces of diseased comb.
- Woodrow & Holst 1942: resistance by hygienic behaviour
- Sturtevant & Revell 1953: proventriculus filters spores
- Rothenbuhler & Thompson 1956: larval resistance

Hygienic behaviour

- Rothenbuhler: two recessive genes, uncapping and removal. Revised later by Moritz, Spivak, Oxley etc.: more complicated genetics.
- Oxley: 6 quantitative loci, 3 for general hyg → 30% of phenotypic variation: 2 loci for uncapping and 1 for removal. 4 genes involved in olfaction, learning and social behaviour, one in circadian locomotion.

Hyg continued

- The quantity of dead larvae (?) or the quantity of bees has little effect on the results of hyg behaviour. The honey yield has an effect.
- Hyg. also effective against varroa and chalkbrood. Not effective against EFB, as it is always infective, not only the spores. Efb might be promoted for some reason when selecting for hyg.

Testing for hygienic behaviour

- Options:
  - Rothenbuhler: potassium cyanide gas!
  - Newton & al.: freezing
  - Newton & Ostasiewski: pin kill
  - ? liquid nitrogen
  - Newspaper removal, etc?

Freezing test

- A round, sharp cylinder with teeth, 50 (43) mm diameter → 100 cells.
- White pupae, purple-eyed
- Frozen and returned after 24h
- Counting after 24, 48, etc hours, as needeed

Cutting the test piece

20.07.2013 Kooru — Mesinike vabariiklik suvine teabepäev


The perforated comb
20.07.2013 Koeru
Mesinike vabariiklik suvine teabepäev


The test piece

Counting the freezing test area

- Count capped and unremoved.
  - Unremoved = still remnants of pupa in the cell
- Do not count empty cells as the edges are always emptied
- Calculate the empty cells by subtracting unremoved and capped from the average total number of uncut cells

Management of the results

- Significant differences in different conditions.
  - The greatest detected difference in time units was about 4-fold.
- Fortunately, the results can be scaled if the sample size is sufficient.
- After scaling the results can be compared
- Basis for scaling: the speed of the cleaning is constant → the number of cleaned cells / time unit will decrease exponentially

Scaling of the results

- Cells to be cleaned in time x = C_x
  - C_0 = 100
- $C_x = 100e^{kx}$, => $k = \ln (C_x / 100) / x$
- $C_y = 100e^{ky}$, replace k with the above
  - => $C_y = 100 (C_x / 100)^{(y/x)}$

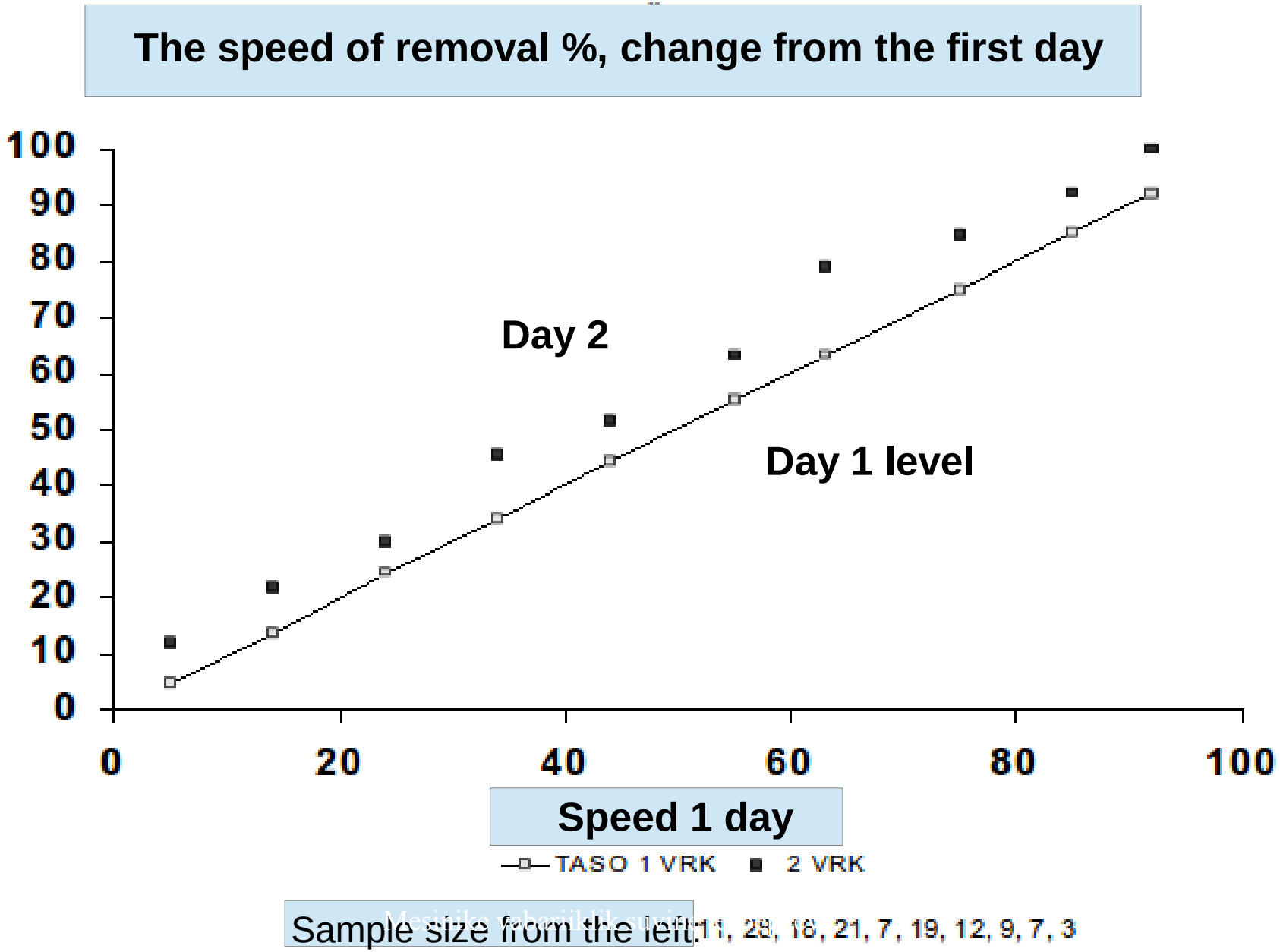
The cleaning speed is constant

- If the cleaning speed is constant in given environmental conditions,
- That is, the speed is constant in percentages:
- => the relation between the geometric mean of the cleaning speed G in conditions a and b is:
- $G(a) = 100(G(b)/100)^{(x_a/x_b)}$
  - x_a/x_b = relation between cleaning speeds

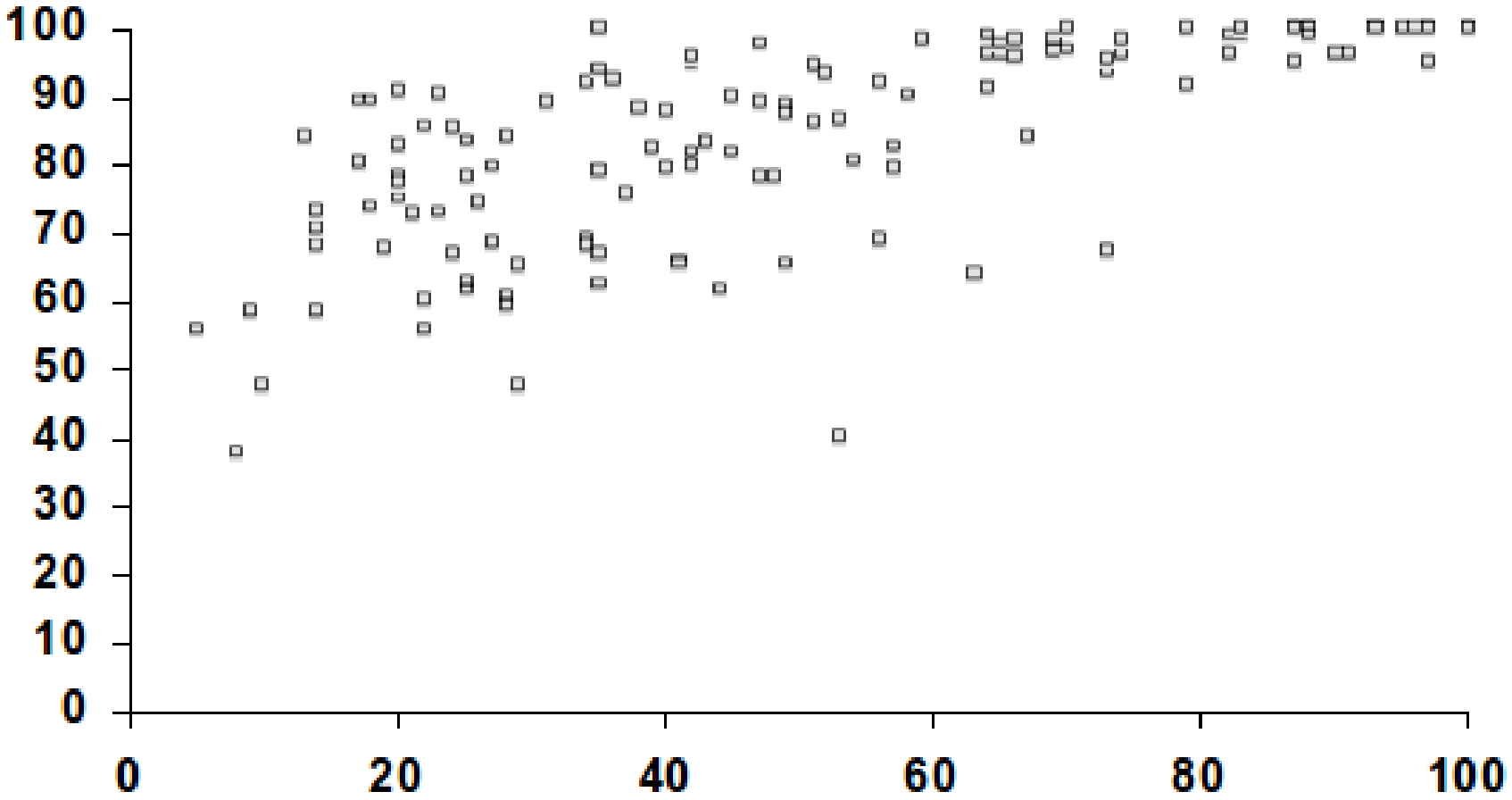
The formula for scaling

- Solve the exponent for all a_i and b_j:
- $$x_a/x_b = \frac{\Sigma(\ln a_i) / n_a - \ln 100}{\Sigma(\ln b_j) / n_b - \ln 100}$$
- $$C_y = 100(C_x /100)^{(y/x)}$$
- x = cleaning speed
- C = number of cells to be cleaned
- Scaling: replace y/x with x_a/x_b

The cleaning speed



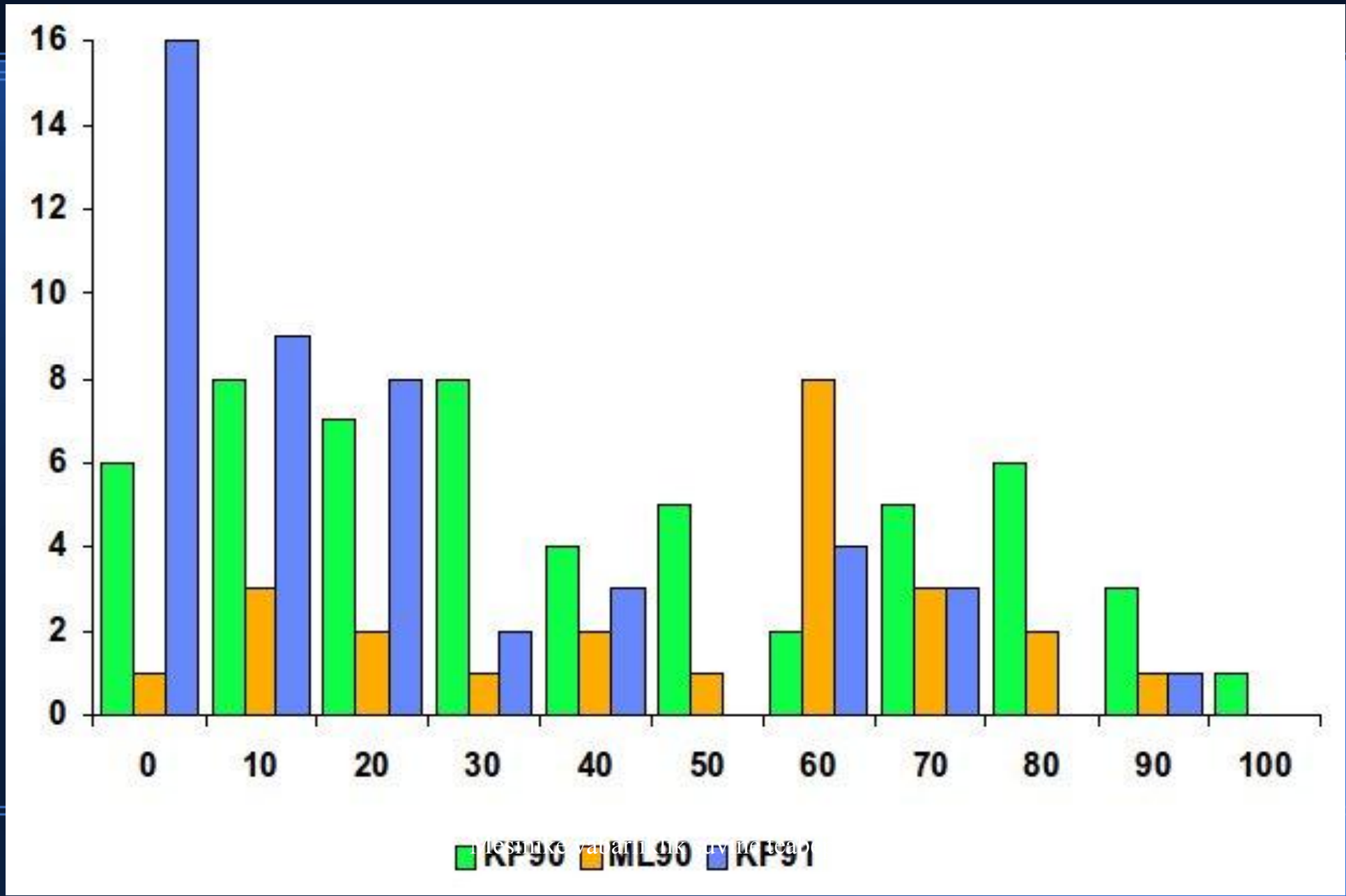
Correlation uncapping-removal



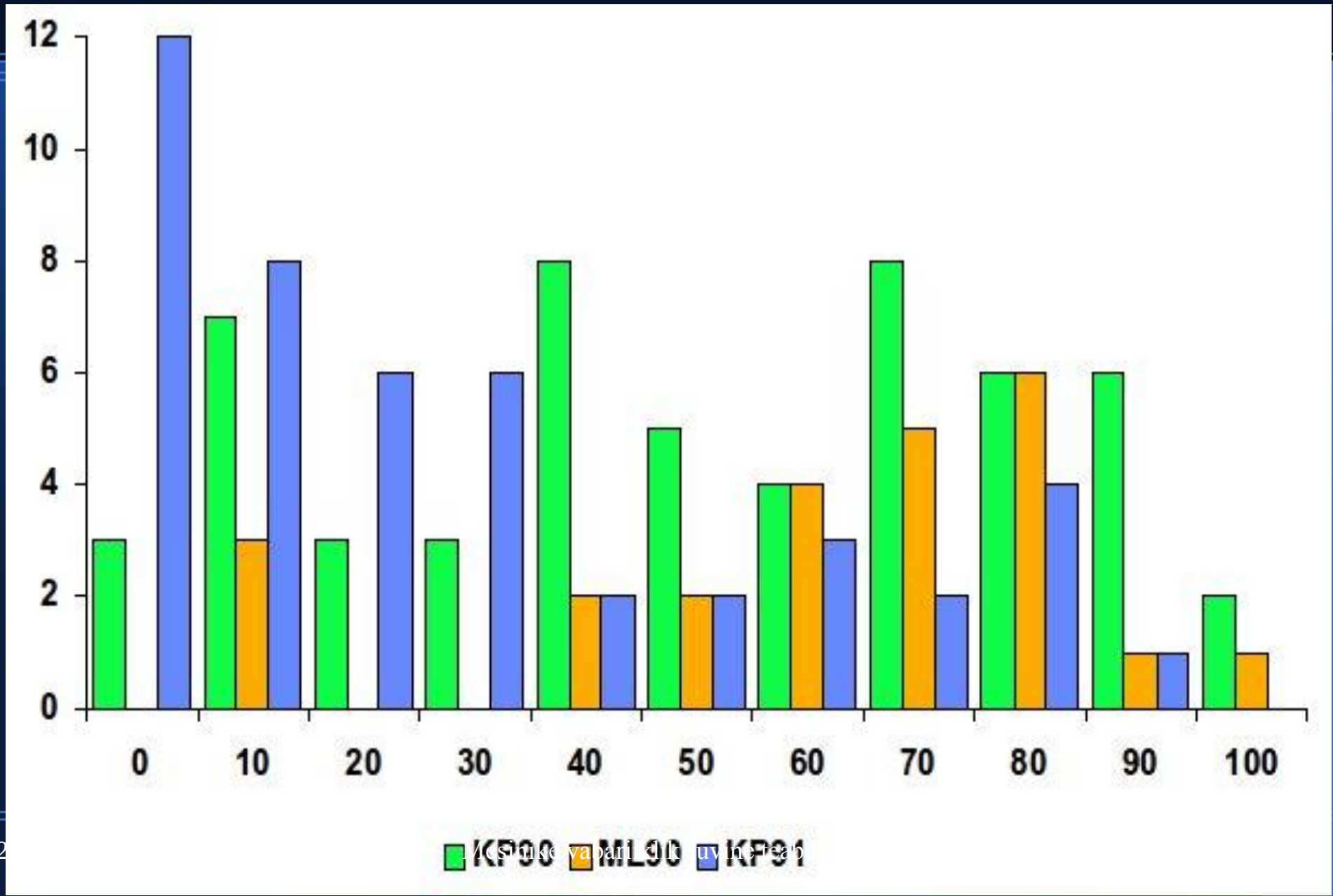
REMOV

SPEARMAN CORRELATION = 0,74

Capped cells, day 2



Unremoved + capped cells, day 2



Practical considerations

- The test piece must be in the center of the brood nest, preferably more than one test.
- Best to count in low speed conditions (not during honey flow) in order to find the best ones more reliably, as well as the bad ones.
- Care has to be taken in not damaging the cutouts
- It suffices to count only the empty cells as resistance arises because of the removal of the diseased pupae, prior to the sporulation

Practical considerations continued

- It is best to count before any (or many) colonies have reached 100% in order to differentiate the colonies better, this is normally after 24h.
- The initiation of cleaning takes some time: a difference of about 6 cells between day 1 and day 2 in my data.
- No significant positive correlation between honey production and hyg in my data.

The pin kill test

- 3 x 21 cells punctured with an insect needle
- Correlation between freeze kill test 0,50***
- The pupa does not always die
- Freeze kill test more accurate
- A tool with several needles can be used
  - Faster, less damage to the cappings

Other mechanisms of resistance

- Resistant larvae
  - Larvae susceptible when young → the larvae have to be tested when young and of the same age.
  - Testing in an AFB-inoculated Petri dish
  - Automatic testing and selection by rearing queens and drones in diseased colonies
- Apparently immune bee strains do exist
- Proventriculus filtering spores?

Instrumental insemination as an aid in breeding for resistance

- Fast results if only a few genes involved
- Single drone insemination
- Insemination with the queen's own drones?

Potential problems

- EFB-susceptibility seems to arise when bees are selected for hyg.
- If only hyg but susceptible larvae → scattered brood but still infested.
- All selection and breeding work leads to more than just the selected change.
- The metabolic price of resistance.