

Honey bee colony vitality

Are we wintering summer bees?

Impact of environment on status of the colony

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WAGENINGEN **UR**
For quality of life

Content

- Vitality / superorganism
- Status description of the honey bee colony
 - Social defence
 - Parasites
 - Nutrition
 - Beekeepers practice
- Study
 - Impact environment, descibed in terms of pollen diversity, on vitality of honey bee colony

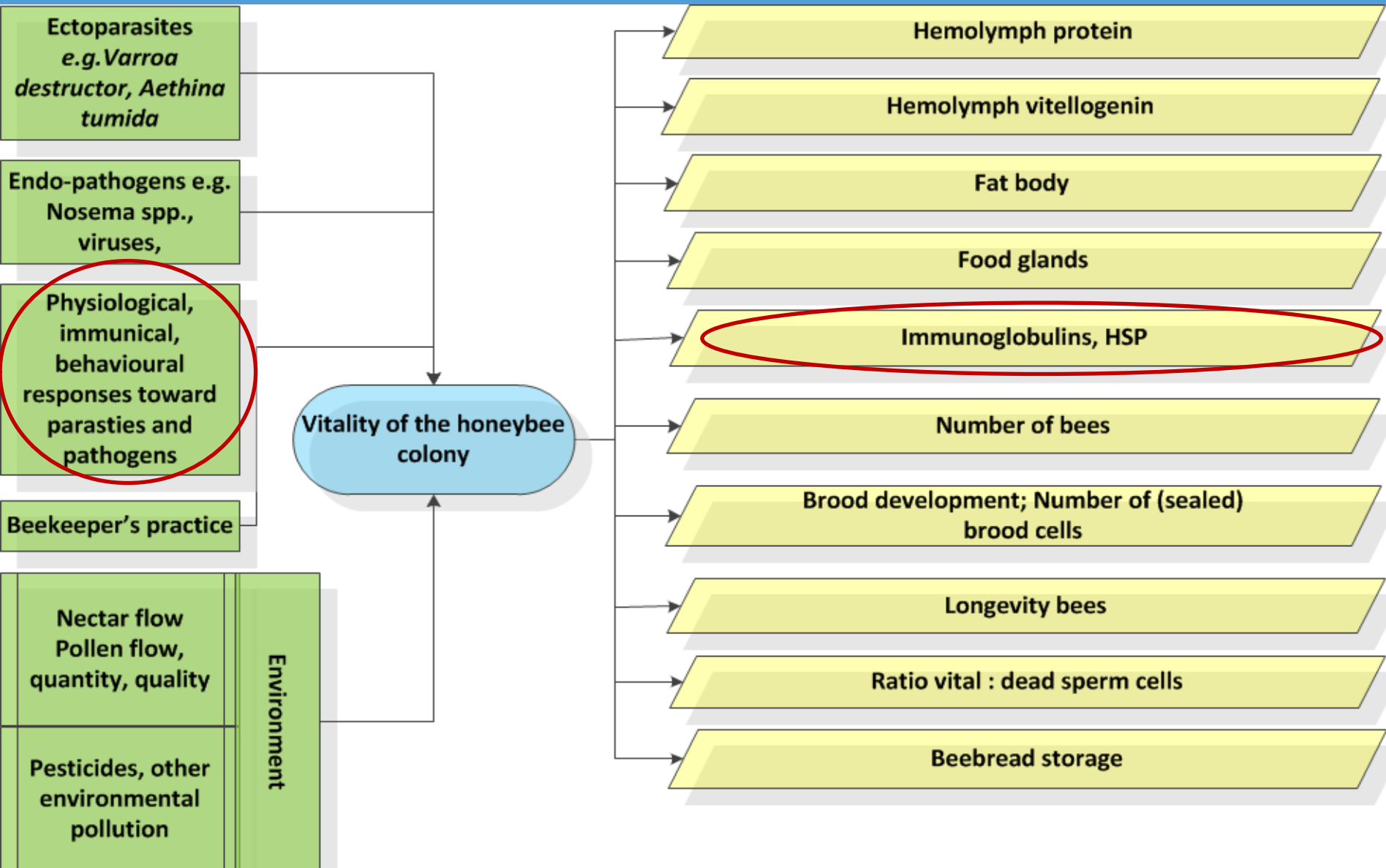


Vitality / health of honey bee colonies

- Not only absence of disease
- Presence of well nourished workers capable of producing progeny
- Resistance stressors: parasites, infection, insecticides
- **Having the status that corresponds with the course of the bee season**

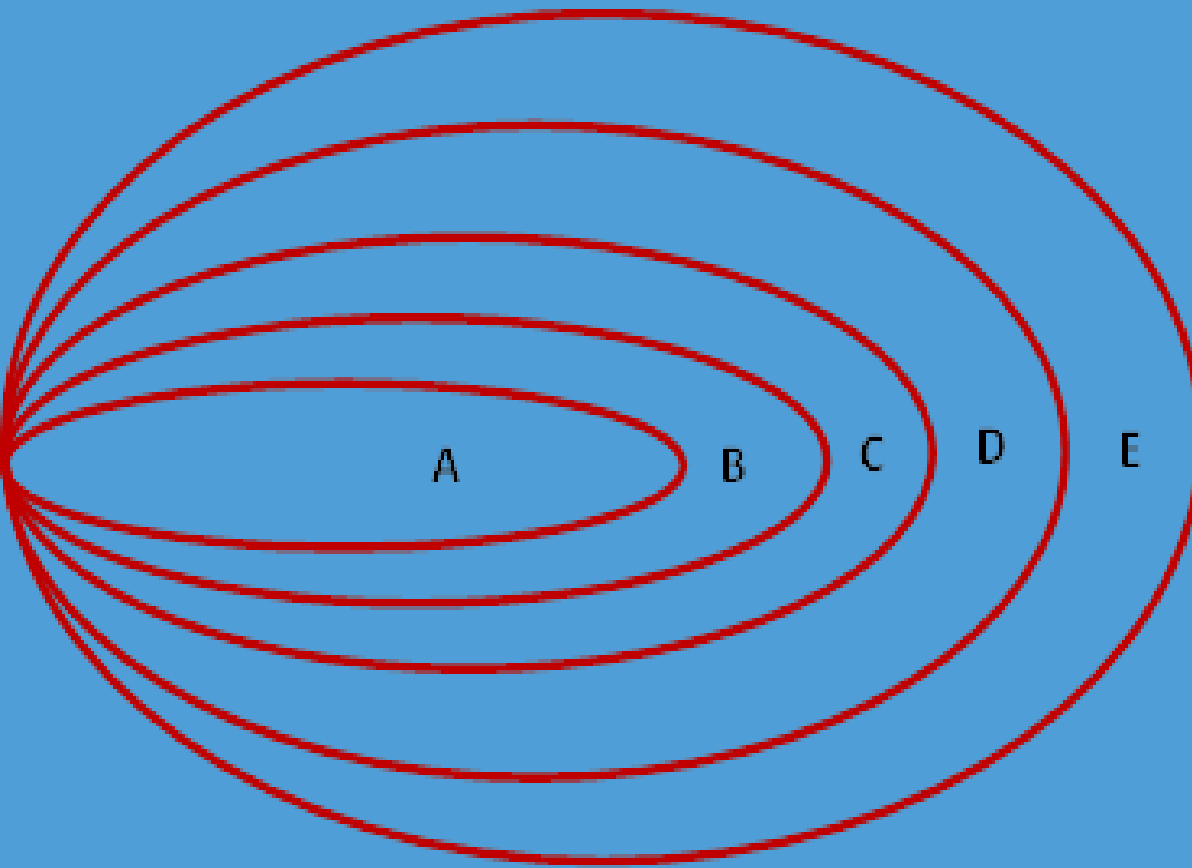


Status description of the honeybee colony



Disease barriers

Defence levels

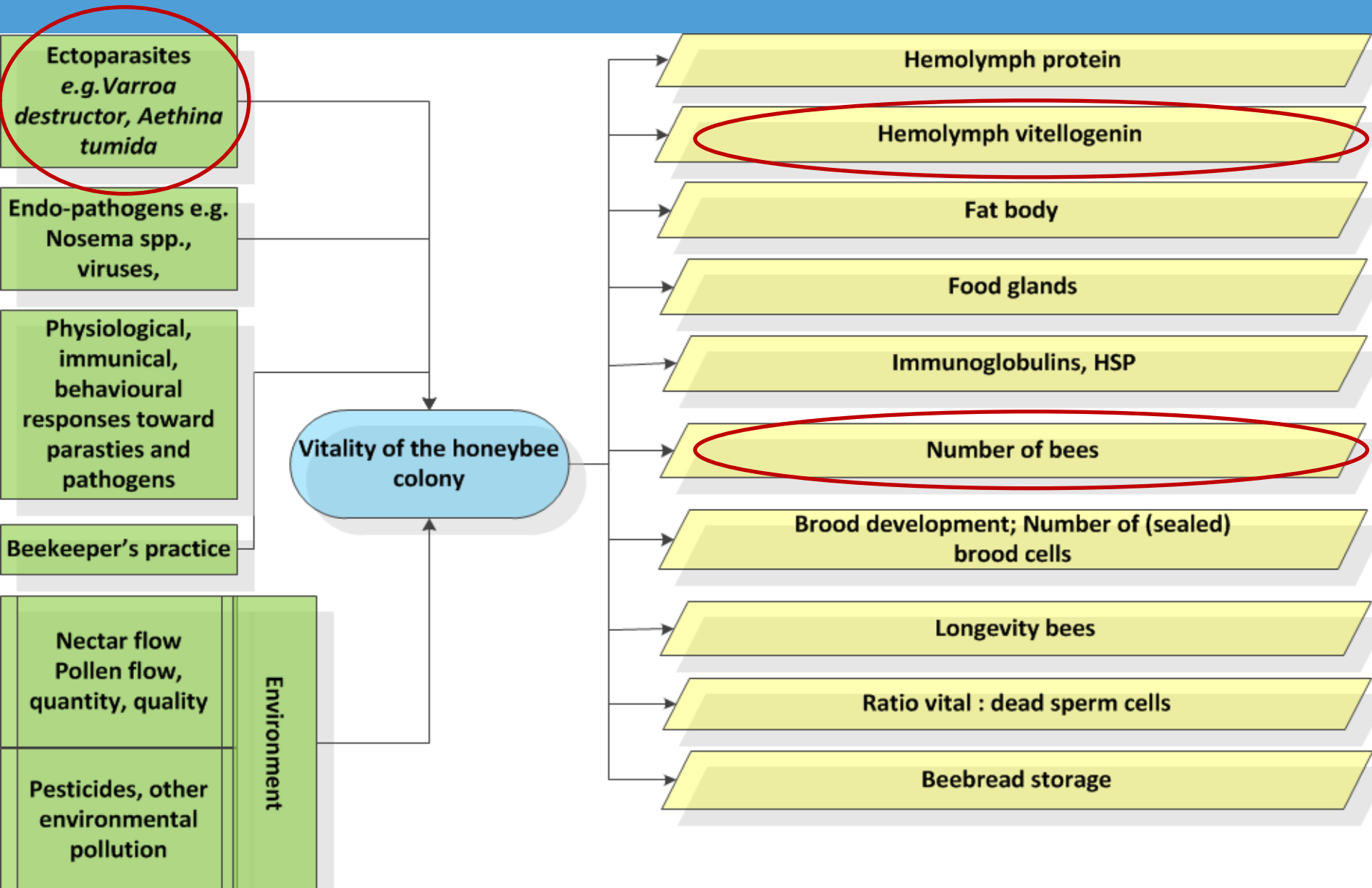


- A: individual
- B: pairwise (grooming)
- C: colony defence (task differentiation)
- D: Minimizing infectious agents
- E: colony shielding (propolis / resins)

Disease barriers

- Mechanical, physiological, immune and behavioural defence
 - Resisting/tolerating: compensating energy costs
 - Mechanical: cuticle and epithelial layers (e.g. *Nosema* spp)
 - Physiological: change gut condition (e.g. pH)
 - Immune response:
 - *Humoral: recognise signals > protein and metabolisms for parasite inhibition
 - *Cellular: Blood cells (phagocytosis)
 - Behavioural:
 - * grooming (auto / allo)
 - * Hygienic behaviour: brood removal, undertaking

Status description of the honeybee colony

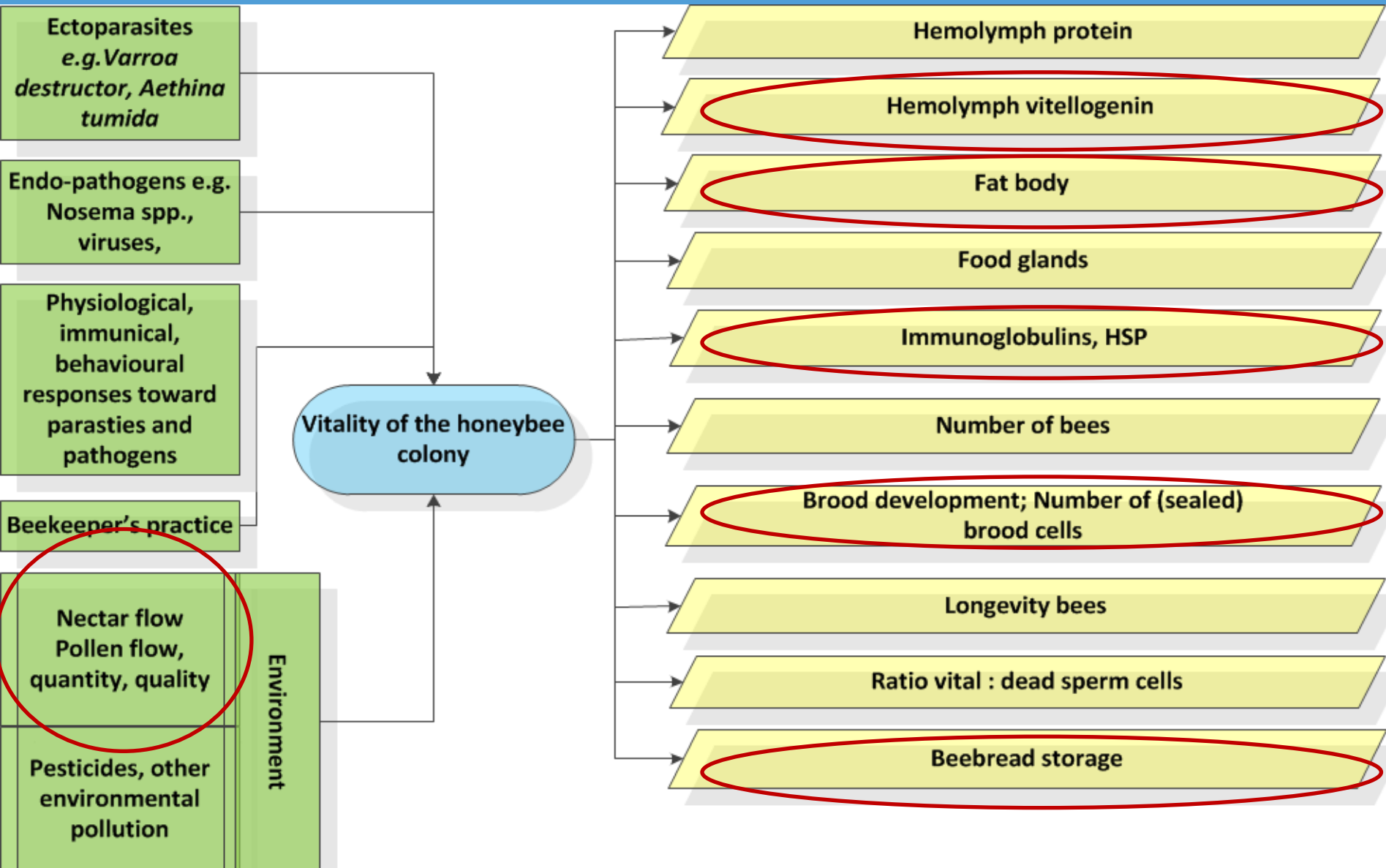


Varroa destructor

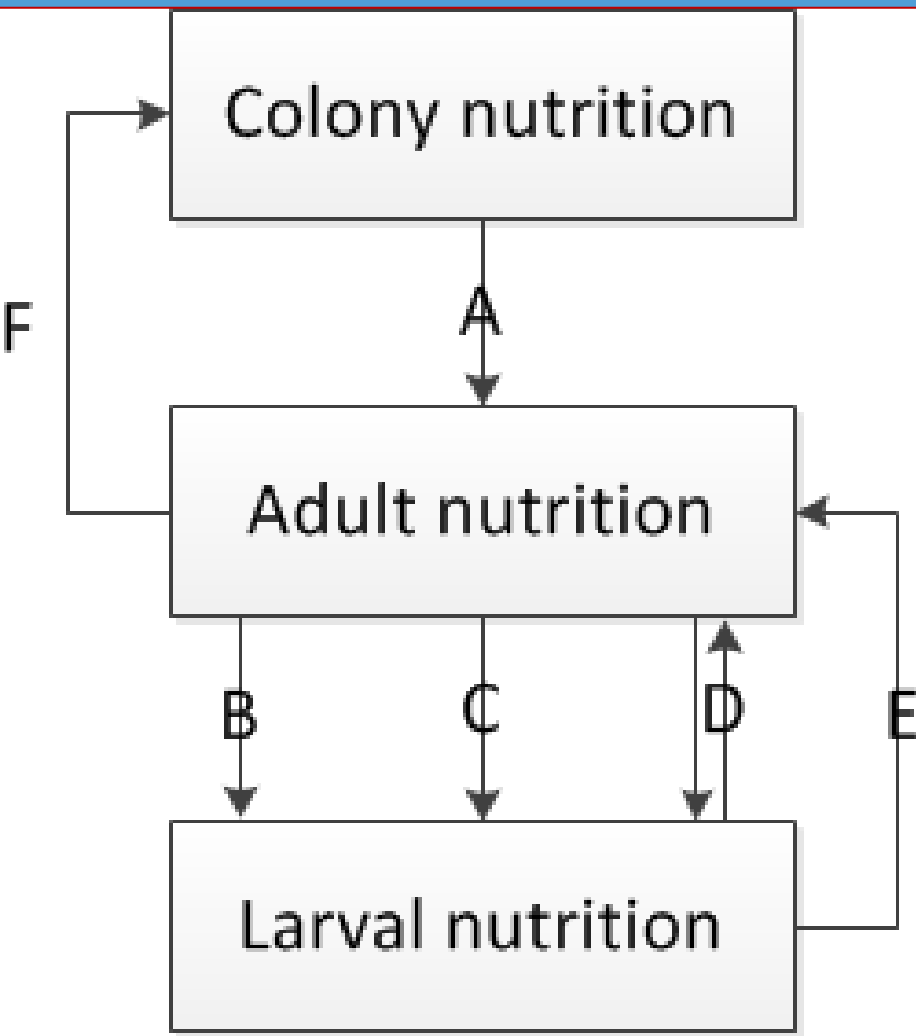
- Ectoparasite of adults and brood
- Impact on
 - bio synthesis of vitellogenin in adults
 - decrease hemocytts in adults (precocious forager features)
 - Elevated ecdysteroids , impact on winterbees?
 - Failure to develop key physiological characteristics of long living winter bees.



Status description of the honeybee colony



Nutrition



- A: dependance of adults on colony stores
- B: investment in larval quality
- C: regulation of larval number
- D: cannibalism
- E: impact of larval nutrition on next generation
- F: impact of adults on colony nutrition

Diet effects

Malnourished colony

- pollen shortage > beebread > body reserves > cannibalism young larvae, brood stop, starvation

Malnourished adults

- Protein shortage: delay maturation flight muscle
- Development fat body, food glands , ovaries, correlated with protein consumption

Malnourished larvae

- Impaired lifespan and protein, reduced body size, ovaries

Weight and protein at emergence

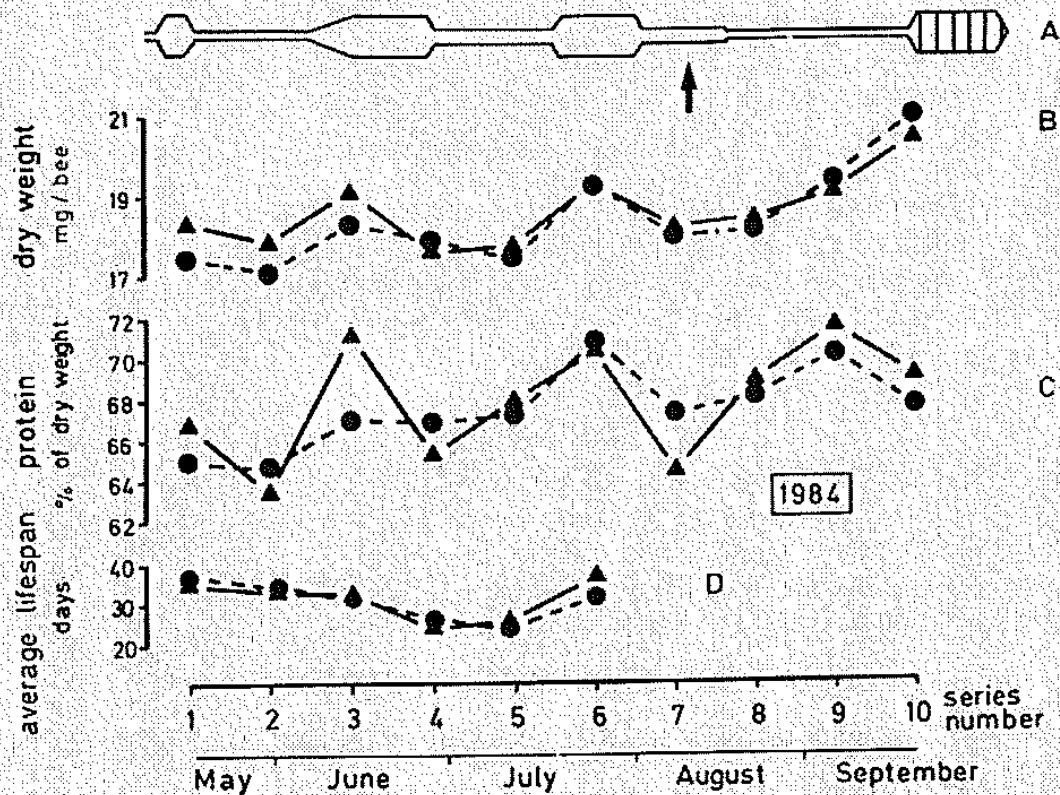


FIG. 3. Protein content and weight of emerging bees and lifespan in 1984.

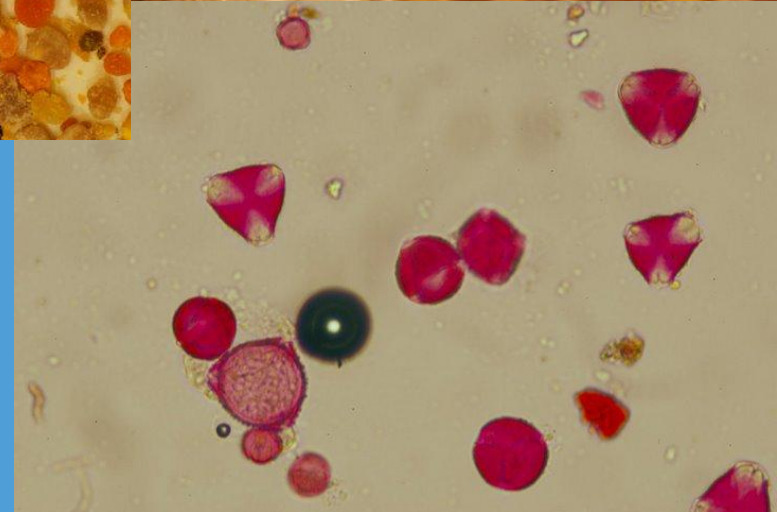
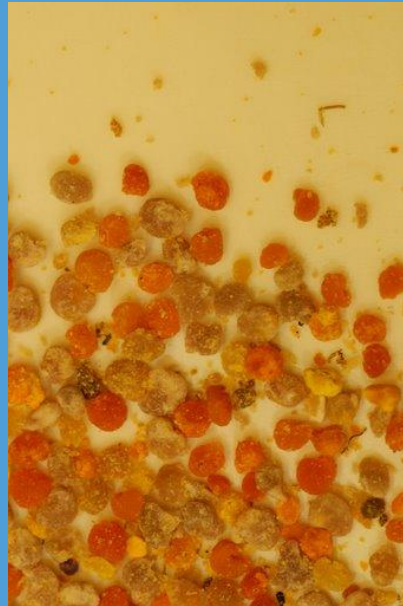
Abscissa shows the time scale of egg-laying.

(A) Estimated food conditions outside the hive. Vertical thickness of the bar shows relative availability of nectar and pollen; the striped segment indicates winter feeding and the arrow, the moment of honey harvest. (B) Dry weight. (C) Protein as a percentage of dry weight. (D) Average lifespan in relation to season. To see the food conditions under which bees with certain lifespans lived as imagos, the lowest curve must be transferred 3 weeks (time from egg-laying to emergence) to the right. (▲) hive I; (●) hive II.

Pollen

Essential amino acids

- Threonine
- Valine
- Methionine
- Leucine
- Iso-leucine
- Phenylalanine
- Lysine
- Histidine
- Arginine
- Tryptofaan



Essential amino acids in pollen, perc protein and ratio amino acids

Plant	% protein	Threonine	Valine	Methionine	Leucine	Isoleucine	Phenylalanine	Lysine	Histidine	Arginine	Tryptophan
ideal		3.0	4.0	1.5	4.5	4.0	2.5	3.0	1.5	3.0	1.0
Rape seed	23.8	4.9	5.1	2.3	7	4.6	4.3	8.2	2.1	5.1	nd
pear	26.2	4.4	5.4	2.4	6.9	4.1	4.2	6.4	2.6	4.8	nd
Corn	14.9	5.1	5.9	1.6	6.8	4.8	3.8	5.6	1.9	4.7	nd
Lucerne	20	3.3	3.3	1.4	5	2.7	3.1	5.6	3.2	4.5	1.6
White clover	25.9	4.6	5.3	2.2	7	4.4	4.3	5.9	2.5	4.7	nd

Pollen

- Have all pollen all the essential amino acids?
 - No, bees forage on as many pollen sources as available
 - Example essential amino acids deficiency
 - Dandelion pollen have no tryptofaan and no fenylalanine and few arginine.
 - malfunctioning food glands
 - have a reduced life span



Herbert et al. 1970. The brood-bearing capability of bagged honey bees fed dandelion and mixed pollen Diets. J. ECONOM ENTOMOL 63 (1): 215-218

Lifespan bees fed

sugar	14 days
Sugar plus dandelion pollen	20 days
Sugar plus Rubus pollen	42 days

Manning et al., 2007. Lipid-enhanced pollen and lipid-reduced flour diets and their effect on the longevity of honey bees (*Apis mellifera* L.). *Australian Journal of Entomology* (2007) **46**, 251–257

Maize

- Impact of solely maize pollen on honey bee colony
 - Maize pollen contain
 - the entire essential amino acids spectrum*
 - Fatty acids?
 - Minerals?
 - Zn/P interaction ?
 - impact of soil conditions
 - Monofloral maize diet results in
 - Increased pollen consumption*
 - Reduced brood rearing *
 - Reduced longevity*
 - No impact on immunocompetence (humoral)*

*Höcherl et al. 2012 Evaluation of the nutritive value of maize for honey bees. J. Insect Physiol 58: 278-285

Minerals in bee collected pollen

mineral	mg/kg
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Cu	8.7
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Mn	11.1
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Zn	33.9
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Fe	39.2
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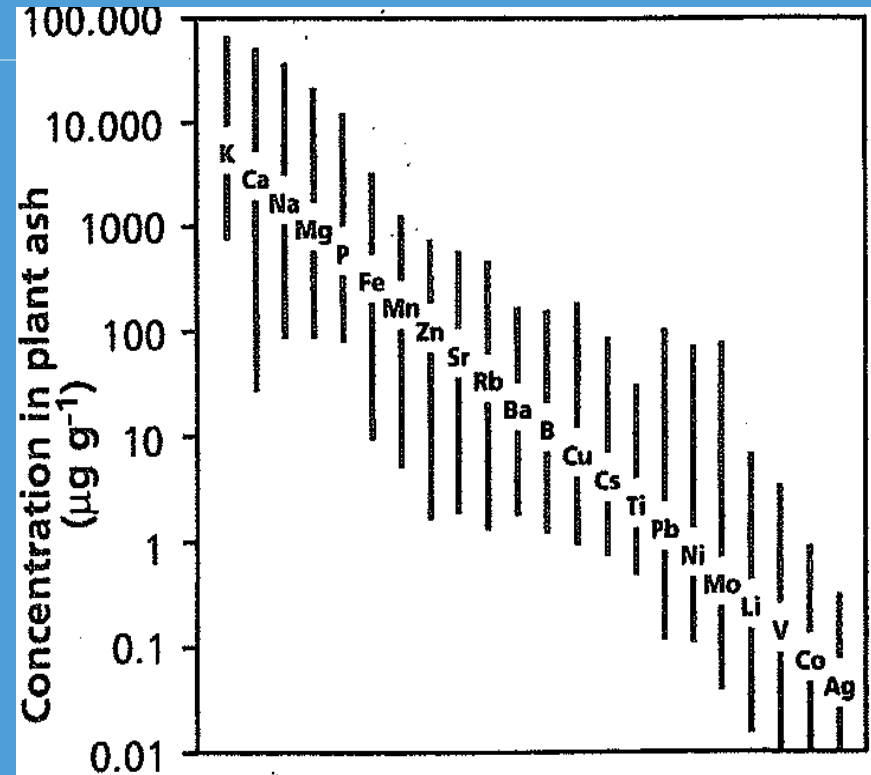
Mg	432.2
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Ca	491.3
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P	581.6
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Na	1301
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K	4153
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Metals in pollen. Lambers et al 1998 Plant Physiology and Ecology

Derived from Nutrient composition and microbiological quality of honeybee-collected pollen in Spain 1997. J.Serra Bonhevi et al. Food Chem 1997. 47: 725-732

Zinc in honey bees (part of Vg molecule and hemocyt)

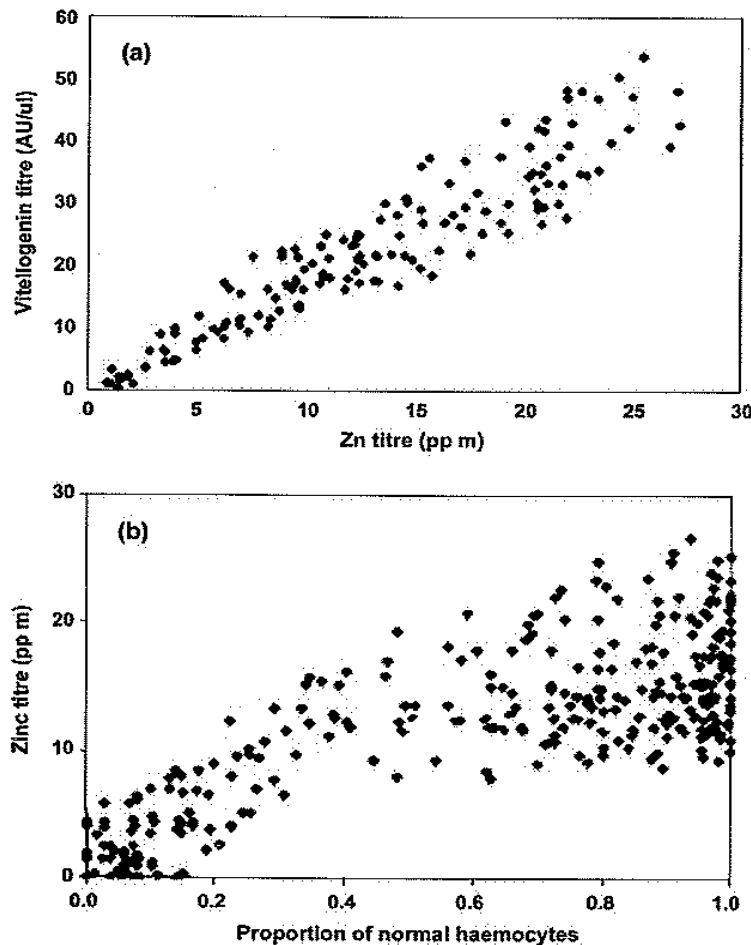


Fig. 1. (a) The relationship between vitellogenin titre and zinc level in honeybee worker haemolymph ($n = 115$). The data plot includes samples from hive bees ($n = 36$), foragers ($n = 18$) and winter bees ($n = 61$). Vitellogenin titre is expressed in arbitrary units (AU) (see Section 2 for further information). (b) The relationship between the proportion of normal haemocytes and the zinc level in honeybee haemolymph ($n = 303$). The plot includes data from hive bees ($n = 134$), foragers ($n = 41$) and winter bees ($n = 128$).

metal	Maas tricht	Buggen um	HvH
Zn	74.70a	79.59a	66.70a

Derived from J. van der Steen 2011 Spatial and temporal variation of metal concentrations in adult honeybees (*Apis mellifera* L.) Environ Monit Assess

Amdam et al 2004. Hormonal control of the yolk precursor vitellogenin regulates immune function and longevity in honeybees. Experimental Gerontology 39: 767-772

Zinc in Maize pollen

- High levels of phosphorus result in immobilization of Zinc in Maize
 - Immobilization in roots and nodes of stem

Dwivedi et al. 1975. Phosphorus –zinc concentration, sites of immobilization of zinc in maize at high level of phosphorus. Plant and Soil 43: 639-648

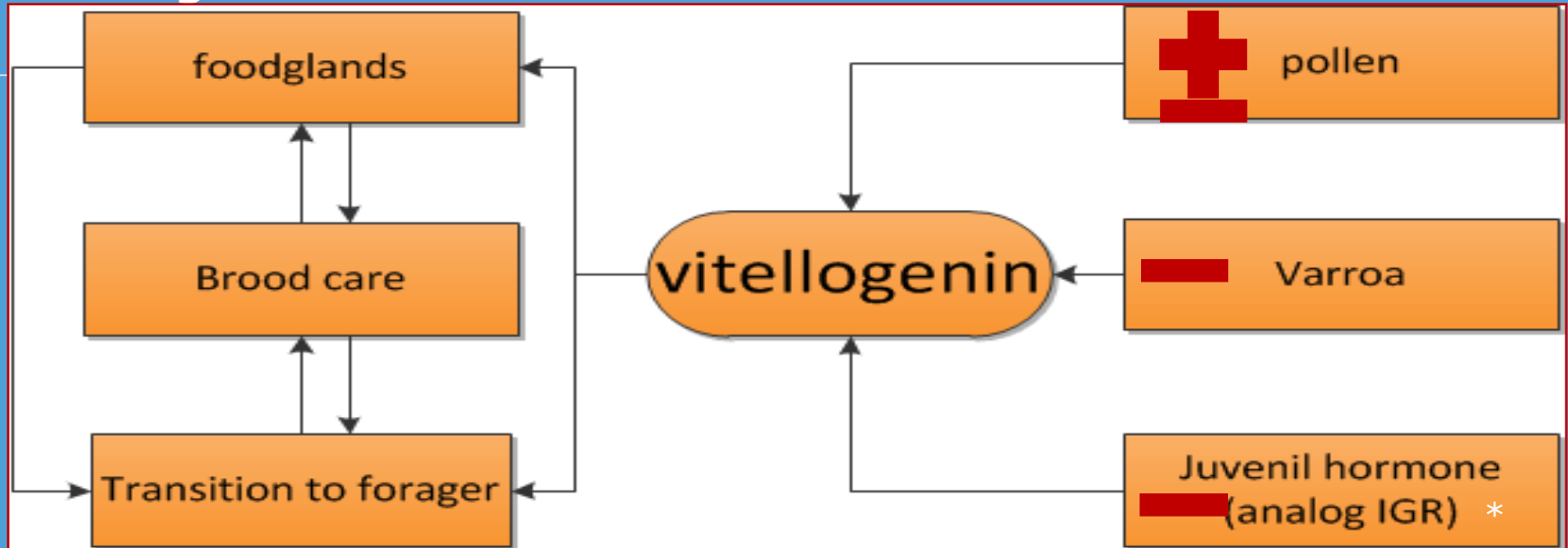
Maize plants grown with low zinc from initiation show a 79% reduction of Zn in the pollen grains

Sharma et al. 1990 Zinc deficiency and pollen fertility (Zea mays) Plant and Soil 124: 221-225



Vitellogenin: Key protein.

Vitellogenine titer: combination of factors



- E.g. feed/protein, food glands, lifespan bees, sealed brood cells, parasites / disease interact.
- Status factors alter in the course of the bee season
- Therefore, for interpretation of colony vitality parameters these interaction and the bee season must take into account

* Pinto et al. 2000. Inhibition of vitellogenin synthesis in *Apis mellifera* workers by a juvenile hormone analogue, pyriproxyfen. J. Insec, Pathol. 46: 153-160

Diet and hemolymph protein / vitellogenin

- Diets and resulting total hemolymph protein and vitellogenin (lab test, 6 day bees)

Diet	µg protein , % vitellogenin / µl hemolymph
Bee bread	27.57 µ g, 68.76% / µl hemolymph
Soya/yeast	24.06 µg, 47.40% / µl hemolymph
Fresh pollen	11.36 µg, 26.85% / µl hemolymph
Corn pollen	3.98 µg, 10.96% / µl hemolymph
No protein	2.17µg, 5.48%% / µl hemolymph

Cremonez, T.M., De Jong, D., Bitondi, M.M. 1998. Quantification of hemolymph proteins as a fast method for testing protein diets for honey bees. J. Econ. Entomol. 91: 1284-1289

Vitellogenin and *Varroa destructor*

Bees, parasitised as pupa are less capable to synthesise vitellogenine

Vitellogenine in bees 15 days old

Parasitised	50 µg / µl hemolymph
Not parasitised	80-90 µg / µl hemolymph

Relation protein nutrition and immunocompetence

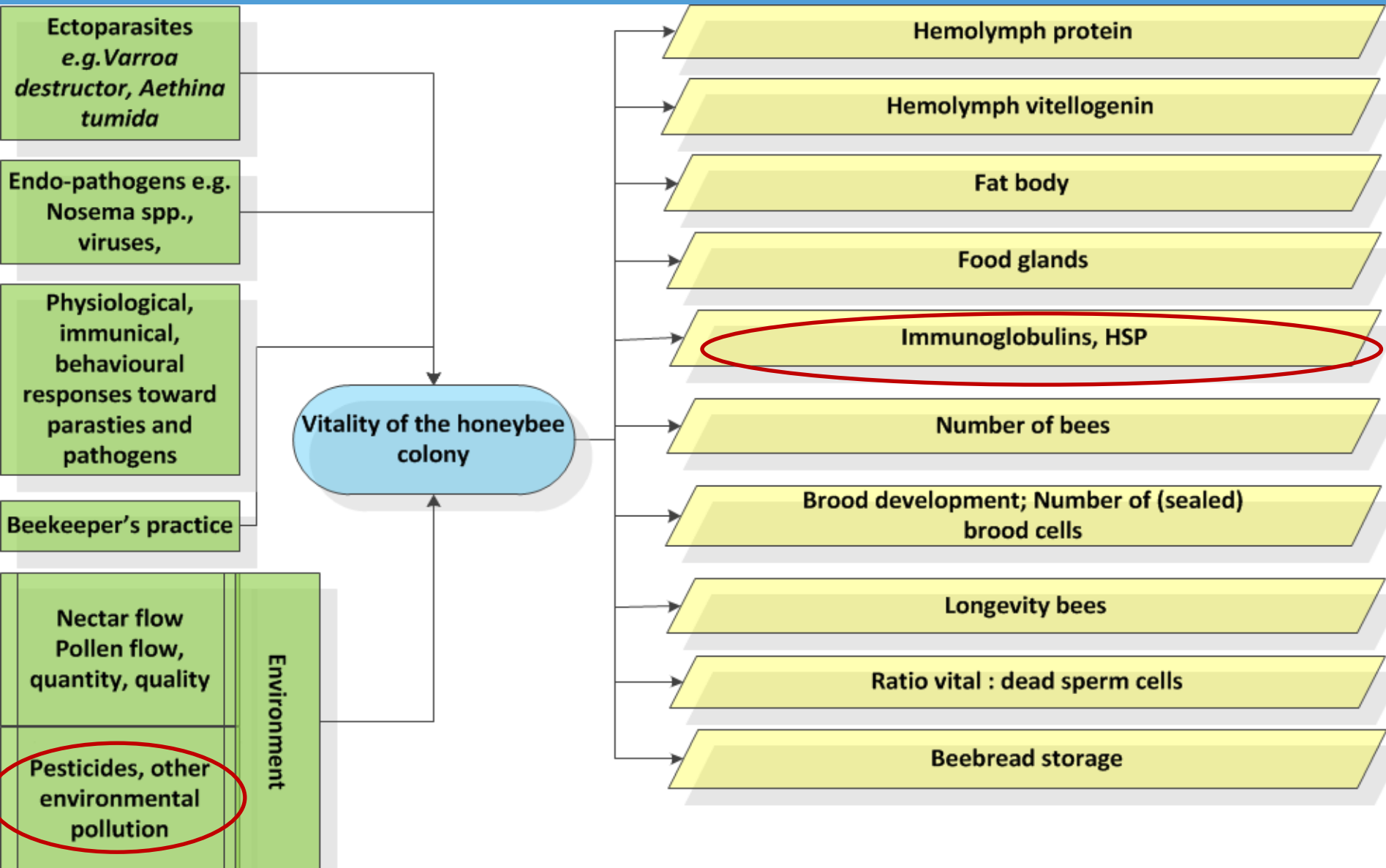
Monofloral and polyfloral pollen diet

- Parameters immunocompetence (IC)
 - Individual IC: Hemocyte concentration, phenyloxidase activity (PO)
 - Colony IC: glucose-oxidase (GOX)

Result:

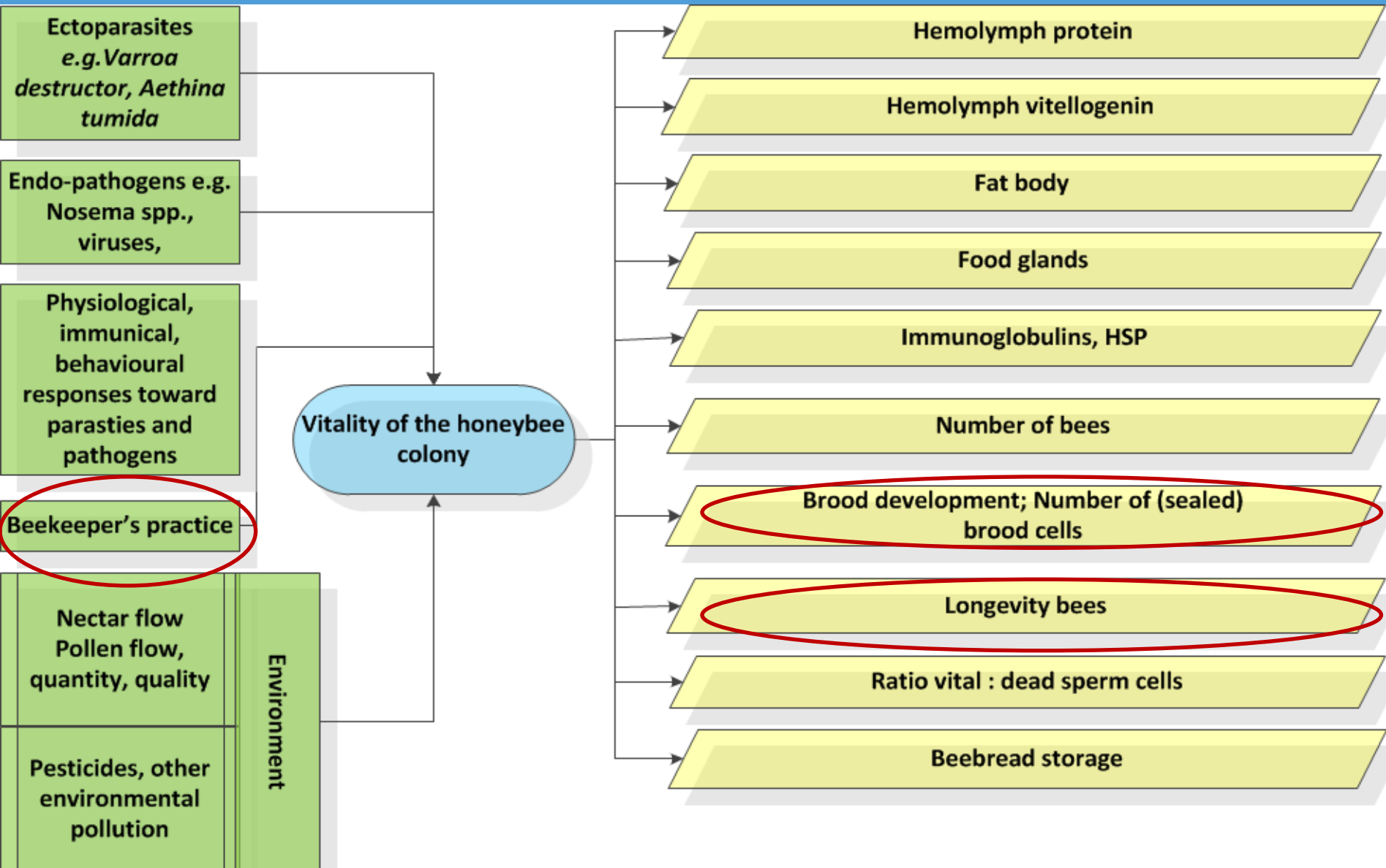
- no impact on PO and hemocyte concentration
- Significant decrease GOX (sterilizing tool colony and brood feed)

Status description of the honeybee colony



Poster Tosi, Medrzycki, Sgolastra. Italy, University of Bologna; Agricultural Research Council (Coloss / Eurbee Halle) sept '12)

Status description of the honeybee colony



Beekeepers practice, timing of winterbees

- Summer / winter bee status
 - Winterbee: longevity, low JH, high Vg, hypertrophoid HPG, enlarged fatbody
 - Winterbee colony: long living bees, few – no brood
- It takes, independently from the start similar length of time (weeks) for the transition from summer – winter colony*
- Depends on cues within the colony: Beekeepers practice
 - Prolonged brood rearing > later (too late?) transition to winter colony
 - Due to longer brood period young queen*
 - Other factors: FEED, VARROA, MORE?

* Matilla et al. 20021. Timing of production of winter bees in honey bee colonies, Insectes Soc 48: 88-93

Study



Impact environment, in terms of diverse and low-divers pollen in flow, on colony status (2010)

Objective: Impact of large scaled agricultural landscape or poor natural landscapes, both providing low pollen diversity on colony development

Parameters **colony status** description

number of bees and capped brood cells in Juni, July and Sept

Mean colony vitellogenin



Parameters bees, capped brood

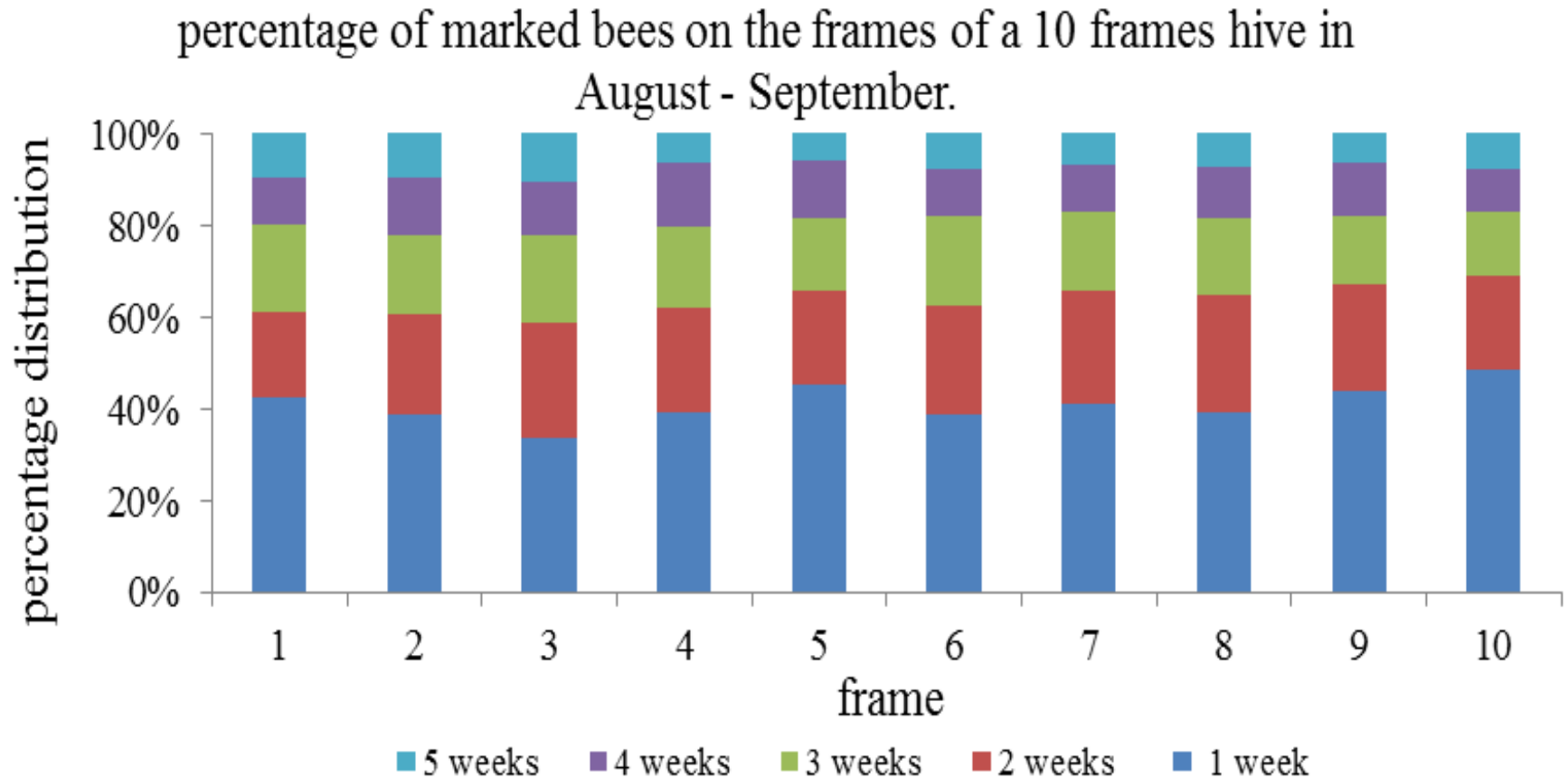


Digital assisted photo analysis using Image J. software

- $1 \text{ cm}^2 = 1.25 \text{ bees}$
- $1 \text{ cm}^2 = 4 \text{ cells}$



Distribution age classes over the frames



Number of bees sampled for mean colony Vg

(individual / pooled sample)

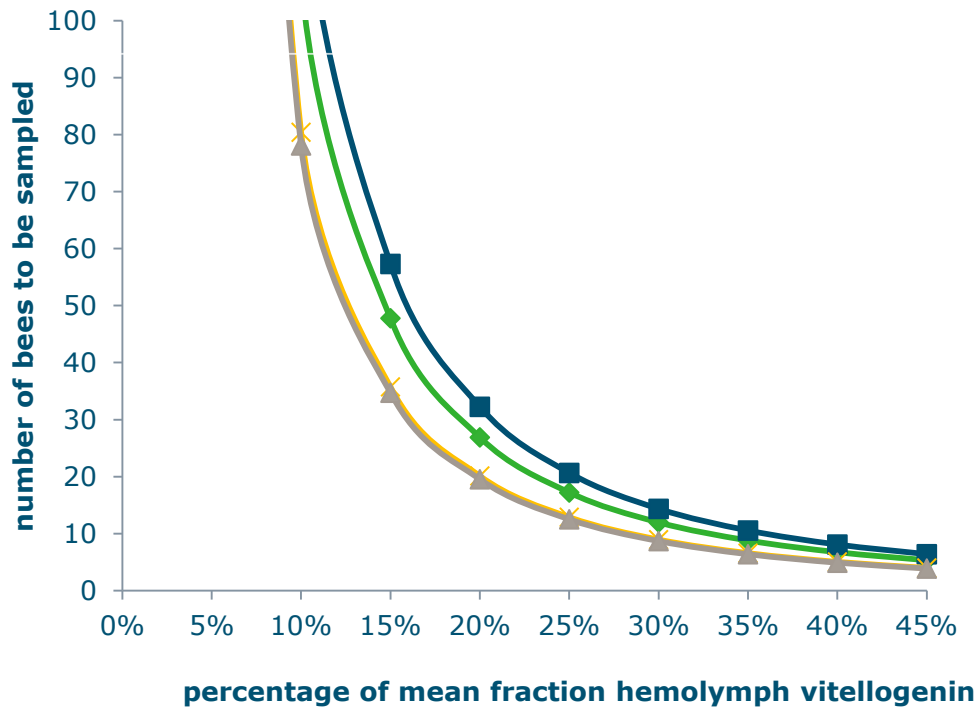
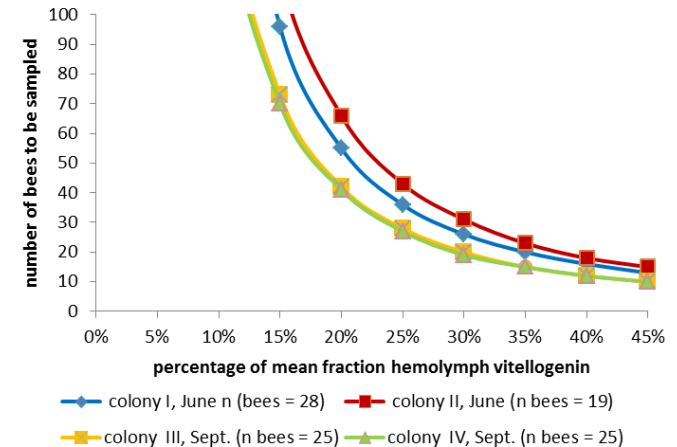


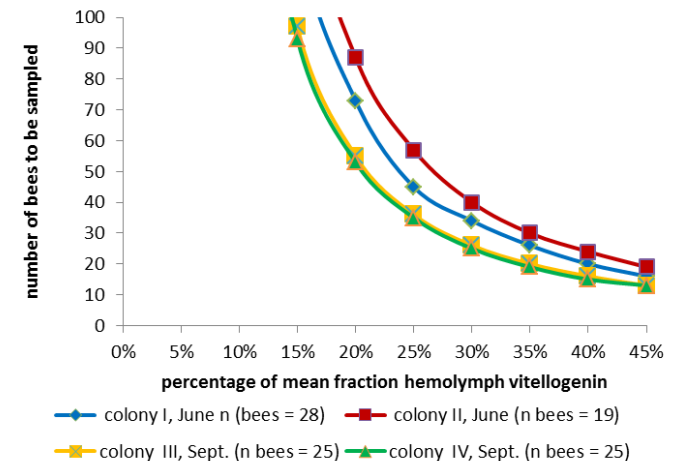
Figure 1. $\pm$ Percentage of the mean (Y axis) at given numbers of replicates (bees)(X axis) ($P = 0.05$) calculated with the formula $\sqrt{\text{replicates}} = 2 * \text{sd} / \text{to be detected}$.

Sampling 20 bees results in detectable percentage of the mean of approximately 18% - 25% 2-sided; mean $\pm$ 9% - 12.5% of the mean.

Sampling 30 bees results in detectable percentage of the mean of approximately 16% - 22% 2-sided; mean $\pm$ 8% - 11% of the mean.

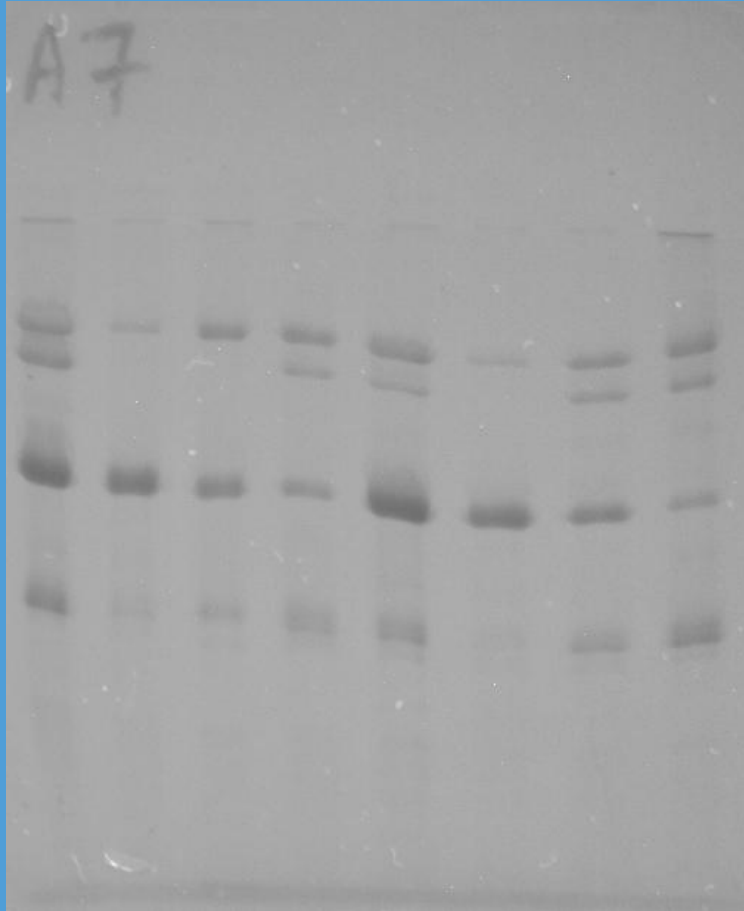


n bees (Genstat p 0.05 power 80%)



n bees (Genstat p 0.05 power 90%)

Hemolymph eiwit / vitellogenine analyse



Phast
electrophoresis
system

← vitellogeninsystem

← standardssystems



Results

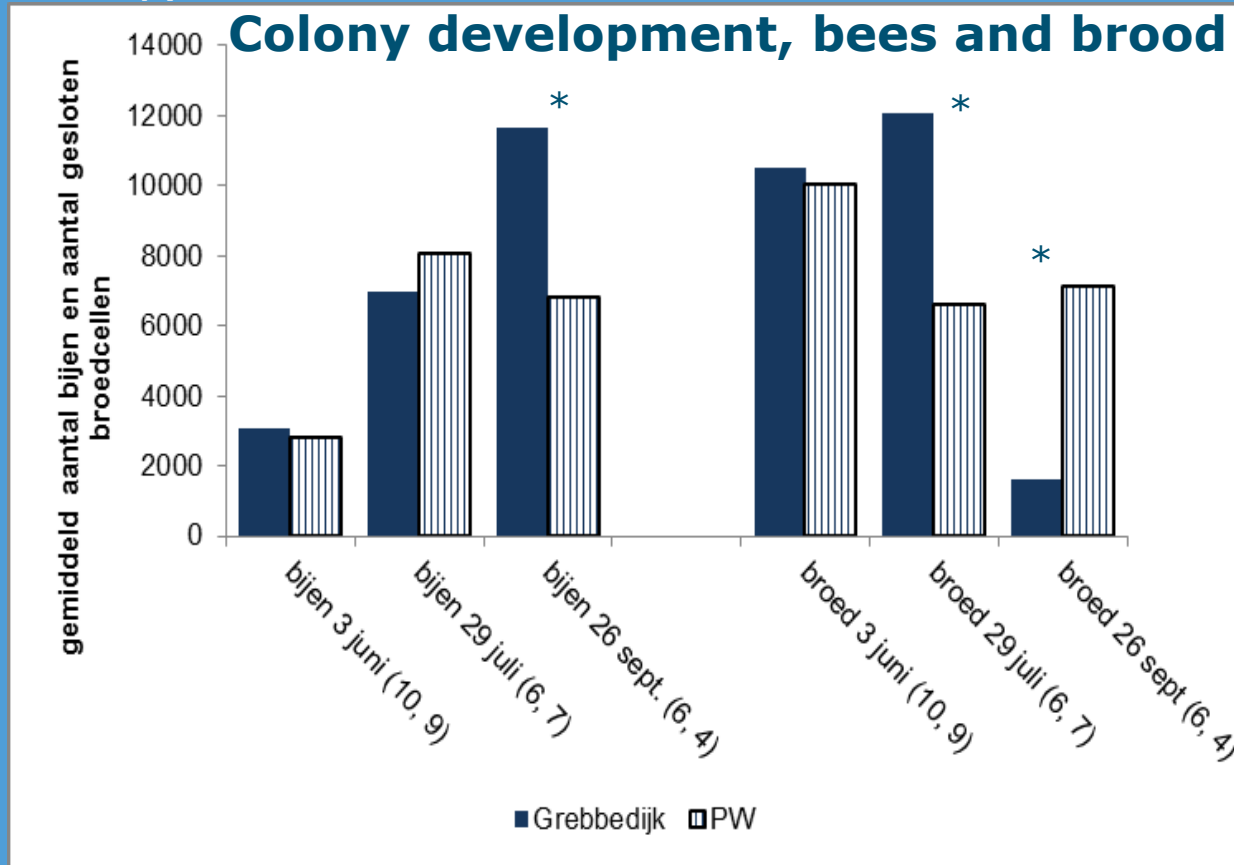
- Significant difference in pollen diversity in the pollen traps

Pollen diversity



Results

Colony development is affected by environment apiary (factor tested: pollen diversity)

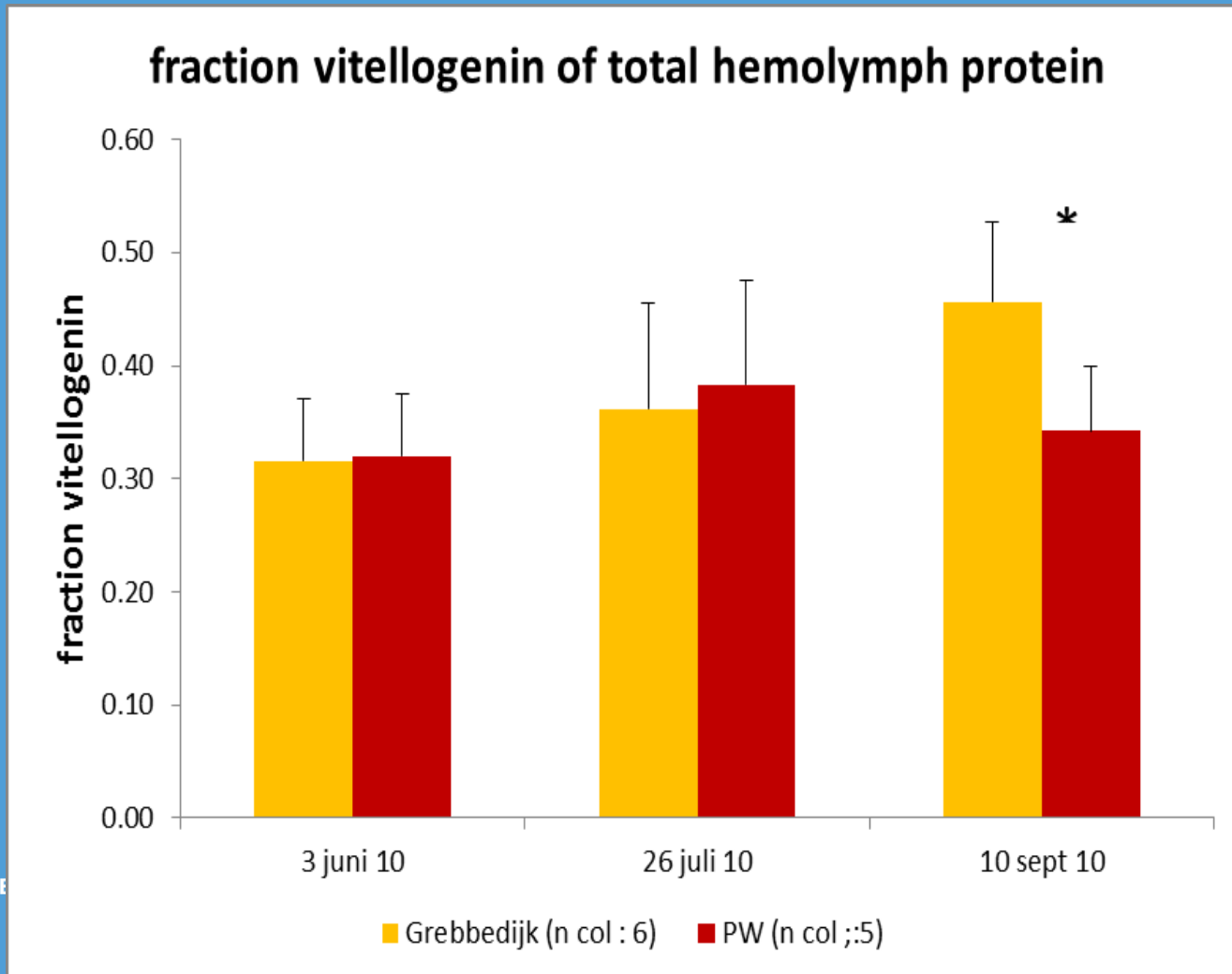


Status September: G: pollen divers > winter colony; PW: poor pollen diversity > summer colonies



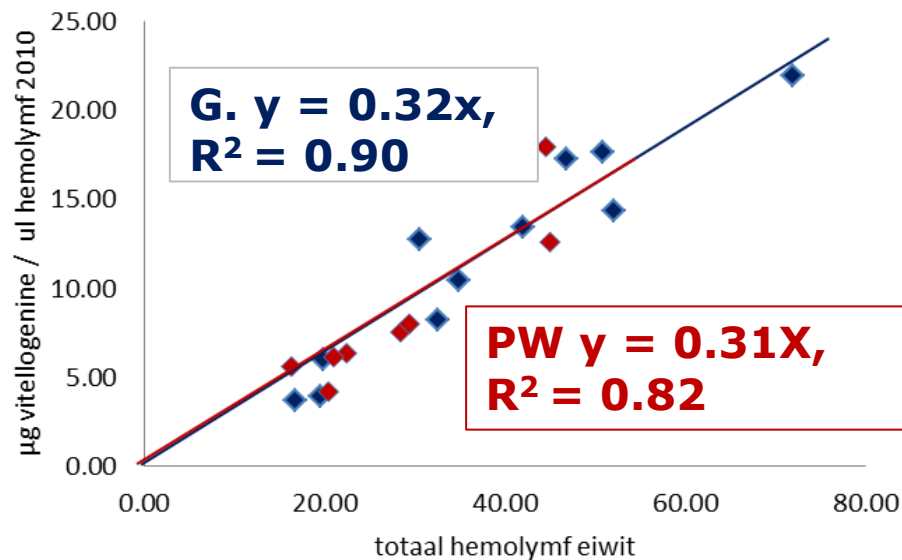
Results

- Fraction vitellogenin in September differs significantly : G. wintercolony, PW summer colony

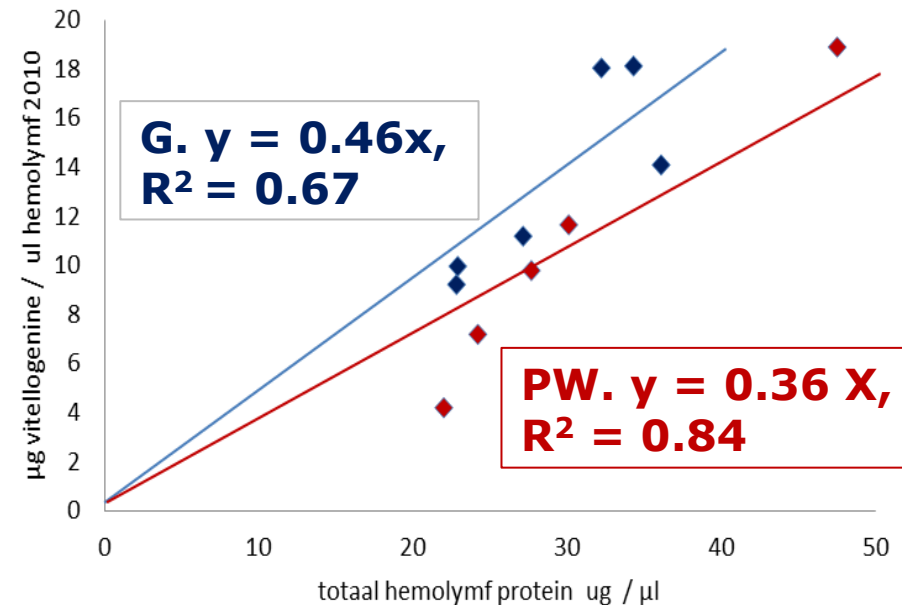


Results

3 June 2010 Correlation total hemolymph protein / Vg



10 Sept 2010 Correlation total hemolymph protein / Vg

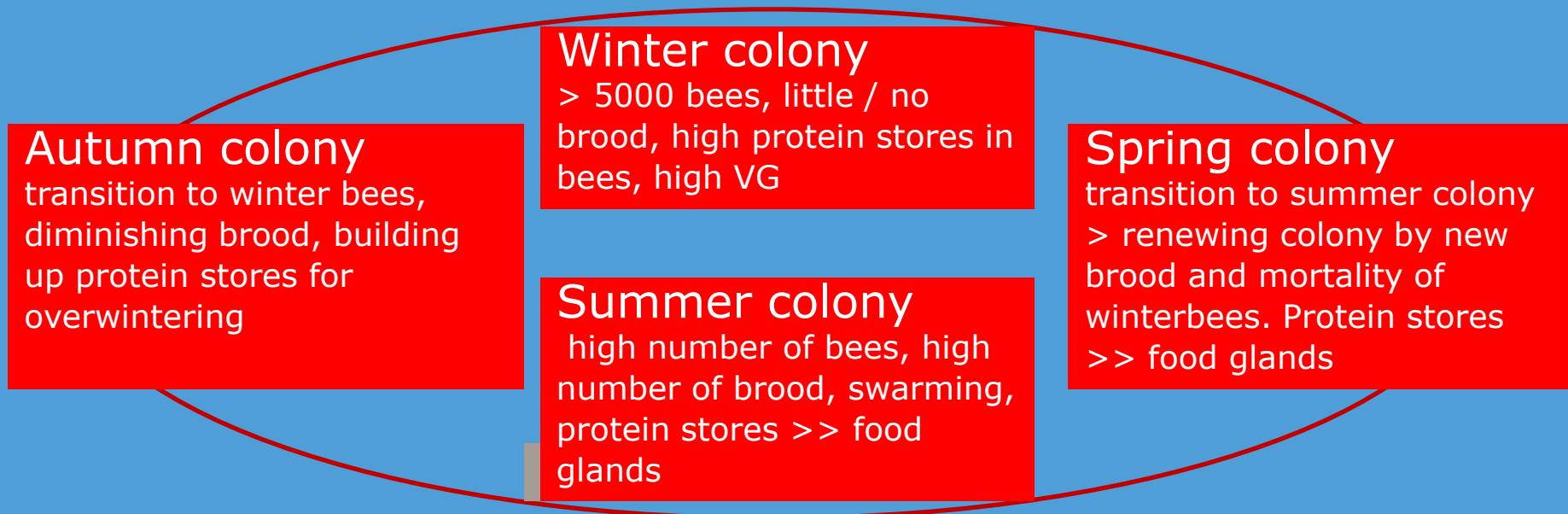


Status September: G: pollen divers > winter colony; PW: poor pollen diversity > summer colonies



Summary

- Status of a honey bee colony alters in the course of the bee season



This normal alteration can be disturbed by
quality pollen feed



Questions?



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