

Proteins, Honey Bee Nutrition and “Amino-B Booster™”

Jan, 2011

Honey bees get protein by digesting pollen grains in the midgut. Different pollen types have different kinds and amounts of protein.

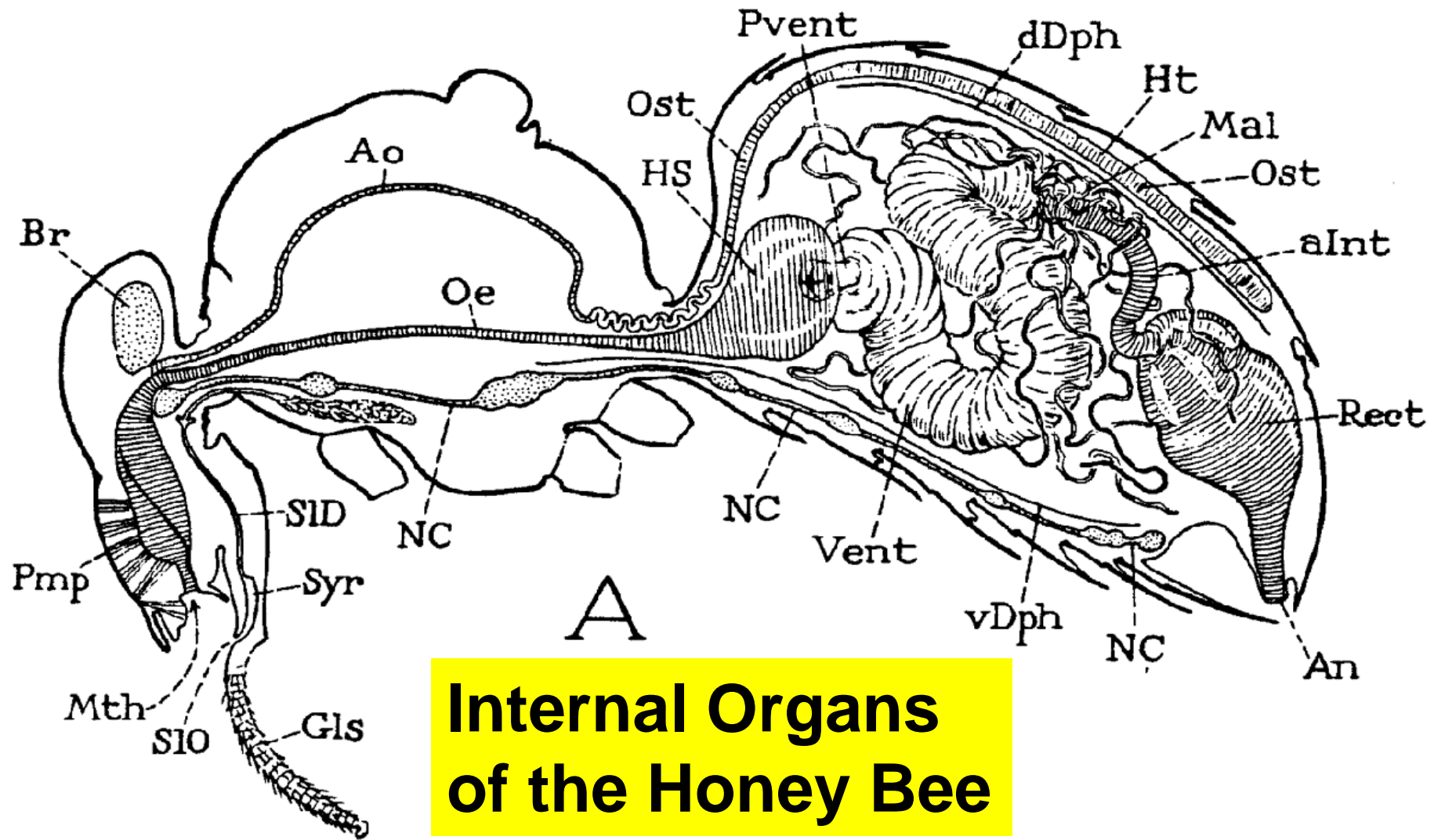
Pollens that are relatively rich in protein come from plants that are usually bee-pollinated: dandelions, apples and other fruit trees, clovers, alfalfa, false indigo, and many others.

Pollens that are poor in protein include most grasses, sedges, conifers, ragweeds, and other plants that are wind pollinated.

But, in all cases, bees obtain their protein from digestion of pollen grains in the midgut.

The bees must secrete enzymes into the midgut, these must penetrate the tough pollen grains, then the various proteins, if present, are digested into fragments called peptides and polypeptides until finally, individual amino acids are released. These are then able to pass into the midgut cells where they are quickly assimilated and passed to various insect blood cells, or hemocytes, which circulate throughout the body, supplying the valuable protein to growing tissues, glands, muscles, etc.

A peptide is a molecule containing 2 or more amino acids.



Internal Organs of the Honey Bee

Modified from: *The Hive and the Honey Bee*, p. 147, fig. 17A. Ch. 4, *The Anatomy of the Honey Bee*, by R.E. Snodgrass and E.H. Erickson. After: Snodgrass, R.E. 1956. (1st edition.) *The Anatomy of the Honey Bee*. Comstock Publishing - Div. of Cornell Univ. Press. 334 pp.

alnt-anterior intestine, or anterior hind gut [starts where Mal are attached]

An-anus

Ao-Aorta, vessel from heart to the head and brain

Br-Brain

dDph-dorsal diaphragm, isolates the heart and some fat body organs

Gls-glossa, the bees tongue

HS-Honey Stomach or Crop, lined with cuticle

Ht-Heart, has multiple, linear chambers; each can contract separately

Mal-Malpighian Tubules, the equivalent of the mammalian kidney

Mth-Mouth

NC-Nerve Cord; has several enlarged nerve centers called ganglia

Oe-oesophagus, leads from the mouth to the honey stomach

Ost-ostia, valve openings in each heart chamber; allows fluids to enter when it relaxes

Pmp-cibarial Pump, muscles that expand the cibarium, the chamber leading to the oesophagus

Pvent-proventriculus, a valve from the honey stomach to the ventriculus or midgut.

Rect-rectum, muscled chamber that collects matter for excretion, absorbs water.

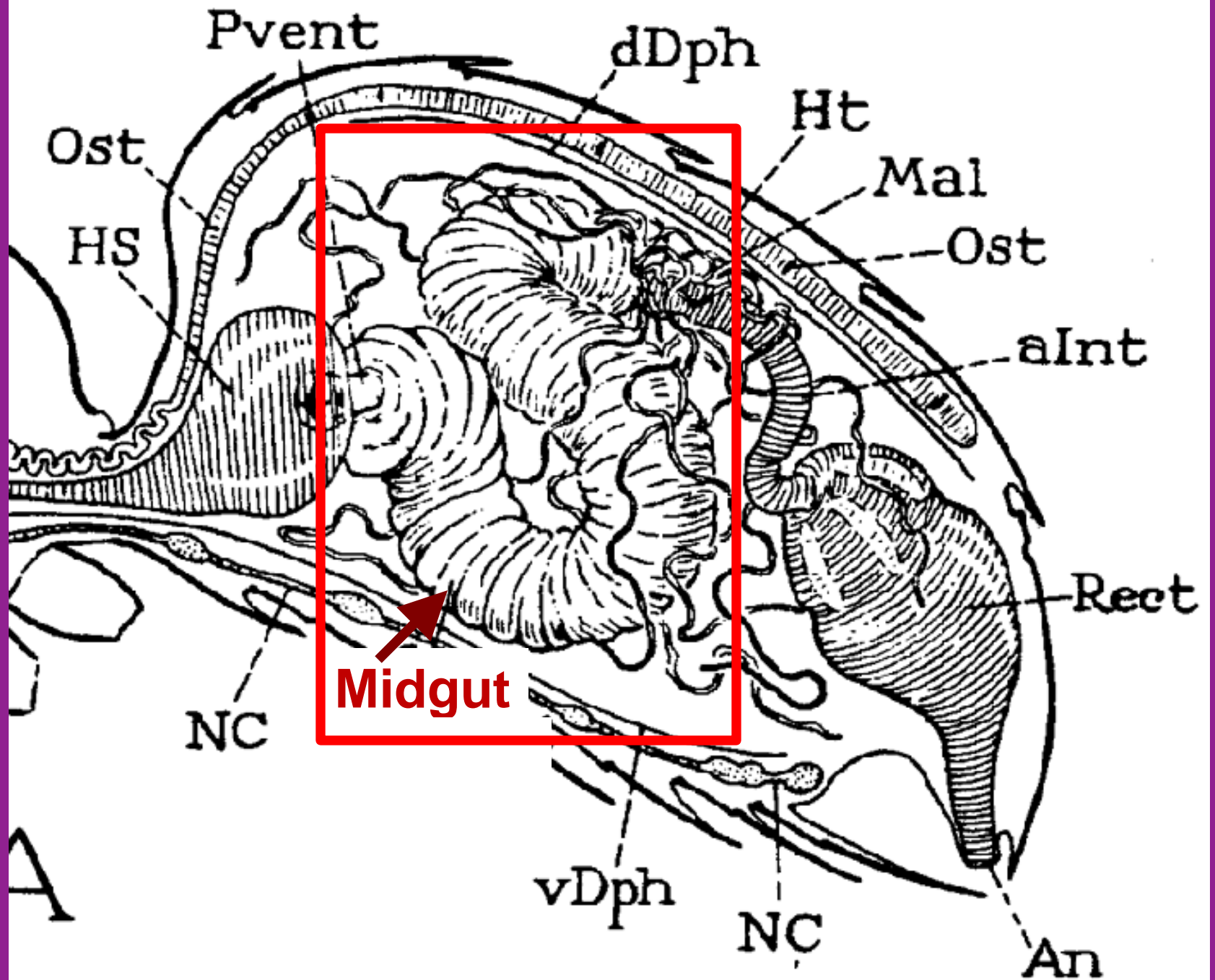
Sld-salivary duct, bee adds invertase to nectar, and other secretions

Slo-salivary duct opening

Syr-syringe, chamber that holds the salivary secretions

vDph-ventral diaphragm, isolates the nerve cord

Vent-Ventriculus or Midgut.



The midgut is where pollen grains are digested. The midgut cells secrete enzymes which are large protein catalysts: they accelerate or moderate chemical reactions, like digestion of protein.

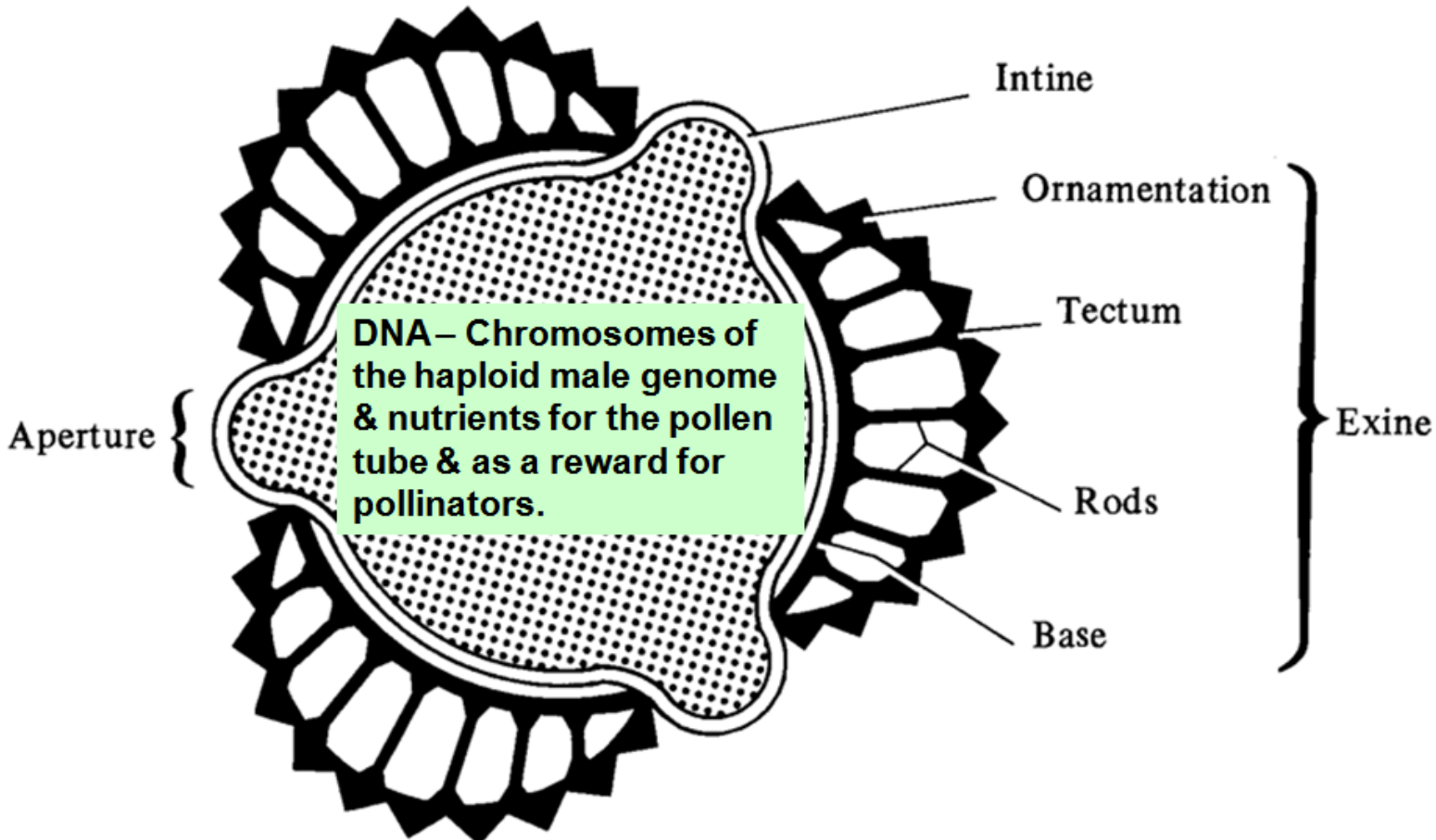
The enzymes must penetrate the pollen grains to reach the nutrients inside in order to digest them. For most pollens only part of the proteins are digested. For example, the essential amino acid (AA) isoleucine as a product of digestion must be equal to 4 mg for each 16 mg of protein present before it can be a substantial nutrient for the bees. If the pollen protein contains less, then this essential amino acid is not available in sufficient quantity for the bees, and the colony suffers 'protein stress'. Feeding amino acids to the bees in syrup with Honey-B-Healthy© (HBH) bypasses this restriction.

Once the protein has been digested to free amino acids or small di- and tri-peptides, these AA can freely enter the midgut cells and be delivered to hemocytes (blood cells) or to the hemolymph. Unlike humans, insect hemolymph or 'blood' has a significant quantity of AA present in order to maintain proper pH and ionic levels. There are no AA in human blood (i.e., **normally**); in humans, carbonic acid and bicarbonates perform this role.

Eating the pollen, secreting the enzymes, digesting the protein (and other nutrients) and absorbing the AA all require an input of energy and take time. The quality and presence of the AA diminishes as pollen ages; fresh pollen is always best.

Feeding free amino acids in a sugar syrup with HBH is a great advantage for the bees; they simply absorb the AA they need and deliver the AA to growing tissues. The syrup, because of the HBH as a preservative, can be stored in cells in the pollen area as amino jelly, and fed to the brood and bees as needed.

Detail of a Triporate Pollen Grain.



The exine is the tough outer layer of the pollen grain (heavy black); it resists chemicals and environmental extremes. Enzymes must penetrate the next layer, the intine, at the pores. Liquids must accompany the enzymes in order to reach the nutrients (eg., protein). Bees need a variety of pollens in order to get adequate nutrition. Plants that are pollinated by insects have larger, often sticky pollen grains which are more nutritious.

Pellets of Honey Bee Pollen from Cumberland, MD Sep 2002 (Bob Noel, Pollen Trap).



Pollen Stored Dry in vials



Black Pollen



Microscope Light



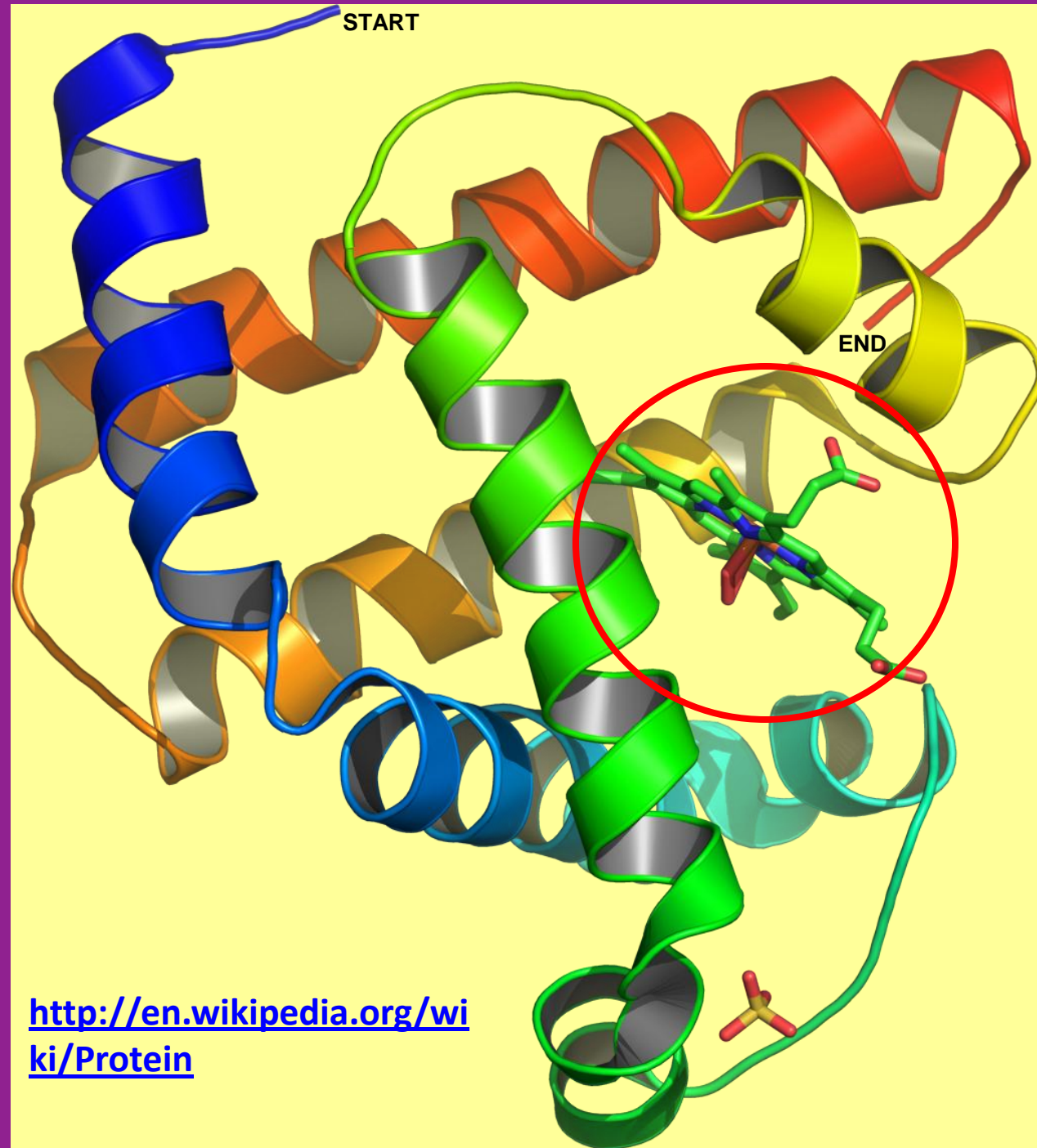
Room Light

These are the 21 amino acids (AA) that are the building blocks of all biological proteins; 8 are absolutely essential to humans and honey bees; several of the others may be essential at various times or situations or for specific organisms.

<u>Essential AA</u>	<u>Nonessential AA</u>
<u>Isoleucine</u>	<u>Alanine</u>
<u>Leucine</u>	<u>Asparagine</u>
<u>Lysine</u>	<u>Aspartic Acid</u>
<u>Methionine</u>	<u>Cysteine</u> *
<u>Phenylalanine</u>	<u>Glutamic Acid</u>
<u>Threonine</u>	<u>Glutamine</u> *
<u>Tryptophan</u>	<u>Glycine</u> *
<u>Valine</u>	<u>Proline</u> *
	<u>Selenocysteine</u> *
	<u>Serine</u> *
	<u>Tyrosine</u> *
	<u>Arginine</u> *
	<u>Histidine</u> *

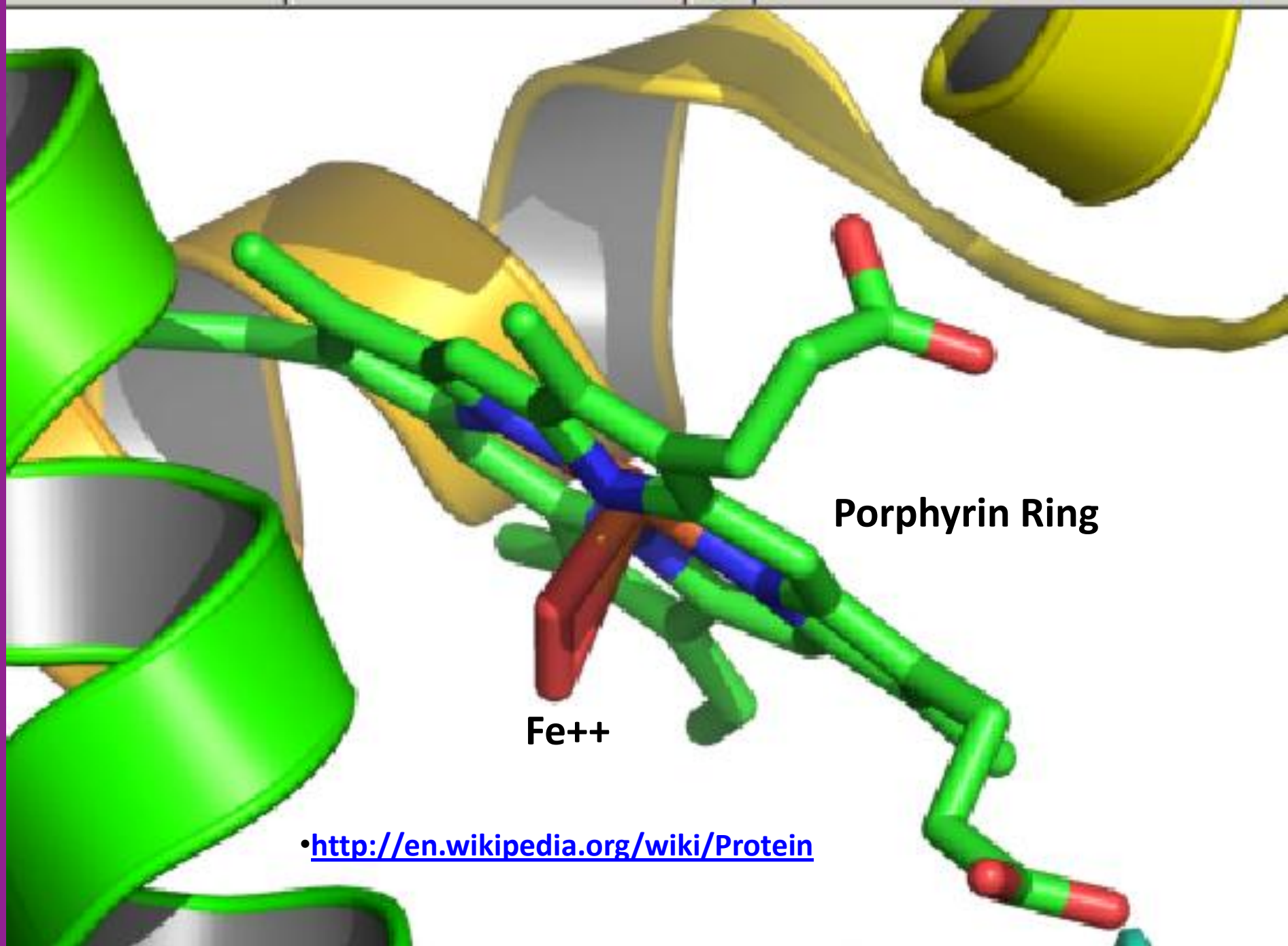
• = amino acids that are essential in specific situations or for specific organisms.

• http://en.wikipedia.org/wiki/Amino_acid



A model of the 3D structure of the protein, myoglobin (the primary oxygen-carrying pigment of muscle tissues), showing colored alpha helices. This protein was the first to have its structure solved by X-ray crystallography. It contains 154 amino acids; mw = 16,700 daltons. Myoglobin contains a porphyrin ring (red circle) with an iron center. There is a *proximal histidine* group attached directly to the iron center, and a *distal histidine* group on the opposite face, not bonded to the iron.

<http://en.wikipedia.org/wiki/Protein>



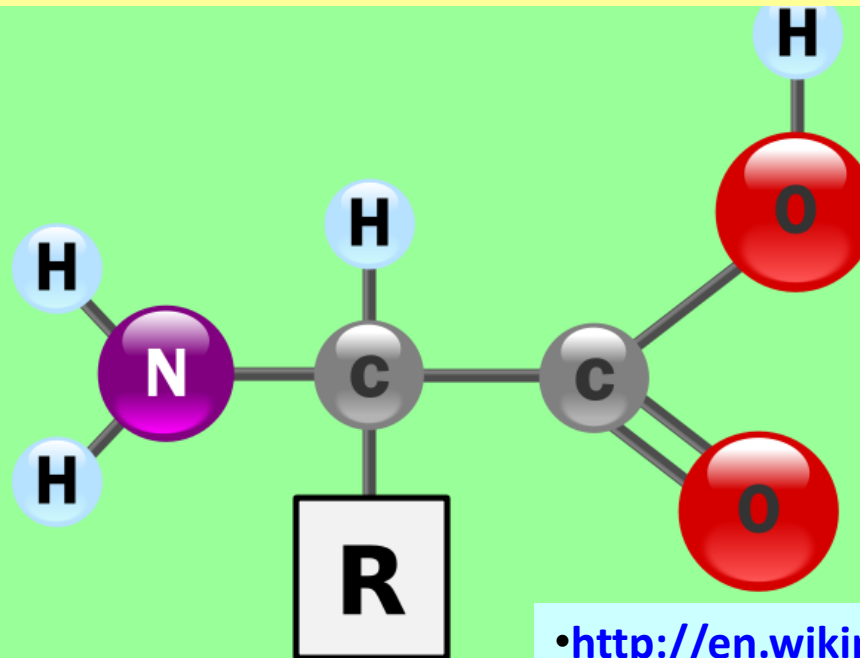
Porphyrin Ring

Fe⁺⁺

• <http://en.wikipedia.org/wiki/Protein>

Proteins (also known as polypeptides or complexes of polypeptides) are very large organic molecules made from the polymeric combination of 21 different amino acids arranged in a linear chain and often folded into some form – globular, sheet, etc. Different types of protein are muscle and muscle fibers, skin, hooves, claws, fingernails, hair, antibodies, mucous, enzymes, etc. There are millions of types of proteins; many are specific for very special functions. But all proteins have amino acids as basic building blocks, forming peptides, polypeptides, subunits and proteins.

Amino acids are molecules containing an amine group (NH_2), a carboxylic acid group (COOH) and a variable side chain (R) of various carbon based molecules that varies in structure between the 21 different amino acids. The side chains can be neutral, positively or negatively charged, and hydrophobic (water resistant) or hydrophilic.

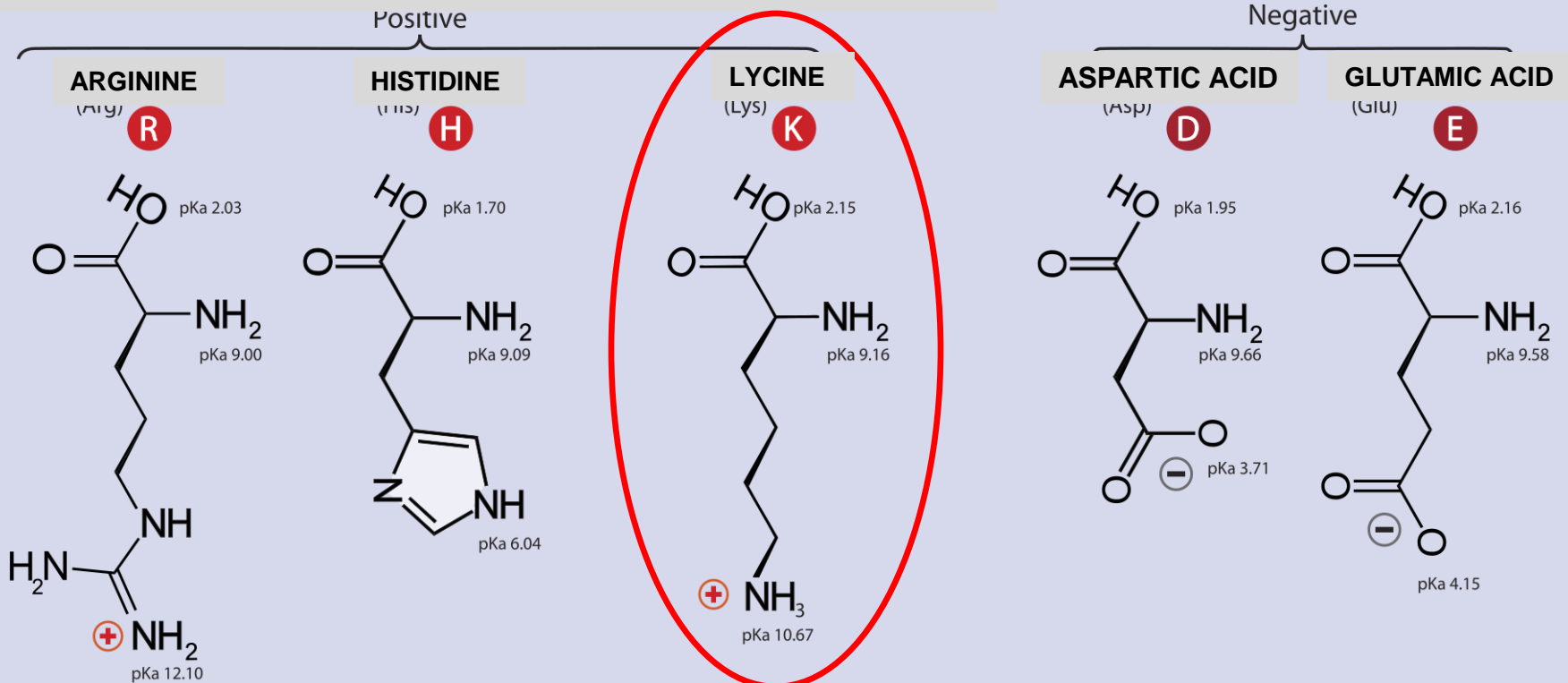


The Twenty-one Amino Acids

Side Chain Charge (pH 7.4):

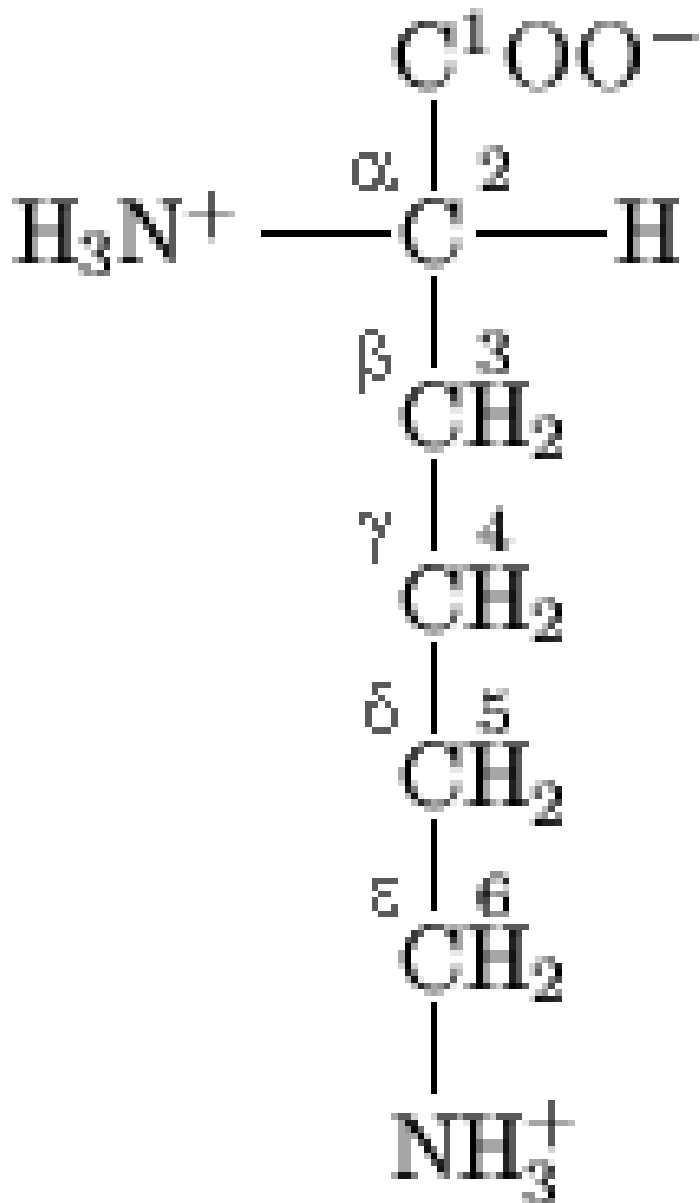


A. Amino Acids with Electrically Charged Side Chains [R].



http://en.wikipedia.org/wiki/Amino_acid

Next Slide



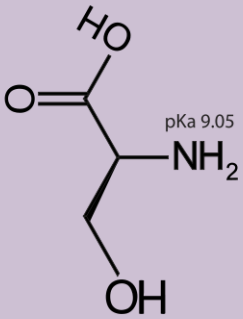
The amino acid lysine with the atoms identified and the carbon atoms labeled from alpha to epsilon, using Greek letters, and numbered from 1 to 6. The carboxyl group is negatively charged and the amine group is positively charged.

B. Amino Acids with Polar Uncharged Side Chains [R].

SERINE

(Ser) **S**

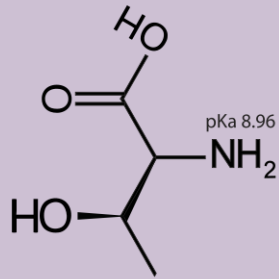
pKa 2.13



THREONINE

(Thr) **T**

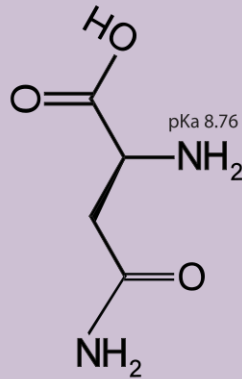
pKa 2.20



ASPARAGINE

(Asn) **N**

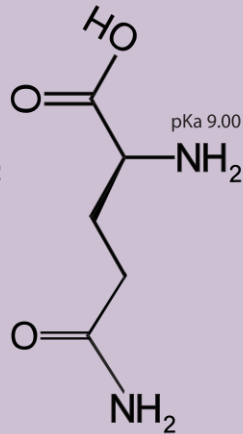
pKa 2.16



GLUTAMINE

(Gln) **Q**

pKa 2.18

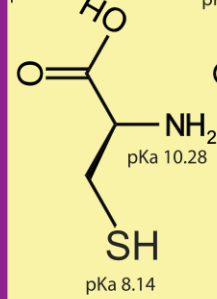


C. Amino Acids with special cases.

Cysteine

(Cys) **C**

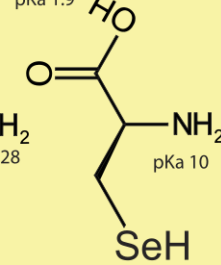
pKa 1.91



Selenocysteine

(Sec) **U**

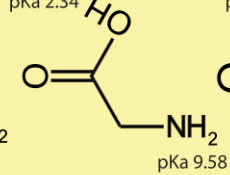
pKa 1.9



Glycine

(Gly) **G**

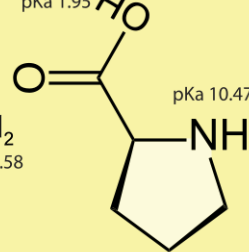
pKa 2.34



Proline

(Pro) **P**

pKa 1.95



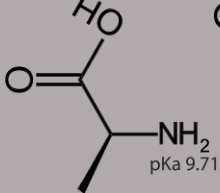
Selenocysteine is a relatively rare AA that incorporates Selenium for specific enzymes.

D. Amino Acids with hydrophobic side chains.

ALANINE

(Ala) **A**

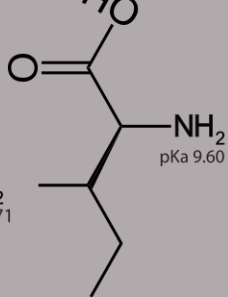
pKa 2.33



ISOLEUCINE

(Iso) **I**

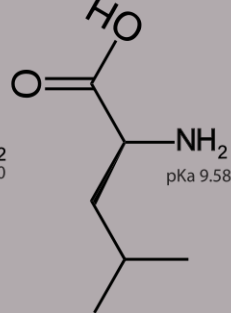
pKa 2.26



LEUCINE

(Leu) **L**

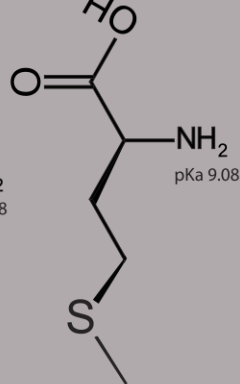
pKa 2.32



METHIONINE

(Met) **M**

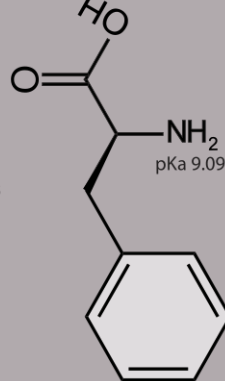
pKa 2.16



PHENYLALANINE

(Phe) **F**

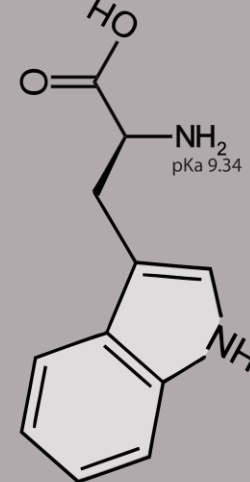
pKa 2.18



TRYPTOPHANE

(Trp) **W**

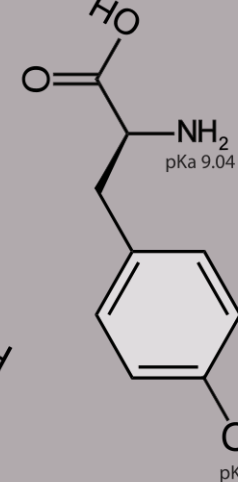
pKa 2.38



TYROSINE

(Tyr) **Y**

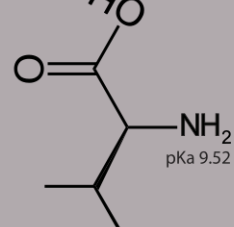
pKa 2.24



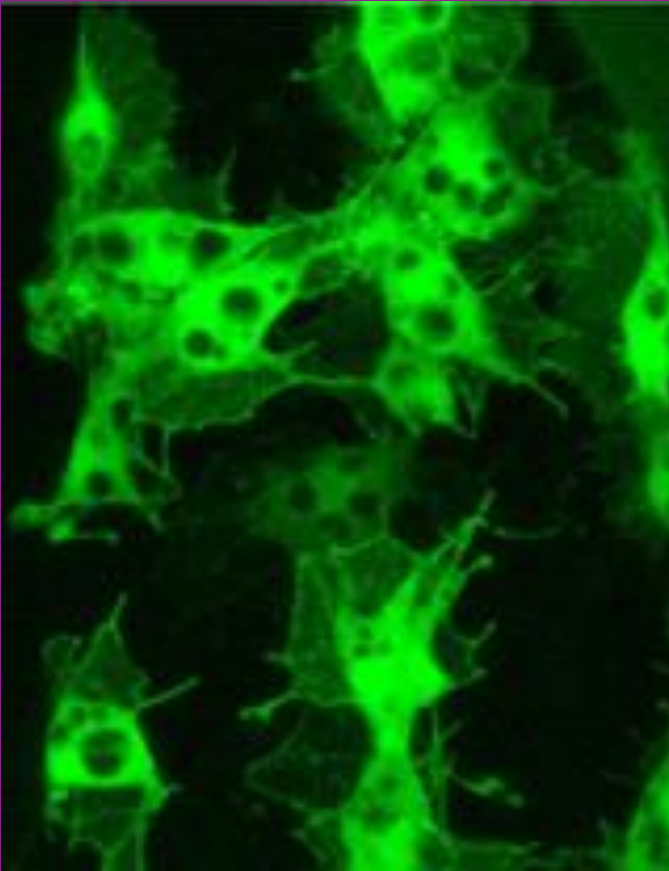
VALINE

(Val) **V**

pKa 2.27



Insect and honey bee hemolymph [insect ‘blood’], unlike humans, has free amino acids that act to buffer the hemolymph pH during physiological processes. Free amino acids resulting from digestion of pollen in the gut lumen can pass directly into midgut cells then directly into the hemolymph. Some AA are packed into specific hemocytes, which may contain numerous granules of the concentrated amino acids. These hemocytes then move to areas where tissues are growing.



Embryonic Hemocytes of *Drosophila* (fruit fly) (Diptera: Drosophilidae) [using confocal microscopy]. (Will Bath.)

<http://www.bath.ac.uk/bio-sci/research/profiles/wood-w.html>

Some of the inclusions (dark bodies) are packets or granules of amino acids.

The natural sequence of protein movement in worker bees is:

1. Build up of food glands in nurse bees: the mandibular and hypopharyngeal glands in the head produce bee milk and royal jelly in workers aged 1 to 12 days; the more rapidly they assimilate free amino acids, the larger the food glands and the higher the quality and quantity of the bee milk and royal jelly.
2. Protein from the food glands is then sequestered [broken down to amino acids for transport] and moved to the wax glands in the abdomen in order to produce wax to cap brood and honey - in workers aged 12 to 18 days. Again, the more quickly they can assimilate free amino acids, the larger the wax glands and the quantities and quality of the resulting wax will be higher.
3. The wax gland protein is then sequestered and moved to the flight muscles in bees >18 days old, for foraging and flight activity. Availability of free amino acids will result in more rapid muscle development.

All of these protein uses can be reversed as needed by the colonies.

The new product, “Amino-B Booster”™ is a specially formulated apiary blend of amino acids that can be mixed with sugar syrups (works well in either sucrose or fructose mixtures), and mixed with Honey-B-Healthy© which enhances its attractiveness to bees and prevents fermentation.

As a blend of amino acids, Amino-B Booster™ is assimilated rapidly, directly through the midgut to the bee hemolymph and hemocytes, then transported to the sites where protein is needed for growth of the bee, both as larvae and as adults.

Amino-B Booster™ was formulated to help build up honey bee colonies when protein was missing from the diet, as in midwinter when no pollen is produced and when, on warm days, bees flock to bird feeders looking for anything that resembles pollen. The old stored pollen may be low in quantity and/or deficient in protein or certain essential amino acids, and the bees are desperate for any protein. Some stored pollens may be toxic to bees, such as that of Camas lilies, and the bees need clean protein sources to recover from poisoning.

In early spring, 2009, experiments conducted by Bob Noel in Cumberland, MD, and by the apiculture class at WVU showed that colonies fed Amino-B Booster syrup with HBH produced larger, healthier workers and drones than did colonies producing bees from old, deficient pollen stores.

Fall honey bees can contain as much as 67% protein, including vitellogenin (a special protein-usually a yolk precursor), relative to their body weight. In addition to the fats and stored glycogen, these nutrients help the wintering bees survive the winter.

When brood is produced in January-February, workers can sequester much of their own protein to produce bee milk (in the food glands) to be fed to young bee larvae. Bees can utilize their protein for brood rearing until their protein content drops to 20% of body weight (they take protein from muscle, glands, endocuticle, fat body, etc.)

This is when Amino-B Booster™ can greatly aid workers in producing brood - early in the season when protein is in short supply.

When feeding Amino-B Booster™ in sugar syrup with Honey B Healthy, we have seen:

Bees swarm over jars containing Amino-B Booster™ + HBH compared to jars with syrup + HBH or with syrup + Amino-B Booster™ alone.

The syrup with Amino-B Booster (amino acids) + HBH is stored in the same region as fresh pollen: in a band around the brood.

The syrup with Amino-B Booster is concentrated into an “amino jelly” which is preserved by the HBH.

Bees can utilize this amino jelly, containing 20 amino acids, at any time during the fall, winter and early spring.



**HBH + Sugar
Water**

**Sugar Water +
Amino-B Booster**

**HBH + Sugar
Water + Amino-B
Booster**

**More Bees Than
The Other Two**

**The bees seem to sense there
is something extra in this jar.**

Three half quarts of feeding mixes placed on top of a colony. Within a short time bees were all over the HBH/A-BB sugar water jar on the right with only a few bees on the A-BB sugar mix center and HBH sugar mix at left.

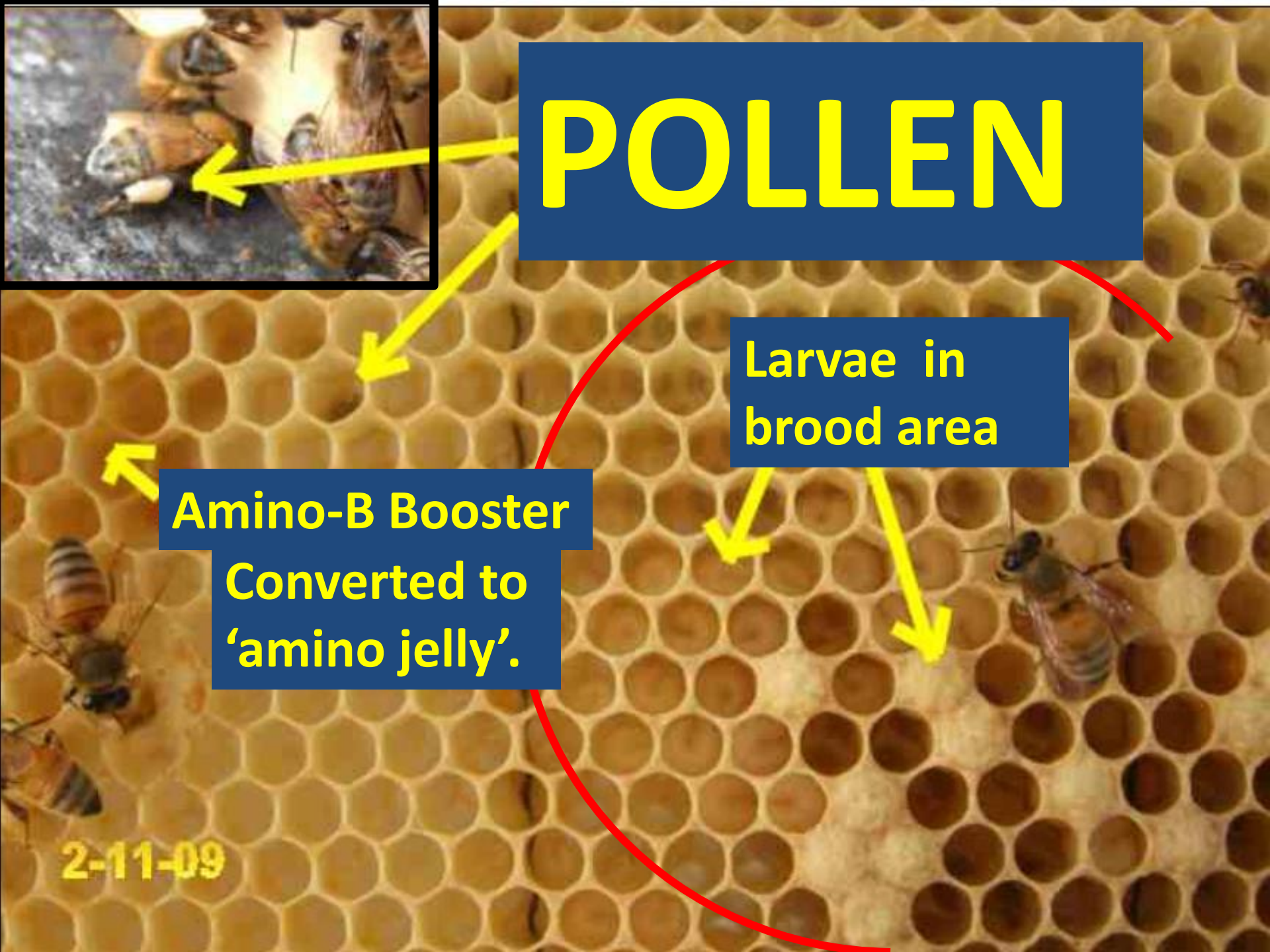
12-28-09

POLLEN

Larvae in
brood area

Amino-B Booster
Converted to
'amino jelly'.

2-11-09





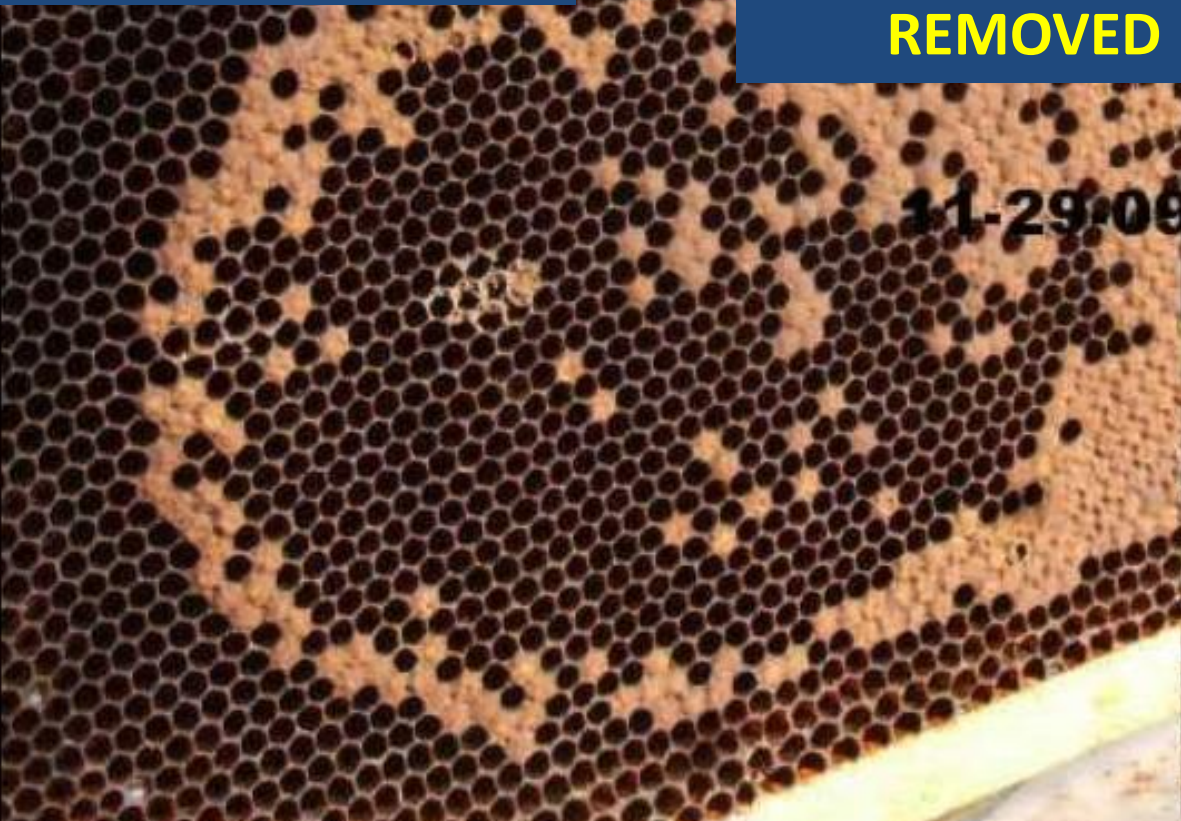
11-29-09

CLOSE-UP OF BEES



11-29-09

**FRAME WITH BEES
REMOVED**



11-29-09



11-29-09

FRAME WITH BEES

Feeding Amino-B Booster can benefit bees and beekeepers in the following situations:

- 1. Early brood development for beekeepers producing strong colonies for pollination, or for producing new colonies or nucs.**
- 2. Feeding bees protein during dearths of pollen in late winter, mid-summer or during droughts.**
- 3. For beekeepers producing queens: Amino-B Booster will provide all of the amino acids needed for healthy nurse bees and for production of high quality bee milk and royal jelly.**
- 4. To assist beekeepers to produce early strong colonies to take advantage of early blooming honey plants, such as autumn olive in WV.**
- 5. To build and strengthen weak colonies; especially following *Nosema ceranae* attack or after pesticide injury.**

6. To simulate a nectar flow and/or pollen bloom when using 1:1 sugar syrup containing 5ml/l (1tsp.qt) Amino-B Booster with HBH.

7. Can be used as a 'Protein drench' when bees are taking pollen substitutes very slowly: spray or drizzle the Amino-B Booster and HBH into the brood area; bees will clean it up, store it and use it.

8. Can be applied during instances of 'protein stress' when bees are producing brood and available pollen sources are limited in quantity and quality of protein or essential amino acids (e.g., 'lucerne stress' in Australia; Stace 1996).

Precautionary Statements:

The mixture of the three components, 1:1 [ratio can vary] sucrose sugar syrup [cane or beet sugar], Amino-B Booster™ and Honey-B-Healthy© is synergistic. That is, the mixture of all three components produces a greater effect than any one or two component(s) alone.

Using Amino B Booster with sugar syrup alone should not be done; the mixture will ferment and may harm your bees. Always add Honey-B-Healthy, the real thing, which greatly reduces or inhibits fermentation. Poorly made imitation products stated to be the same as Honey-B-Healthy will not protect the mixture of syrup and Amino-B Booster; we know, we have tested them. The mixture will ferment and may harm your bees.

Remove honey supers when feeding Amino-B Booster™.

Feeding Amino-B Booster™ in the fall is not recommended, unless you know what you are doing and you have surplus honey on the bees. Having several frames of brood all winter in northern climes will cause the bees to consume honey at a high rate, and may contribute to the death of weak or stressed colonies.

However, if you want to pollinate almonds in February, and you have sufficient honey to feed the bees, this is an excellent way to add more frames of brood to your colonies.

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Stace P. 1996. Protein content and amino acid profiles of honey bee collected pollens.
<http://www.honeybee.com.au/Library/Pollenindex.html> ISBN 0-7310-2867-8, Bees 'N Trees Consultants, NSW Australia 2480.

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<http://rnoel.50megs.com/2000/index.htm>

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