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Pollen nutrition affects honey bee stress resistance

Zachary Huang

Department of Entomology, Michigan State University, East Lansing, Michigan 48824, USA
bees@msu.edu
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Summary

The honey bee, *Apis*, is perhaps the most beneficial insect to humans because most of our fruits and vegetables depend on them for pollination. Yet these iconic insects have been plagued by many types of stresses. This paper reviews many lines of evidence pointing to the importance of pollen nutrition in honey bee health. In laboratory studies that used caged honey bees, poor pollen nutrition led to a reduction of worker bees' resistance to the microsporidian, *Nosema apis*, an increase of bee's sensitivity to pesticides, and an increased titer of bee virus. On the other hand, polyfloral pollen made bees more resistant to stresses by enhancing their immune related enzyme activities. At the colony level, good pollen nutrition increased honey bee's resistance to *Nosema ceranae* or the ectoparasitic mite, *Varroa destructor*. The effects of both transportation and habitat changes on honey bees seem most likely mediated via decreased diversity, or amount, of pollen to the colonies. Pollen nutrition, therefore, might work together with other factors in reducing the bees' resistance and exacerbate the effects of viruses, pesticides, or parasites, eventually resulting in Colony Collapse Disorder. Besides paying attention to all of these other factors, pollen nutrition should be an important focus in the future for maintaining healthy bee colonies.

Keywords

Pollen nutrition; honey bee; *Apis mellifera*; colony collapse disorder; essential amino acids; pollen substitutes; *Nosema*; *Varroa*; immunosuppression; habitat destruction

Introduction

Honey bees are unequivocally the most important insect pollinator for humankind (Fig. 1). Honey bee pollination of commercial crops was valued at nearly $15 billion USD per year in U.S. alone (Morse and Calderone, 2000). This is likely an underestimate, since in the state of Michigan alone, fruits, vegetables and filed crops have already been added to almost $1 billion USD per year (Huang, 2010). For world agriculture, more recent estimates also suggest honey bees are worth much more. Costanza et al. (1997) estimated the value of all pollination ecosystem services to be $117 billion USD per year, while Richards (1993) estimated that the value of pollination in global agriculture alone amounts to $200 billion USD per year. Gallai et al. (2009) estimated

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Figure 1. A worker honey bee collecting pollen from a spiderwort (*Tradescantia virginiana L.*, Commelinaceae*)*, a commonly cultivated garden flower. Photo by Zachary Huang.

the economic value of the pollination services to agriculture worldwide to be 153 billion euro. The majority of insect pollination was done by the managed Western honey bees (*Apis mellifera* Linnaeus). The importance of honey bees to world agriculture is simply indisputable.

Honey bees in the U.S. have been long plagued by introduced pests/diseases. These include the tracheal mites, *Acarapis woodi* (Rennie) in 1984 (Wilson et al., 1997), *Varroa* mites, *Varroa destructor* (Anderson and Trueman), in 1987 (De Jong et al., 1997), and the small hive beetle (Nitidulidae), *Aethina tumida* (Murray), in 1996 (Neumann and Elzen, 2004). A microsporadia, *Nosema apis* (Zander), probably came with the first batch of honey bees with the early settlers, but we are not sure when *Nosema ceranae* (Fries) came into the U.S., we only know that it was present in samples as old as 1995 (Chen et al., 2008).

Recently, Colony Collapse Disorder (CCD) emerged to attack honey bees in the U.S. and has caused 30%-40% loss of bee colonies each year since the fall of 2006 (vanEngelsdorp et al., 2009). CCD-affected colonies have greatly reduced adult bee populations, leaving only a few hundred workers and the queen, yet with many frames of brood, which suggests rapid depopulation of adults. The cause of CCD remains unknown, but many scientists believe that it may be caused by a combination of factors, such as pesticides, parasites, nutritional stress, and stress from long distance transportation (Oldroyd, 2007; Huang, 2008). Poor nutrition can weaken the immune system at both the individual and colony level, thus exacerbating effects of other stresses. In this review, I concentrate on how protein nutrition affects honey bee stress resistance and its relation to the CCD crisis. A recent review by Brodschneider and Crailsheim (2010) dealt with carbohydrate, protein and other nutrition of honey bees.

The importance of pollen

Similar to other animals, ten amino acids are essential to honey bee development, brood rearing and reproduction (reviewed by Keller et al., 2005). Amino acid content in nectar is negligible, so pollen is the only source of these amino acids. Pollen is therefore essential to individual bees and colony development.

Haydak (1967) found that colonies lacking pollen had high worker mortality, no interest in queen-caring and remained weak. Early studies showed that the development of hypopharyngeal (HP) glands relies on pollen feeding (reviewed by Haydak, 1970). When honey bees were provided with insufficient pollen, or pollen with low nutritional value, brood rearing decreased (Kleinschmidt and Kondos, 1976; 1977), and workers lived shorter (Knox et al., 1971). These effects ultimately affected colony productivity. Shortages of pollen during rainy seasons have also been found to cause colony decline or collapse (Neupane and Thapa, 2005). Recent studies have shown that spring pollen supplement can work as an insurance, when weather is poor, for faster spring buildup and higher honey yield (Mattila and Otis, 2006a), and can reduce the effects of *Varroa* parasitism (Janmaat and Winston, 2000) and *Nosema* infection (Mattila and Otis, 2006b). Honey can be replaced by sucrose to feed bees, while a complete substitute for pollen has not been found. Therefore, pollen becomes a limiting factor in maintaining good colony health.

Measurements of pollen quality

Pollen quality can be measured by two methods: crude protein levels and the composition of amino acids. Ten amino acids have been found to be "essential" for honey bees (De Groot, 1953), meaning that bees cannot synthesize or even convert other amino acids to acquire them. Bees therefore must obtain them directly from food, either as free amino acids or digested from protein. These ten amino acids are listed in Fig. 2. The crude protein level tells us how much protein a particular plant pollen has. Higher crude protein levels are better than lower ones. However, if the ten amino acids are not balanced, bees cannot fully use what is available in a type of pollen. For example, Fig. 2 shows that honey bees need 4% isoleucine from the total available amino acids. If a certain type of pollen contains only 2% isoleucine, then bees can only use 50% of the total protein available in the pollen because isoleucine becomes the limiting factor (Stace, 1996). This forces bees to ingest twice the amount of total pollen to obtain the needed isoleucine, essentially wasting half of the total protein (De Groot, 1953).

The amino acid profiles of major plants that provide bees with pollen were investigated in France (Rasmont et al., 2005) and Australia (Stace, 1996; Sommervile, 2004; Somerville and Nicol, 2006). However, this has not yet been done in the U.S. Roulston et al. (2000) measured 377 plant species from North and Central America, but only

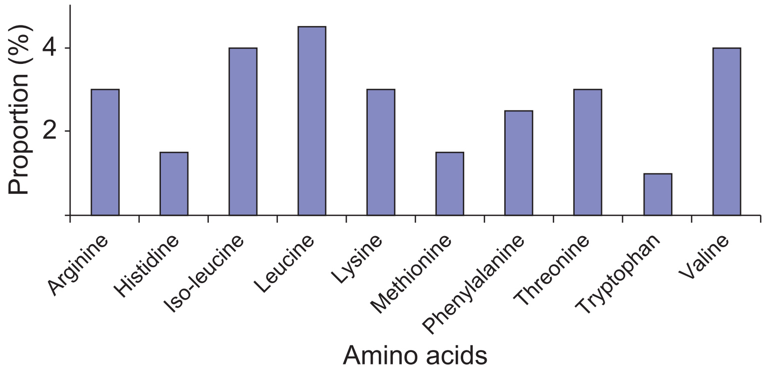

Figure 2. Proportion (%) of the 10 essential amino acids needed by honey bees (after De Groot, 1953).

for total protein concentrations and not for profiles of the ten essential amino acids. I have been actively pursuing funding to do this for the U.S. pollen plants but have not been successful to date.

Nutrition to honey bees is plant-dependent

Pollen from different plant species has different nutritional values to honey bees. The protein content of pollen ranges from 2.5% in cypress (*Cupressus arizonica* Greene, Cupressaceae) to 61.7% in Padre's shooting star (*Dodecatheon clevelandii* G., Primulaceae) (reviewed by Keller et al., 2005). A protein level around 20% -25% in pollen meets the honey bee colony requirements readily, otherwise longevity of workers and brood rearing are affected by the lack of the protein (Schmidt et al., 1987). Schmidt et al. (1987) studied the nutritional value of 25 species-pure pollen by feeding caged bees and observing their survival. Both pollen consumption rates and crude protein levels were correlated with the ability to improve longevity. Pollen that decreased worker longevity included ragweed (*Ambrosia sp*, Asteraceae), rust spore (*Uromyces*, Pucciniaceae), cattail (*Typha*, Typhaceae), and Mexican poppy (*Kallstroemia*, Zygophyllaceae). Those that slightly improved worker longevity included terpentine bush (*Haplopappus*), desert broom (*Baccharis*), and dandelion (*Taraxacmu)*, all from the family Asteraceae. The best pollens are these from Mormon tea (*Ephedra*, Ephedraceae), mesquite (*Prosopis*, Fabaceae), blackberry (*Rubus*, Rosaceae), and cottonwood (*Populus*, Salicaceae). Mixed pollen consistently performed very well. In another study, Schmidt et al. (1995) concluded that bees foraging in the fields of sesame (*Sesamum indicum* L., Pedaliaceae) and sunflower (*Helianthus annuus* L., Asteraceae) should be supplemented with other pollen, but rapeseed (*Brassica napus* L., Brassicaceae) pollen is highly nutritious to bees and does not need supplementation. Through these studies, Schmidt concluded that factors contributing to increased bee longevity include 1) the presence of attractants and phagostimulants, so that bees will readily consume large amounts of pollen; 2) lack of toxic compounds; and 3) a good nutrient balance. So far no studies have tried to correlate the amino acid profile of a type of pollen with its ability to improve worker longevity.

Pollen substitute for bees

The amount of pollen available to bee colonies is a limiting factor in apiculture. Honey bee colonies normally have very limited pollen stores during winter (Maurizio, 1950; Fluri et al., 1982). To make a strong colony, pollen substitutes have been used to supplement pollen during the off-season. Honey bees usually prefer pollen over pollen substitutes, not because pollen is more nutritious, but because there are substances in pollen making it more attractive. There is a strong correlation between the amount of food consumed and the size of brood area and adult population (Herbert and Shimanuki, 1982; DeGrandi-Hoffman et al., 2008). Herbert et al. (1980) showed that adding lipid extracts from pollen can increase food consumption and brood rearing in the honey bee colony. Utilizing soy bean (*Glycine max* Merrill, Fabaceae) and lupin (*Lupinus*, Fabaceae) flours as pollen substitutes reduces the longevity of the honey bee (Manning and Rutkay, 2007), probably due to the presence of stachyose in them. Stachyose is toxic to honey bees unless it is diluted to below 4% of total sugar using 50% sucrose (Barker, 1977).

Schmidt et al. (1995) suggested that a good pollen substitute for honey bees should have the same features as good pollen: 1) palatability: bees will readily consume it, 2) digestibility: it is easily digested by bees, and 3) balance: it has the correct the amino acid balance and enough crude proteins. Currently there are four commercial pollen substitutes for honey bees in the U.S.: Bee-Pol®, Bee-Pro®, Feed-Bee®, and MegaBee®. It appears that Bee-Pro® is soy-based, and Feed-Bee® and MegaBee are non-soy-based. There is insufficient information available for Bee-Pol. Cremonez et al. (1998) fed caged bees various diets and used hemolymph protein titer to assess their quality, with a higher protein titer suggesting a higher quality. Six-day-old bees had protein concentration of 27.6, 24.1, 11.4, 3.98, and 2.22 μg/μl, for bees fed with bee bread, soybean/yeast, pollen, corn meal and sucrose only, respectively. De Jong et al. (2009) used the same assay to assess the quality of commercial pollen substitutes. They found that bees feeding on Feed-Bee®, Bee-Pro®, pollen, *Acacia* pod flour diets and sucrose had hemolymph titers of 9.42, 8.95, 6.26, 6.0 and 3.56 2 μg/μl, respectively.

Gregory (2006) reported the longevity of bees fed different diets within small colonies, ranked by superiority: fresh pollen > Feed-Bee® > Bee-Pro® > old pollen. In cage studies, bees fed with Feed-Bee® had hemolymph protein levels similar to those fed with fresh pollen. This study also suggested that higher hemolymph protein in workers would allow them to live longer, although the full study is not published and the abstract did not provide a regression analysis between the two variables.

DeGrandi-Hoffman et al. (2008) evaluated three diets, Bee-Pro®, Feed-Bee®, and MegaBee®, in two separate trials. In both trials, Bee-Pro® and MegaBee® patties were consumed at rates similar to pollen cake, but Feed-Bee® was consumed significantly less. Higher food consumption was significantly correlated with increase in brood area and adult population size. According to this study, MegaBee® appeared to be superior to both Bee-Pro® and Feed-Bee® in terms of producing more brood or adult population.

Poor pollen nutrition reduces stress resistance, may cause CCD

Pollen nutrition affects Nosema *resistance in caged bees*

In an early study, Rinderer and Kathleen (1977) found that bees provided with pollen lived more than two times longer than those without (group 2 vs. 4, Fig. 3). However, when bees were inoculated with *N. apis*, there was also a significant reduction of lifespan, in both bees with pollen (group 2 vs. 1, Fig. 3), and bees without pollen (group 4 vs. 3, Fig. 3). *Nosema apis* infection therefore exerted its adverse effect regardless of pollen presence. Although the relative reduction was higher when pollen was present (46.8 to 21 days) compared to when pollen was absent (21 to 14 days), when a colony had a *Nosema* infection and no pollen, the workers lived the shortest (14 days) if these results could be extrapolated to a colony setting. In a more recent study (Z.Y. Huang, unpublished data), *N. ceranae* was used instead and the same four treatments were used (Fig. 4). Here when pollen was provided to caged bees, *N. ceranae* caused a significant shift in the survival curves, but when no pollen was provided, the difference between infected bees and non-infected bees was smaller, with *Nosema*-infected bees living slightly longer. In both studies, the absence of pollen alone seemed to have caused a larger reduction in worker lifespan than the *Nosema* infection itself. *N. ceranae* was thought to be a key player for colony collapse in honey bee population in Spain (Higes

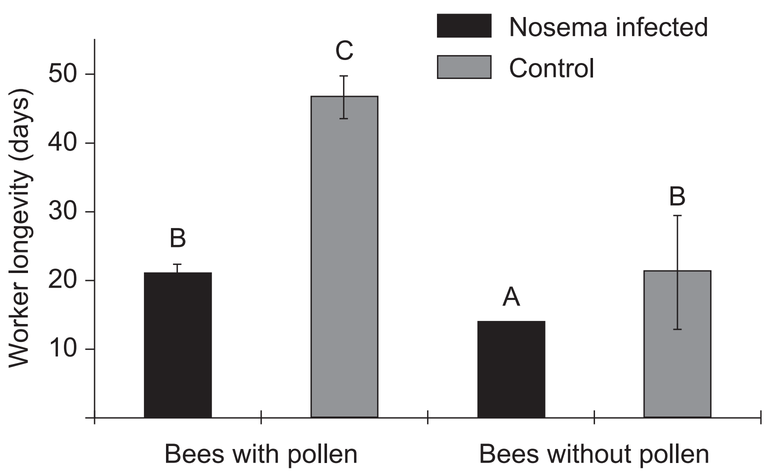

Figure 3. Longevity (days ± SE) of caged worker bees that were fed with pollen, without pollen, and then either infected with *Nosema apis* (black) or not infected as a control (grey) (data from Rinderer and Kathleen, 1977, Table 1, Experiment 1). Bars with different letters were significantly different (P<0.05) by LSD after ANOVA.

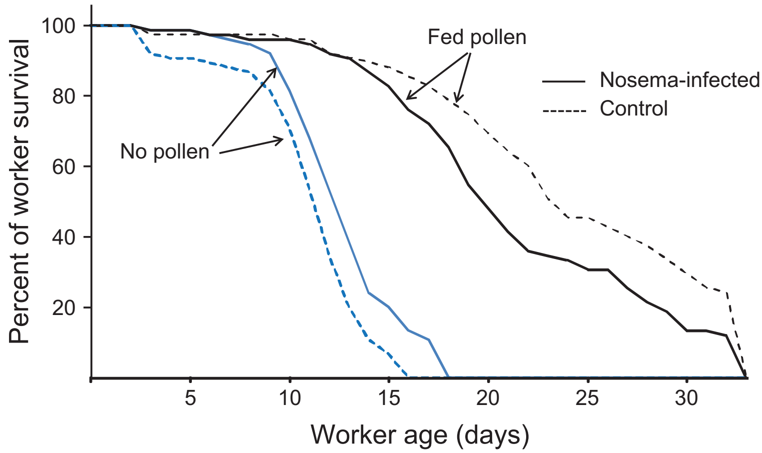

Figure 4. Survival of caged honey bee workers that were fed with pollen (black), or without (blue), and then infected with *Nosema ceranae* (solid lines), or not infected with *Nosema ceranae* (broken lines) (Z.Y. Huang, unpublished data).

et al., 2008), but several studies concluded that *Nosema* alone was not the cause of CCD in the U.S. (vanEngelsdorp et al., 2009), Germany (Genersch et al., 2010) or Canada (Guzmán-Novoa, 2010).

Pollen nutrition affects sensitivity to pesticides in caged bees

Wahl and Ulm (1983) found that the amount and quality of pollen ingested in the first few days of life in workers affected their sensitivity to most of pesticides tested. Bees fed adequate high quality pollen were less sensitive than those fed inadequate pollen, inferior pollen or pollen substitute. Table 1 shows that for all six pesticides, LD 50 (the dose that causes 50% mortality in bees) shifted to a lower number (i.e. becoming more sensitive) when protein was not provided. These results suggest that workers provided with good protein nutrition are more resistant against the toxic effects of pesticides. We do not have data on how protein nutrition affects all the newer pesticides used today (e.g. pyrethroid and neonictinoid pesticides).

Pollen nutrition affects resistance to viruses in caged bees

DeGrandi-Hoffman et al. (2010) fed bees with pollen, a protein supplement (MegaBee®), or a protein-free diet of sugar syrup and then measured the amount of protein in heads, hypopharyngeal gland sizes and virus titers in worker honey bees. Bees fed sugar syrup alone had lower protein amounts in heads and smaller hypopharyngeal glands compared with the other two fed with pollen or the supplement. Deformed wing virus (DWV) titers increased as bees aged and were the highest in those fed sugar syrup and lowest in bees fed pollen. These results suggest that good protein nutrition can increase the resistance of bees to DWV.

Table 1. Effect of protein nutrition on honey bee resistance to pesticides. All six listed pesticides showed a shift to lower LD50, indicating that bees were becoming more sensitive to pesticides when protein was deficient. Data from Wahl and Ulm (1983).

Pesticide	LD 50 (µg/bee)	
	Normal feed	Protein deficient
Tormona 80	709.3	482.3
Hedonal	147.2 - 151.9	113.3 - 117.0
U 46 KV	92.6 - 122.5	64.8 - 85.8
Thiodan 35	61.7	31.45
Cupravit	32.0 - 47.9	18.9 - 28.3
Rubitox	16.85	11.45

Pollen nutrition affects gene expression in caged bees

Alaux et al. (2011) studied the molecular mechanisms underlying the nutritive impact on honey bee health by pollen. They determined whether there was an interaction between pollen and *Varroa destructor* in affecting the transcriptome of worker bees, because *Varroa* is shown previously able to suppress immunity and decreasing lifespan in *A. mellifera* (Yang and Cox-Foster, 2005) and *Apis cerana* (Fabricius) (Zhang et al., 2010). They analyzed digital gene expression (DGE) of bee abdomens in four experimental groups: control bees without pollen, control bees fed with pollen, *Varroa*-parasitized bees without pollen and *Varroa*-parasitized bees fed with pollen. Comparing the transcriptome of bees fed with pollen and bees restricted to a sugar diet, they found that pollen activated nutrient-sensing and metabolic pathways. Pollen also had a positive effect on genes affecting longevity and those coding for antimicrobial peptides. *Varroa* parasitism increased virus titers and caused a decrease in metabolism by inhibiting protein metabolism essential to bee health. Unfortunately, this harmful effect was not reversed by pollen intake.

Polyfloral pollen healthier for caged bees

Many studies have shown that polyfloral pollen is healthier for honey bees than single single species of pollen. Schmidt and colleagues conducted a series of studies and convincingly showed that in general, mixed pollen given to caged bees made bees live longer than those on a single species of pollen (Schmidt, 1984; Schmidt et al., 1987, 1995). In a more recent study, Alaux et al. (2010) showed that polyfloral pollen enhanced immune functions (especially glucose oxidase activity) compared with monofloral diets, suggesting that diversity in floral resources provides bees with better in-hive antiseptic protection. These studies suggest that caged bees feeding on a single type of pollen are not as healthy as those on a variety of pollens. The mechanism behind this remains unclear. Is it a lack of balance in amino acids? Or a lack of other nutrients such as vitamins or minerals in the monofloral pollen? Do these effects remain the same at the colony level when bees are free flying? If so, modern agriculture— growing increasingly larger areas of mono-cultured crops— may have been adversely affecting honey bee health for decades.

Pollen nutrition affects resistance to Nosema *or* Varroa *in colonies*

Some colony studies suggest that good nutrition at the colony level can prepare colonies for better disease or pest resistance. Eischen and Graham (2008) used 28 overwintering honey bee colonies infected with *N. ceranae* (average of 2.4 million spores per bee) that were then either 1) fed pollen supplement, 2) treated with fumagillin and fed pollen supplement or 3) untreated control. They found that the two fed groups ended up with significantly larger adult bee populations than unfed control colonies. In another study, Eischen et al. (2008) determined the interaction of nutrition and *Varroa* infestation and used four treatments. They found that colonies that were fed BeePro + 4% pollen and with a low-mite infestation (0-5 mites/200 bees) had a 77.4% success rate in meeting the size criterion (more than 6 frames of bees) for almond pollination, while unfed/low-mite; fed/high-mite, and unfed/high-mite colonies had 45.2, 48.4, and 19.4% of the colonies meeting the same criterion, respectively. The fed/high-mite and unfed/high-mite colonies were quite different (48.4 vs. 19.4%), suggesting pollen fed colonies were not impacted as much by high *Varroa* populations. This is in contrast to the cage study, which did not show a pollen effect in reversing the damage caused by *Varroa*.

When winter is mild, natural pollen and nectar are available, and *Varroa destructor* is maintained at low levels, supplementing bees with pollen or sugar, or treating bees with fumagillin are not necessary because no differences in colony strength, brood survival or colony mortality were observed among those supplemented with protein or vitamin, or those treated with fumagillin (Pajuelo et al., 2008).

Transportation affects bee health, possibly via pollen nutrition

Despite the need for transportation of bee colonies to provide pollination services and honey production, we understand little of the stresses on colonies from long distance transportation. My laboratory conducted three trials to study the effect of long distance migration on honey bee physiology (K. Ahn, X. Xie, J. Riddle, J. Pettis and Z.Y. Huang, in preparation). Newly emerged bees from one colony were split into two groups and introduced into two colonies, one experiencing migration (M) and another one stationary (S). Each trial consisted of moving the M colonies for 2-3 days over more than 2,000 km. Results showed that the acini sizes of hypopharyngeal glands were the only reliable indicator for transportation stress. They were significantly smaller in migratory colonies than stationary ones in all three trails. Other measured parameters such as juvenile hormone titers, total protein in head or thorax, and lipid content in abdomen were not significantly different. These results suggest that bees during transportation had trouble finding pollen to fully develop their hypopharyngeal glands, which is important to produce the "jelly" that is fed to adult bees as well as to larvae (Crailsheim, 1991; 1992). The smaller glands should have lower protein producing abilities (Huang and Otis, 1989) and may have implications at the colony level because vitellogenin, a component of the jelly, has shown to play a role in aging and immune response (Amdam et al., 2004). What are the mechanisms of transportation affecting pollen consumption? Do the smaller glands translate into poor brood rearing? Does this reduce resistance of larvae to diseases? These are unanswered questions and are currently being investigated at the author's laboratory.

Habitat change affects colony health

Urbanization and development also cause loss of habitat of many plants, which in turn could affect honey bee health. Naug (2009) tested the hypothesis that nutritional stress due to habitat loss may have played a role in the CCD. He showed that the percentage of colony loss by each state was significantly predicted by the ratio of its open land area divided by developed land area. He also found a significant positive correlation between honey yield per colony and the open land area. Finally he provided data showing the declining numbers of total colonies in the U.S. were also significantly correlated with the decline of rangeland for the past 25 years (Fig. 5). This study highlights the importance for preserving natural areas in maintaining healthy honey bees.

In a more recent study, Morimoto et al. (2011) used microarray to understand the gene expression changes in honey bees used for long-term pollination in greenhouses. They discovered that the greenhouse environment changed gene expression profiles and induced immune-suppression and oxidative stress in honey bees. They then used quantitative PCR (polymerase chain reaction) to verify if indeed immune-related and

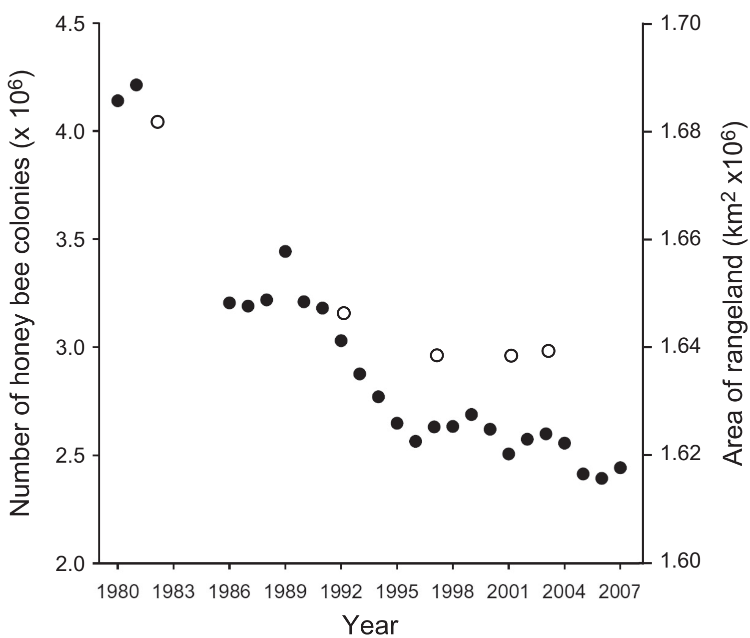

Figure 5. Correlation between honey bee colony numbers (millions, solid circles) and areas of rangeland (million km2, open circles) (modified from Naug, 2010).

oxidative genes were down-regulated in colonies which had been placed in green houses for 42 days. They verified the decreased amount of mRNAs for PGRP-S2, a peptidoglycan recognition protein involved in detecting bacteria, and antimicrobial peptides including abaecin, apidaecin, defencin-1, and hymenoptaecin. Similarly, mRNAs levels were also decreased for enzymes involved in antioxidation such as glutathione S-transferase 1, glutathione S-transferase S1, microsomal glutathione transferase, peroxiredoxin 2540, thioredoxin reductase 1, catalase, and ferritin 1 heavy chain. Coupled with these, they found increased numbers of *Nosema ceranae* spores and protein carbonyl content in honey bees. Increased carbonyl content reflects stronger effects of oxidant damages. This study therefore indicates that honey bee colonies, when stressed inside green houses, show physiological changes prior to colony collapse. It is not clear whether honey bees colonies at different landscapes would show differences in the expressions of these same genes.

Honey bee learning and CCD

Naug (2009) suggested that nutritionally deprived bees left the hive and had trouble returning home if they were weakened by other factors, resulting in the CCD symptoms. It is possible that similar to the earlier studies, bees without adequate pollen nutrition became more sensitive to pesticides (Wahl and Ulm, 1983). The pesticides (either from inside the hive or gathered from the field) could reduce bee's learning ability, which rendered them unable to return home. A recent study found that when worker bees were exposed to sublethal doses of fluvalinate, a commonly used acaracide against *Varroa*, and imidacloprid, a neonictinoid insecticide that bees might be exposed to as foragers, they did not learn some landscape features so that they could not return home as well as the controls (Z.Y. Huang and S. Zhang, unpublished data). This effect was not seen when each pesticide was used alone at twice the dose, suggesting a synergistic effect between the two pesticides. It is possible that such effects are more profound when workers are not receiving adequate pollen nutrition, thus further reducing the percentages of foragers that are able to return home. Whether pollen nutrition can exacerbate the effects of pesticides on honey bee learning and/or the degree of synergism is being investigated in my laboratory. In addition, bees would not learn as well when they were weakened by stresses such as viruses (Iqbal and Mueller, 2007), *N. ceranae* (Kralj and Fuchs, 2010), *Varroa* (Kralj et al., 2007), or pesticides (Weick and Thorn, 2002). Reduced learning ability could affect honey bee homing ability. How these factors (pathogens, parasites and pesticides) interact with one another and affect honey bee physiology and behavior is another topic of research in my laboratory.

Conclusions

Honey bees can obtain all of their nutrients naturally if bees are in a natural setting. Unfortunately, modern agriculture has necessitated large scale mono-cropping which can be harmful to honey bees. This is mainly because each plant species has a specific nectar or pollen characteristic. Much like humans, a lack of variety in foods can cause problems in honey bees, although we do not fully understand why. Many studies have shown poly-floral pollen diets are superior to a single species of pollen, with perhaps one exception (rape seed pollen alone can be excellent). The lack of pollen diversity by itself probably did not cause CCD, but it seems to reduce honey bee resistance to all the major stresses (*Varroa, Nosema ceranae*, and pesticides). We urgently need to understand the impact of each mono-culture crop on honey bee health. For example, how much stress do bees experience when feeding exclusively on almond nectar and pollen for the 3-4 weeks needed for pollination? How long do they need to (or can they?) recover after the stressful period? Are there "supplemental" crops available to reduce or eliminate such a stress? By understanding these questions and providing solutions to them, we will be able to make bees as healthy as possible.

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