The Wisdom of Honeybee Defenses Against Environmental Stresses

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As one of the predominant pollinator, honeybees provide important ecosystem service to crops and wild plants, and generate great economic benefit for humans. Unfortunately, there is clear evidence of recent catastrophic honeybee colony failure in some areas, resulting in markedly negative environmental and economic effects. It has been demonstrated that various environmental stresses, including both abiotic and biotic stresses, functioning singly or synergistically, are the potential drivers of colony collapse. Honeybees can use many defense mechanisms to decrease the damage from environmental stress to some extent. Here, we synthesize and summarize recent advances regarding the effects of environmental stress on honeybees and the wisdom of honeybees to respond to external environmental stress. Furthermore, we provide possible future research directions about the response of honeybees to various form of stressors.

Keywords: honeybee, environmental stress, abiotic stress, biotic stress, defense mechanism

INTRODUCTION

Honeybees (Hymenoptera: Apidae), highly eusocial insects, first emerged ∼120∼130 million years ago, coinciding with the appearance of early angiosperms (Engel, 2001). With continued study, it is discovered that honeybees are essential to the agricultural economy because of their efficient pollination of many agricultural crops worldwide. Estimates suggest that crop yields will decrease by more than 90% without honeybee pollination (Klein et al., 2007). Honeybees are also significant for many wild plant communities (Potts et al., 2010). Some plants even have unique reproductive structures that, through the process of evolution, only allow pollination by honeybees. Moreover, honeybees can make important contributions to human life through their ability to produce honey, propolis, bee venom, bee wax, and royal jelly.

Although the important roles of honeybee in agricultural productivity, wild plant communities and human livelihoods, there is clear evidence of marked regional population decreases in honeybee populations due to colony failure in recent years (Potts et al., 2010; Kulhanek et al., 2017). In particular, from the winter of 2006 to the spring of 2007, adult bees suddenly underwent mass disappearances in outwardly healthy colonies within ∼2–4 weeks in parts of Asia, Europe and the United States, leading to alarming levels of colony failure, termed colony collapse disorder (CCD) (Vanengelsdorp et al., 2009). Such catastrophic colony losses may seriously influence wild plant diversity, terrestrial ecosystem stability, crop production, global food supply, and human welfare. A general consensus now exists that CCD is a product of environmental stress, including multiple

> **BOX 1 | Social behavior.**
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> Honeybees can use many social behaviors to address microbial infection. For example, the honey produced by honeybees contains specific ingredients with antimicrobial activity, including methylglyoxal, sugar, H_2O_2, and defensin1, which can be used by honeybee to defend against pathogens and parasites. Honeybees can also collect certain compounds that possess potential antimicrobial properties, such as resins, plant pollens, and complex plant secretions. These compounds can be further used by honeybee to produce propolis to coat colony interior and thereby decrease pathogen loads in the colony (Cremer et al., 2007; Simone et al., 2009; Wang et al., 2015; Wang K. et al., 2016). Moreover, honeybees can perform allogrooming and hygienic behavior, change worker-worker interactions, switch from in-hive tasks to foraging, and remove ectoparasites and diseased brood to reduce the transmission of pathogens and parasites between individuals in the hive (Rueppell et al., 2010).

forms of biotic and abiotic stress (**Figure 1A**) that function singly or synergistically (Potts et al., 2010; Ratnieks and Carreck, 2010; Vanbergen, 2013; Kerr et al., 2015; Perry et al., 2015). Though high levels of environmental stress will lead to honeybee brought to their knees to it, honeybees can cope with low levels of environmental stress through their "wisdom," such as decreasing the natural mortality of foragers, reducing the forager recruitment level and increasing queen's laying rate (Ratnieks and Carreck, 2010; Evans and Schwarz, 2011; Vanbergen, 2013; Booton et al., 2017). Moreover, we previously found that many genes and signaling pathways can also be employed by *Apis cerana cerana* for defense against environmental stresses (Yu et al., 2011; Meng et al., 2014; Yao et al., 2014; Li et al., 2016a,b; Zhang J. et al., 2016; Zhang Y. Y. et al., 2016; Zhao et al., 2018).

BIOTIC STRESS IN HONEYBEES

Biotic stresses in honeybee mainly include fungi, bacteria, virus and parasites (**Figure 1A**), which can infect different developmental stages of honeybees, resulting in various diseases and pathogenic characteristics (**Table 1**; Schmidhempel, 1998; Randolt et al., 2008; Albert et al., 2011; Evans and Schwarz, 2011; Gatschenberger et al., 2013; Collison et al., 2016). The honeybee genome lacks many typical innate immunity genes that enable other insects to cope with biotic stress (Schmid-Hempel, 2005; Evans et al., 2006; Lemaitre and Hoffmann, 2007; Rolff et al., 2009). However, this does not mean that honeybees are defenseless against pathogens and parasites. Instead, they have evolved a suite of efficient defensive measures to fight the invasion of various pathogens and parasites, including social behavior (**Box 1**), energy metabolism, innate immune response (Glossary), and physical barriers such as gut epithelium and cuticle (**Figure 1B**; Cremer et al., 2007; Randolt et al., 2008). Not only that, honeybees can use many other mechanisms to respond to biotic stress, and the defense mechanisms of honeybee against multiple stress agents exhibit different characteristics.

Fungi

The main fungi that threaten honeybee colonies include *Ascosphaera apis* and *Aspergillus flavus*, which trigger chalkbrood

disease and aspergillosis, respectively (**Table 1**). Infection with these fungi often accompany rain, humidity and unstable temperature (Mehr et al., 1978; Chen, 2013a). Foundation wax contaminated with *A. apis* spores is a likely source of chalkbrood disease in honeybee colonies (Flores et al., 2005). The lethality of *A. apis* is dose dependent and can be exacerbated by environmental humidity and temperature (Aronstein and Murray, 2010).
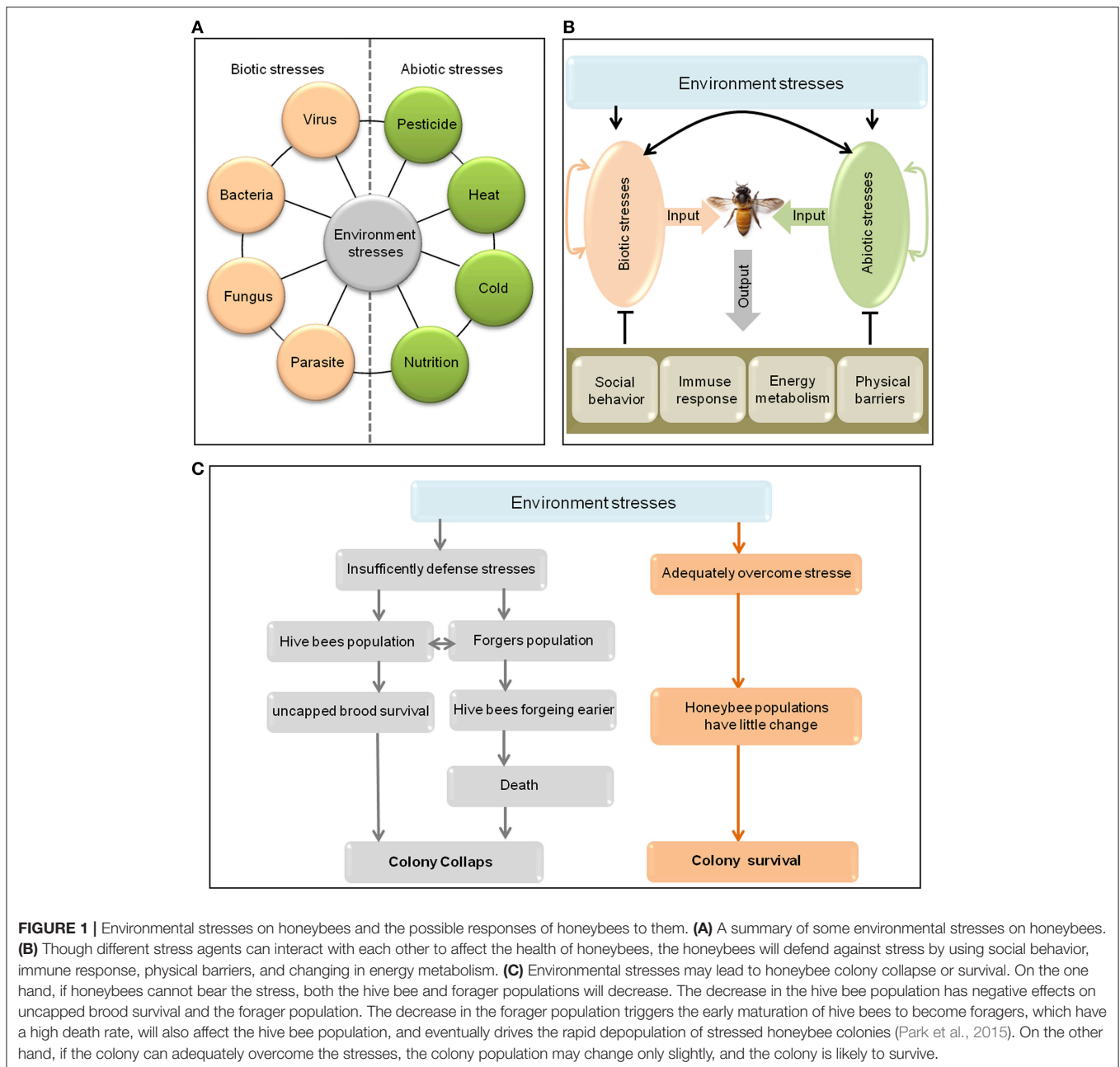
Transcriptome sequencing analysis reveals many pathways involved in cellular immune, humoral immune-related, MAPK signaling, Toll-like receptor signaling, and part of the nuclear factor-κB (NF-κB) signaling are upregulated in the gut of *Apis mellifera ligustica* infected with *A. apis* (Chen et al., 2017), indicating that honeybees can employ these signaling pathways to cope with *A. apis* infection. In addition, 3-acyl dihydroflavonols (a component of poplar resins) and the extracts of many plants, such as *Allium sativum*, *Piper betle*, *Syzygium aromaticum*, *Amomum krervanh*, *Piper sarmentosum*, *Cinnamomum* sp., and *Piper ribesioides* have been proved to exhibit inhibitory effect on *A. apis* by utilizing antimicrobial activity assays (Chaimanee et al., 2017; Wilson et al., 2017). These substances can be used by beekeepers to prevent chalkbrood disease in the future. It is worth mentioning that the larval mortality caused by *A. apis* is increased by cooling the larvae for 24 h after inoculation (Vojvodic et al., 2011), suggesting that cold may facilitate *A. apis* infection. Of course, further research is needed to confirm the interaction between *A. apis* infection and cold stress.

Regarding *A. flavus*, an omega-class glutathione S-transferase has been found to participate in the response to *A. flavus* infection in *Apis cerana cerana* (Zhang Y. Y. et al., 2016). The acetone, hexane and petroleum ether extracts from thyme and santonica plants at 1,000 parts per million completely repress the growth of *A. flavus* (Ali, 2007), and thus may be used by beekeepers to control *A. flavus* infection in honeybee colonies.

Bacteria

The main bacterial pathogens that infect honeybees are *Paenibacillus larvae*, *Melissococcus pluton*, *Spiroplasma melliferum*, *Pseudomonas,* and *Salmonella paratyphi A*, which can cause American foulbrood, European foulbrood, honeybee spiroplasmosis, septicemia, and paratyphoid disease, respectively (**Table 1**; Morse and Nowogrodzki, 1978; Mouches et al., 1983; Clark et al., 1985; Evans and Schwarz, 2011). These diseases have long infection times and fast propagation speed, and cause serious degree of harm that severely influences the growth and development of honeybees (Evans and Schwarz, 2011).

For the first 48 h following eclosion, honeybees are susceptible to infection by *P. larvae*. However, honeybees become progressively more resistant to this bacterial pathogen with age (Brødsgaard et al., 1998). That may be caused by the age-dependent development of the gut epithelium, which is a barrier for *P. larvae*, and too small synthesis of antimicrobial compounds to produce adequate amounts of antimicrobial peptides in first instar larvae (Yue et al., 2008; Gatschenberger et al., 2013). Based on mass spectrometry-based proteomics analysis, the levels of chaperones, immunity proteins, and certain metabolic proteins are found to be upregulated in the hemolymph of 5-day-old

FIGURE 1 | Environmental stresses on honeybees and the possible responses of honeybees to them. **(A)** A summary of some environmental stresses on honeybees. **(B)** Though different stress agents can interact with each other to affect the health of honeybees, the honeybees will defend against stress by using social behavior, immune response, physical barriers, and changing in energy metabolism. **(C)** Environmental stresses may lead to honeybee colony collapse or survival. On the one hand, if honeybees cannot bear the stress, both the hive bee and forager populations will decrease. The decrease in the hive bee population has negative effects on uncapped brood survival and the forager population. The decrease in the forager population triggers the early maturation of hive bees to become foragers, which have a high death rate, will also affect the hive bee population, and eventually drives the rapid depopulation of stressed honeybee colonies (Park et al., 2015). On the other hand, if the colony can adequately overcome the stresses, the colony population may change only slightly, and the colony is likely to survive.

healthy larvae compared with their levels in infected honeybee larvae. These findings reveal that honeybee larvae can not only fight *P. larvae* infection directly, by using immune factors, but also indirectly, by employing energy metabolism pathways to sustain the effort (**Figure 1B;** Chan and Foster, 2008; Chan et al., 2009). Interestingly, a result from immunohistochemical localization technique reveals that heat shock protein 70 (Hsp70) localizes in the cytoplasm and nuclei of hemocytes, midgut cells, and salivary gland cells in honeybee larvae infected with *P. larva* but not in uninfected honeybee larvae (Gregorc and Bowen, 1999), which suggests that Hsp70 may be used as a possible diagnostic criterion for *P. larvae* infection. Many plant extracts,

for example *Chromolaena odorata* and longer acyl groups can be employed to defend against *P. larvae* infection (Chaimanee et al., 2017; Wilson et al., 2017).

Aside from *P. larvae*, honeybees are also susceptible to certain gram-negative bacteria, such as *Escherichia coli* (*E. coli*). Gene ontology analysis shows that canonical immune response pathways, particularly the Notch signaling pathway and the Toll signaling pathway are specifically altered in response to *E. coli* infection in *Apis mellifera* (Richard et al., 2012). These signaling pathways may play important roles in defending against *E. coli* infection in honeybees. Interestingly, the immunocompetence competence of honeybees to *E. coli*

TABLE 1 | A summary of some parasites and pathogens of the honeybees.

Classification	Pathogenic characteristics	Species	Cause of disease	Susceptible developmental stages	References
Virus	Existence with no obvious symptoms until activated under certain environmental conditions, resulting in strong pathogenicity	Black queen cell virus	Black queen cell disease	Larvae and pupae	Ellis and Munn, 2005; Zhang et al., 2012
		Deformed wing virus	Deformed wing disease	Various life stages	Bailey and Ball, 1991
		Israeli acute paralysis virus	Israeli acute paralysis disease	Various life stages	de Miranda et al., 2010
		Sac brood virus	Sacbrood bee disease	Various life stages	Bailey and Fernando, 2010; Park et al., 2016
		Chronic bee paralysis virus	Chronic bee paralysis disease	Adults	Bailey and Woods, 1974
		Kashmir bee virus	Kashmir bee disease	Various life stages	Ellis and Munn, 2005
		Acute bee paralysis virus	Acute bee paralysis disease	Various life stages	Bailey, 1981
		Tobacco ringspot virus	Unclear	At least adult worker bees	Li et al., 2014
Bacteria	Long infection time, fast propagation speed and serious harm	*Spiroplasma apis*	Spiroplasmosis	Adults	Mouches et al., 1983
		Spiroplasma melliferum	Spiroplasmosis	Adults	Clark et al., 1985
		Paenibacillus larvae	American foulbrood disease	Larvae	Morse and Nowogrodzki, 1978
		Melissococcus plutonius	European foulbrood disease	Larvae	Morse and Nowogrodzki, 1978
Fungi	Frequent association with rain, humidity and unstable temperature	*Ascosphaera apis*	Chalkbrood disease	Larvae	Mehr et al., 1978
		Aspergillus flavus	Aspergillus flavus disease	Various life stages	Chen, 2013a
Parasite	Ingestion of nutrients in honeybees	*Nosema ceranae*	Microsporidiosis	Adults	Sak et al., 2004
		Nosema apis	Microsporidiosis	Adults	Sak et al., 2004
		Tropilaelaps	Acariasis of bees	Larvae	Chen, 2013b
		Varroa destructor	Acariasis of bees	Larvae, pupae and adults	Martin, 2001

infection varies between different development stages, different seasons, and different castes honeybees. Honeybee larvae and adult can use immune responses to defend against *E. coli* infection. However, pupae have no immune system, thus they cannot cope with the infection of *E. coli* through immune response (**Figure 2**). Moreover, summer adult honeybees upregulate seven types of immune proteins, namely, defensin1, abaecin, hymenoptaecin, phenoloxidase (PO), carboxylesterases (CEs), peptidoglycan recognition proteins (PGRPs), and immune responsive protein 30 (IRP30), after infection with *E. coli*, while only hymenoptaecin, defensin1 and IRP30 are induced in winter honeybees. It is worth mentioning that winter honeybees have no nodulation reactions as observed in summer honeybees, but they possess an enlarged fat body and many haemocytes, which allow them to kill viable *E. coli* faster and more reliably than summer honeybees. In regard to different castes of honeybees, after infection with *E. coli*, hymenoptaecin, defensin 1, abaecin, PGRPs and lysozyme 2 are detected in drones, while only hymenoptaecin, CEs and IRP30 are found in queens (Randolt

et al., 2008; Albert et al., 2011; Gatschenberger et al., 2012, 2013). Workers infected with *E. coli* tend to increase allogrooming behavior, alter their social interactions, and become more aggressive. However, the defense mechanism of honeybee eggs to *E. coli* remains unclear, and should be the focus of future study.

Viruses

Viruses are widely involved in colony diseases, and are both historically and recently known to be harmful to the physiology, behavior, morphology and learning ability of honeybee (Bailey and Ball, 1991; Chen et al., 2005; Maori et al., 2007; Runckel et al., 2011). More than 20 viruses are reported to infect honeybees (Bailey and Woods, 1974; Bailey, 1981; Ellis and Munn, 2005; Bailey and Fernando, 2010; de Miranda et al., 2010; Zhang et al., 2012; Li et al., 2014). Among these viruses, Deformed wing virus (DWV) infection causes deformed wings, small body size and discoloration in adult honeybees (Bailey and Ball, 1991). Though the viral load of DWV increases slowly before the adult stage of honeybee, and this virus seldom kills
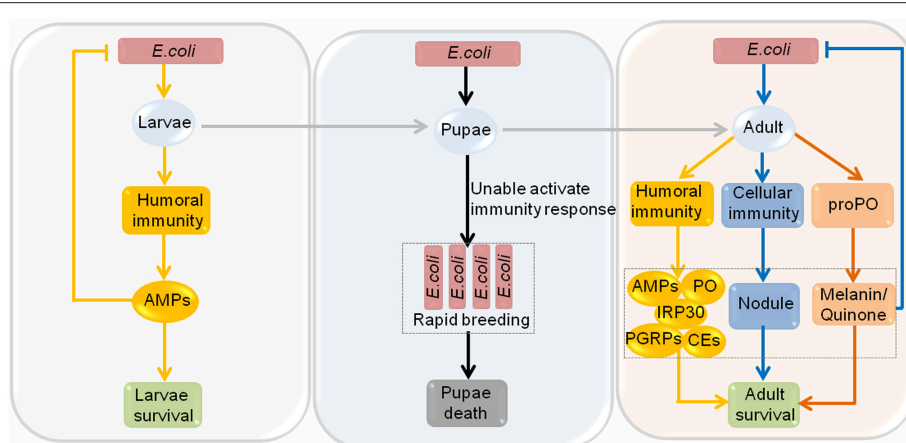
FIGURE 2 | The immunocompetence of honeybees at different developmental stages to *Escherichia coli* infection. The immunocompetence of honeybees to *Escherichia coli* (*E. coli*) infection varies among different development stages. Honeybees go through the larval stage and then the pupal stage before becoming adult. High-dose injection of *E. coli* into honeybee larvae causes a humoral immunity response and then induces the synthesis of antimicrobial peptides (AMPs), including defensin1, abaecin and hymenoptaecin, which can repress the multiplication of *E. coli*, and the larvae can survive. Pupae are completely incapable of activating cellular and humoral immune reactions upon *E. coli* infection, resulting in the rapid proliferation of *E. coli*, ultimately triggering the death of pupae. Moreover, both the humoral and cellular immune responses play important roles when adult honeybees are infected with *E. coli*. Prophenoloxidase is activated, resulting in synthesis of melanin and quinones. In the humoral immune response, AMPs, phenoloxidase (PO), carboxylesterases (CEs), peptidoglycan recognition proteins (PGRPs) and immune responsive protein 30 (IRP30) are detected, and in the cellular immune response, nodules are formed. All of these immune reactions can repress the amplification of *E. coli*, resulting in the survival of adults.

pupae, it decreases the total lifespan of honeybee by acting independently or synergistically with *Varroa destructor* (Dainat et al., 2012). If the copy number of DWV reaches a specific threshold, it will restrain the immune system of honeybee, then accelerates greater replication of DWV (Highfield et al., 2009; Dainat et al., 2012; Wu et al., 2017). Feeding larvae in advance with DWV double-stranded RNA (DWV-dsRNA) reduces wing deformity in adults, and feeding adults with DWV-dsRNA reduces the DWV concentration and increases adult longevity (Dainat et al., 2012). The oral administration of 0.5% β-glucan (Glossary) can enhance the tolerance of *Apis mellifera ligustica* to DWV, possibly by increasing phenoloxidase activity and the number of prohemocytes (Mazzei et al., 2016).

Chinese sacbrood virus (CSBV) causes sacbrood disease in *Apis cerana* with low prevalence and pathogenicity (Gong et al., 2016). In-situ hybridization can be used to detect, diagnose, and locate CSBV (Park et al., 2016). Gel-based and liquid chromatography-mass spectrometry-based proteomic strategies have shown that networked groups connected with the cytoskeleton, development, protein metabolism, protein folding, and energy metabolism are clearly changed in honeybee larvae suffering from sacbrood disease. In addition, the antioxidant defenses of honeybee are overwhelmed by CSBV infection. All these changes severely influence the normal development of larvae, and prevent the metamorphosis from larvae to pupae (Aronstein and Murray, 2010; Han et al., 2013). Recent studies demonstrate that RNA interference (RNAi) is a valuable antiviral strategy for sacbrood disease control. For example, a marked reduction in larval mortality is found after bee ingesting of dsRNA-VP1 that against VP1 gene in CSBV (Liu et al., 2010;

Zhang J. et al., 2016). Therefore, if possible, RNAi can be considered for use in defense against CSBV in beekeeping.

Israeli acute paralysis virus (IAPV) is thought to be closely related to CCD in the US (Cox-Foster et al., 2007). Hou et al. support this view by showing that when infected with high loads of IAPV, the colonies in an apiary present typical CCD characteristics (Hou et al., 2014). *V. destructor* can act as a vector of IAPV, and the IAPV copy number is positively related to the density of *Varroa* mites (Di Prisco et al., 2011). By using mass spectrometry-based quantitative proteomics analysis and gene expression analysis, it is revealed that various pathways related to mitosis, cell division, energy production and the protein biosynthetic machinery and many fundamental cellular processes connected with ribosomal biogenesis and other cellular functions are affected or disturbed by IAPV infection in *Apis mellifera* (Boncristiani et al., 2013; Michaud et al., 2014). Changes in these pathways and cellular processes may severely impact the lives of honeybees, even causing their death. Honeybees fed with dsRNA-IAPV can successfully silence IAPV, resulting in greatly decreased honeybee mortality triggered by IAPV infection (Maori et al., 2009). Therefore, using dsRNA-IAPV in apiculture may be an effective strategy to protect honeybee hives from IAPV and even from CCD.

Recent studies show that a dose of 10^4 virus particles of Acute bee paralysis virus (ABPV) per individual causes typical paralysis and trembling signs, and is followed by death within 48 h in adult bees. The ABPV dose of 10^3 virus particles per individual retards growth, and triggers a change from yellowish-white color to brownish-black followed by the sudden collapse of the infected bee larvae (Randolt et al., 2008; Fedorova et al., 2011). When present on its own, ABPV has a low impact on

the survival of whole honeybee colony, while accompanied by infection with *V. destructor*, ABPV is related to high colony mortality (Genersch et al., 2010). Though the innate immune response plays indispensable roles in counteracting bacterial and some viral infections (Trenczek, 1998; Lemaitre and Hoffmann, 2007), a previous study demonstrates that infection with ABPV does not cause cellular or humoral immune responses in young adult worker bees or honeybee larvae. Instead, proteins involved in translation, antioxidant protection, stress response and energy metabolism are upregulated when honeybees are infected with ABPV (Azzami et al., 2012). These proteins may play important roles for honeybees to withstand ABPV infection. A recent study shows that aRNases D3-12 and Dp12F6 exhibit high cleavage activity of ABPV RNA accompanied by low toxicity and without changing the morphology of ABPV particles, resulting in ABPV inactivation and increased survival rate of adult and larval bees that infected with ABPV (Fedorova et al., 2011). This result, together with the above-mentioned considerable difference in the influence of ABPV on adult and larval bees, as well as the lack of acquired immunity, make bees a possible new experimental model for the identification of antiviral agents (Fedorova et al., 2011). If aRNases D3-12 and Dp12F6 can be used to produce novel vaccines that are extensively used in apiculture in the future, the damage caused by ABPV will certainly be significantly decreased.

Parasites

Some parasites are the carriers of viruses that infect honeybees, and parasite infection leads to physical decline in honeybees (Martin et al., 2012; Wu et al., 2017). *Nosema ceranae*, *Malpighamoeba mellificae* and mites are the most common honeybee parasites, and they can trigger microsporidiosis, loeschiasis and acarine disease, respectively (Martin, 2001; Sak et al., 2004; Chen, 2013b). Among these parasites, *N. ceranae* was first identified in the Asian honeybee (*Apis cerana*) and then transferred to the Western honeybee (*Apis mellifera*) as a host. It can synergistically interact with DWV in a nutrition- and dose-dependent manner (Zheng et al., 2015), suggesting a connection among DWV, *N. ceranae,* and nutrition. The optimal temperature for *N. ceranae* infection is 25°C, and the preferred temperature for its multiplication is 35°C (Woyciechowski and Czekonska, 2014), which may explain the phenomenon that honeybees infected with *N. ceranae* are inclined to congregate in the warmer part of the hive (Moeller, 1956).

Recent researches demonstrated that *N. ceranae* degenerates honeybee gut epithelial cells, increases sugar metabolism, impairs tissue integrity and learning ability, reduces honeybee lifespan and foragers homing ability, modulates hormonal stress and innate immunity pathways (mainly the octopamine pathway), and induces hormonal stress, oxidative stress, and energetic stress by using multiple experimental methods, such as real-time quantitative PCR and mass spectrometry and 2-dimensional differential in-gel electrophoresis (Antunez et al., 2009; Mayack and Naug, 2009; Dussaubat et al., 2012; Wolf et al., 2014; Mayack et al., 2015; Piiroinen and Goulson, 2016). Furthermore, a previous study present that no energetic stress is caused by *N. ceranae* infection in *N. ceranae*-tolerant honeybees (Kurze

et al., 2016). Therefore, breeding *N. ceranae*-tolerant honeybees may be conducive to decreasing the damage caused by *N. ceranae* infection. Interestingly, with increasing levels of infection with *N. ceranae*, honeybees often choose sunflower honey over honeydew honey or black locust honey. This selection may occur because sunflower honey has higher antimicrobial activity due to its higher H_2O_2 concentration (Oelschlaegel et al., 2012; Gherman et al., 2014). Moving honeybee colonies to sunflower-rich sites may be a crucial tactic for healing and improving resistance to nosemosis.

Mites, such as *Tropilaelaps* mites and *V. destructor*, are also familiar parasites in honeybees. Mites cause physiological and physical damage to honeybees by feeding on the its hemolymph, resulting in suppression of honeybee immune function, triggering premature death of pupae, impairing cognitive ability and reducing the nutrient levels (Degrandi-Hoffman and Chen, 2015). *Varroa* mites can transmit bacteria through their connection with worker honeybees in the hive, and enable bacteria to enter the hemolymph when they absorb hemolymph from pupae, which may contribute to bacterial infections, and accelerat their damage to honeybee pupae (Kanbar and Engels, 2003; Gatschenberger et al., 2013). Some subspecies of honeybee are resistant to mites, such as *Apis mellifera* from far-eastern Russia and *Apis mellifera syriaca*. By using next-generation sequencing, proteome analysis and near-infrared cameras, it is found that several factors including inheritance, natural selection, social immunity, higher proportions of drone broods, neuronal and olfactory sensitivity and gene expression regulation are believed to be conducive to their tolerance (Rinderer et al., 2001; Haddad et al., 2016; Hu et al., 2016). Besides, recent research shows that certain doses of α-terpineol can repel female mites from entering into brood cells in hives (Dong et al., 2016), which indicates that α-terpineol may be a potential substance for future use in resisting mites in apiculture.

In addition to *N. ceranae* and mites, the small hive beetle is also a parasitic pest of honeybees. The small hive beetle can weaken and collapse a honeybee colony in a matter of 2 weeks. Gas chromatographic-electroantennographic detection and gas chromatography-mass spectrometric analysis indicate that eight volatiles that are released by worker honeybees, namely, decanal, octanal, nonanal, 2-heptanone, 2-nonanone, hexyl acetate, isopentyl acetate, and isopentyl acetate, are very attractive to the small hive beetle, especially the female small hive beetle. Honeybees release these volatiles more readily under various abiotic or biotic stimuli than in their absence (Wenning, 2001; Torto et al., 2005; Rolff et al., 2009). Thus, decreasing certain type of biotic and abiotic stresses in honeybees may reduce the damage caused by the small hive beetle.

ABIOTIC STRESS IN HONEYBEES

In addition to biotic stresses, many abiotic stresses also contribute to the colony collapse in honeybee. Abiotic stress can be triggered by multiple factors, such as temperature, pesticides and nutrition. These stress factors rarely kill honeybees directly. However,

the effect of one of these stresses may make honeybees more susceptible to other environmental stresses. Honeybees exhibit different defense responses to various abiotic stresses.

Temperature

Unsuitable temperature conditions may result in stress in honeybees. The relative humidity of brood comb is maintained at ~70%, and the temperature of brood comb is in the range of 33–36°C. Humidity in the range of 30–75% has no obvious influence on honeybee survival, whereas temperature change has a stronger effect on honeybee survival, and over a long period of time, honeybee will lose the ability to tolerate high or low temperatures (Heinrich, 1979; Southwick and Heldmaier, 1987; Petz et al., 2004). For example, a previous study reports that a noticeable reduction in honeybee survival is observed at higher temperatures (Abou-Shaara et al., 2012). Extreme temperature can trigger temperature stress for foragers, and foragers do not collect nectar and pollen at temperatures that are too hot or too cold (Park et al., 2015). Honeybees regulate their head temperature at a high ambient temperature, and the thoracic temperature is secondarily stabilized. Nevertheless, the thoracic temperature is regulated at low ambient temperatures (Heinrich, 1979). Many other thermoregulatory behaviors can also be performed by honeybees to defend against temperature stress (**Figure 3**).

When suffering from high temperatures in the honeycomb, honeybees will take many measures to cope with it (**Figure 3**). First, some honeybees move across the combs, fan their wings to generate air currents, and produce convective cooling to the honeycomb. Second, honeybees will collect more water, and use their proboscises to spread water in a film through tongue lashing, which enables evaporative cooling in combination with the fanning wings of other honeybees. Third, many honeybees will evacuate the hive, possibly to make more room for the honeybees that are responsible for evaporative cooling. Last but not least, reallocation of labor will occur when a honeybee colony is suffering from high temperatures. The additional labor needed to address heat stress may be obtained by the switching of honeybees from any other task and by the activation of the reserve labor of honeybees in the middle-aged caste (Southwick and Heldmaier, 1987; Johnson, 2002; Lindauer and Watkin, 2015). In addition to behavioral changes, Hsps, such as Hsp24.2, Hsp23.0, Hsp27.6, Hsp70, grp78, Hsp 80, and Hsp90 are reported to take part in the high temperature stress response of honeybees by increasing their mRNA or protein levels (Johnson, 2002; Elekonich, 2009; Liu et al., 2012, 2014; Koo et al., 2015). Furthermore, under treatment at 43°C, the transcription of *AccHsp22.6* continues to increase from 1 to 5 h in *Apis cerana cerana*. Knockdown of *AccHsp22.6* significantly reduces survival of *Apis cerana cerana* under heat stress (Zhang et al., 2014). Overexpression of *AccHsp22.6* may increase the tolerance of *Apis cerana cerana* to heat, thus enhance their survival under high temperature.

In situations of cold stress, honeybees usually perform rapid contraction to vibrate their thoracic muscles with the wing immobile, and congregate to produce body heat (**Figure 3**; Heinrich, 1980). Interestingly, a type of honeybee called hot

bees has been found by using a modified brood nest and an infrared-sensitive thermal imaging camera in the honeybee hive. Hot bees do not carry out any other work. Their abdomens exhibit rapid and continuous respiratory movements, and their thorax temperatures can range from 34.1 to 42.5°C. This high thoracic temperature is derived from previous warm-up and heating activity on the surface of the comb. Hot bees can firmly press their thoraxes onto the capped surface of sealed brood cells or remain inside empty cells. Within 30 min, they can enhance the temperature of the brood cap by 3°C through heating the brood cap surface, and increase the temperature of adjacent brood cells by 2.5°C by sitting inside empty cells, leaving a "hot spot" in the place that has been warmed (Bujok et al., 2002; Kleinhenz et al., 2003; Dantas, 2016). Glutaredoxin 1, glutaredoxin 2, thioredoxin 1, TGF-β-activated kinase-1, mitochondrial thioredoxin peroxidase gene 3, and thioredoxin gene 2 have been demonstrated to be associated with cold stress regulation in *Apis cerana cerana* (Meng et al., 2011; Gong et al., 2012; Yao et al., 2013, 2014).
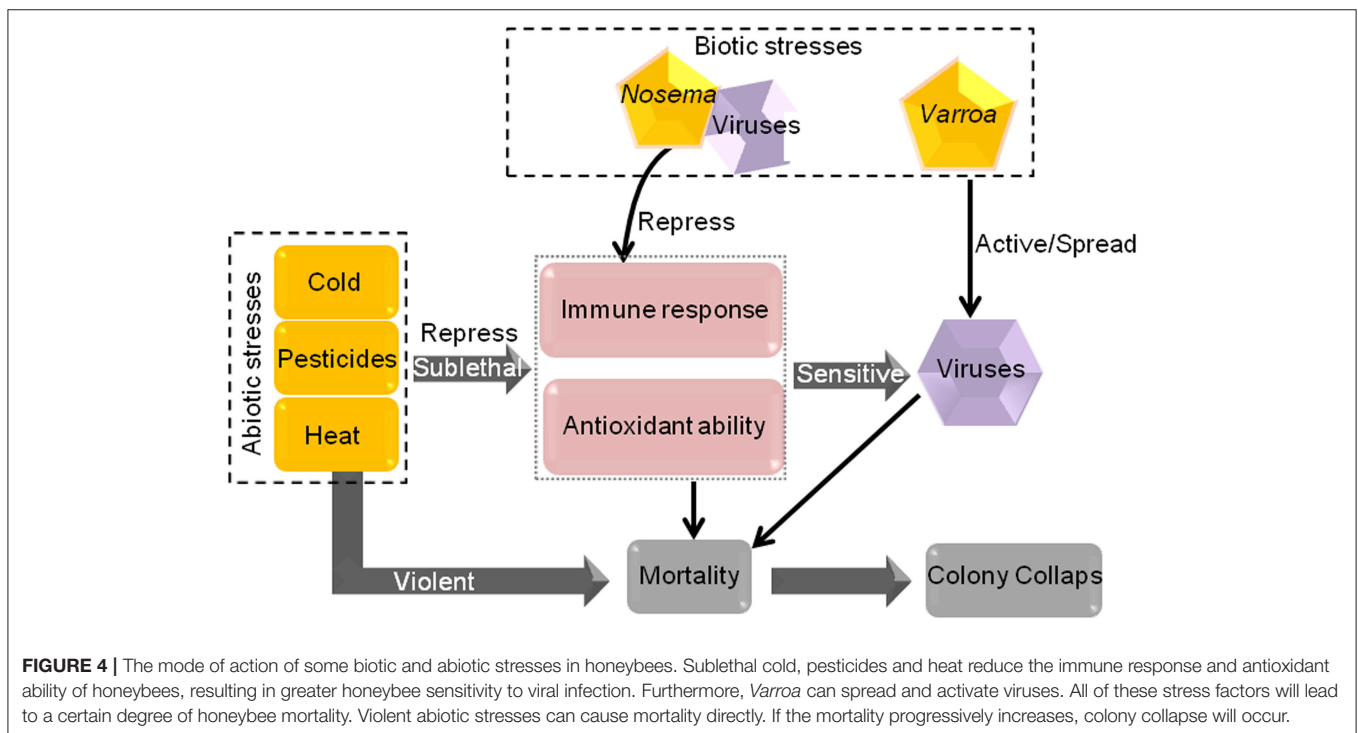
Pesticides

Many pesticides are used in agriculture to reduce crop damage caused by pests and weeds. However, pesticides often trigger considerable damage to honeybees, including influence honeybee behavior, antioxidant ability and immunocompetence, and are linked to honeybee disease through interaction with pathogenic stressors by increasing the sensitivity of honeybees to viral infection (**Figure 4**; Qiao et al., 2005; Chakrabarti et al., 2015; Kakumanu et al., 2016; Sanchez-Bayo et al., 2016). Though honeybees have been found to lack certain detoxification enzymes used by other insects in response to pesticides (Claudianos et al., 2006), many genes and mechanisms are employed by honeybees to defend against pesticide stresses. Notably, different pesticides may cause diverse degrees of damage to honeybee, and honeybees' defense measures in response to them are not identical.

Recent studies, using radiofrequency identification (RFID) and realistic experiment, show that exposure to a sublethal dose of neonicotinoid pesticide impairs olfactory, learning acquisition, and social immune system, influences the mortality of honeybee workers and egg laying by queens, reduces life span, foraging activity and hygienic behavior, increases forging flights, and causes precocious foraging in honeybees (Schneider et al., 2012; Tsvetkov et al., 2017). Neonicotinoids poison honeybees by the upregulation of a leucine-rich repeat protein (Amel\LRR), then negatively regulating an NF-κB immune signaling pathway and actively promoting the replication of DWV in *Apis mellifera* (Di et al., 2013), and by interacting with odorant-binding protein and chemosensory protein 1 to influence honeybee olfactory function (Li et al., 2015, 2017). Tau-fluvalinate can be metabolized by *CYP9Q1*, *CYP9Q2,* and *CYP9Q3*, and the metabolite can then undergo further cleavage by carboxylesterases (Mao et al., 2011). Furthermore, analysis of genome-wide expression patterns in honeybee have identified a total of 1,118 differentially expressed transcripts related to immunity, nutrition, detoxification and behavioral maturation as responding to fluvalinate and coumaphos
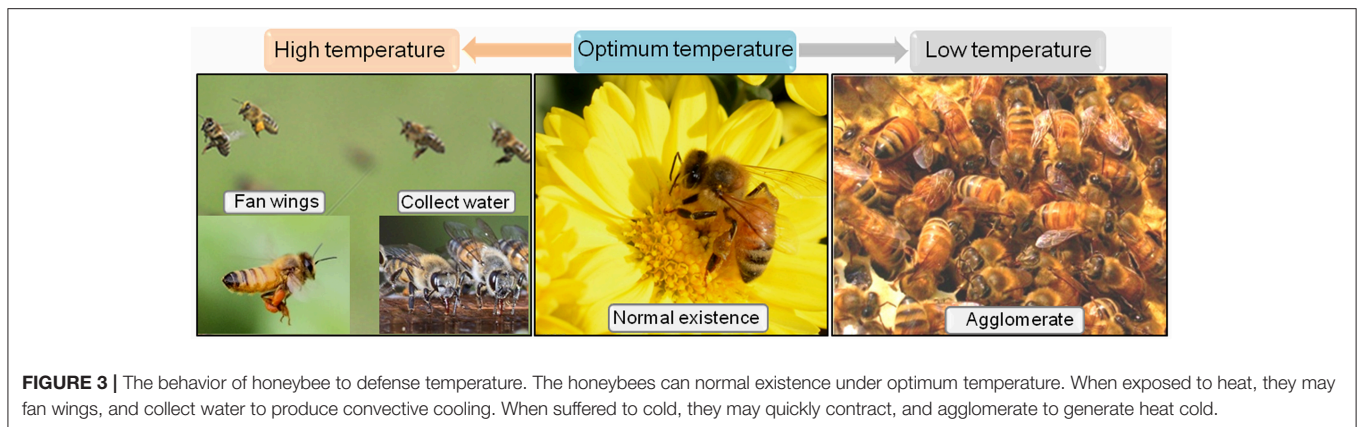
FIGURE 3 | The behavior of honeybee to defense temperature. The honeybees can normal existence under optimum temperature. When exposed to heat, they may fan wings, and collect water to produce convective cooling. When suffered to cold, they may quickly contract, and agglomerate to generate heat cold.

FIGURE 4 | The mode of action of some biotic and abiotic stresses in honeybees. Sublethal cold, pesticides and heat reduce the immune response and antioxidant ability of honeybees, resulting in greater honeybee sensitivity to viral infection. Furthermore, *Varroa* can spread and activate viruses. All of these stress factors will lead to a certain degree of honeybee mortality. Violent abiotic stresses can cause mortality directly. If the mortality progressively increases, colony collapse will occur.

treatment (Schmehl et al., 2014). Many other genes, for example *GSTS1*, *GSTT1*, *GSTO2,* Hsp70, grp78, and Hsp90 are also reported that participate in different pesticide stress reaponse (Yan et al., 2013; Zhang et al., 2013; Koo et al., 2015; Liu et al., 2016). In addition to genes that regulate pesticide stress, abscisic acid (ABA) (Glossary) has been found to enhance the tolerance of honeybees to pesticides. Honeybees can tolerate high concentrations of ABA (1 mM) (Negri et al., 2015). ABA supplementation in syrup solution results in improving wound healing and hemocyte response in non-selfrecognition, increasing plasmatocyte, granulocyte activation, and winter honeybee populations. ABA may therefore be used in apiculture to decrease colony losses to some extent.

In addition to the pesticides used in agriculture, some medicines used to kill pathogens in honeybee colonies can also trigger a stress response in honeybees. For example, formic acid that used by beekeepers to kill *Varroa* mites has a negative effect on brood survival, and reduces honeybee longevity by altering the expression of detoxification and development related genes, immune system components and the c-Jun amino-terminal kinase (JNK) pathway (Fries, 1991; Underwood and Currie, 2003; Di et al., 2013). A recent study also shows that eight immunity-related genes contribute to the regulation of acaricide tratment in honeybees (Boncristiani et al., 2012), indicating that these immune response genes may play essential roles in decreasing the damage from acaricide.

Nutrition

Apart from pesticide and temperature stress, nutrition stress is also an abiotic stress for honeybees. It influences the

immunocompetence and the survival of honeybees upon exposure to other stressors, and nutritional stress triggered by the habitat may be among the reasons for the recent colony collapse of honeybees (Naug, 2009; Archer et al., 2013). Honeybees fed polyfloral diets and protein-rich diets possess higher fat body contents, hemocyte concentrations, GOX oxidase activity and phenoloxidase activity, and dietary protein deficiency increases the susceptibility of organisms to multiple diseases (Alaux et al., 2010). Moreover, providing honeybees with high-quality nutrition (pollen-based diet) reduces the sensitivity of honeybees to one-third of pesticides (Schmehl et al., 2014). Therefore, balanced nutrition is vitally important to maintaining healthy and well-fed colonies, especially in a difficult environment (Brodschneider and Crailsheim, 2010). Particularly worth mentioning is that though adult honeybees have fewer ovarioles and smaller bodies when they are starved at the fifth instar larval stage, the survival of adult bees is increased under starvation condition, because they can better maintain blood sugar levels, decrease energy use and suppress metabolic rate (Wang et al., 2014; Wang Y. et al., 2016). The specific molecular mechanism that involved in honeybees addressing nutrition stress is still not fully explored, and further study should focus on this topic.

Other Abiotic Stresses

Dietary salt is important for honeybees. Ingestion too much or too little salt may cause stress to honeybees in some degree. Honeybees are often found collecting brackish water, dirty water and seawater, possibly because these water sources contain low salt concentration. Foragers are reluctant to collect onion nectar, which contains ~1.3% potassium. The 0.4–0.75% Na_2HPO4, 0–1.5% KCl, and 1.5% NaCl are optimal salt concentration for *Apis mellifera* according to the tests from proboscis extension reflex response (Waller et al., 1972; Lau and Nieh, 2016). Artificially supplying honeybees with salt water at optimal concentrations may contribute to the health of honeybee. Nevertheless, very little is known about the specific genes and signaling pathways that participate in salt stress regulation. RNA sequencing and proteome analysis can be used to reveal the specific molecular mechanisms of the impact of salt stress in honeybees in the near future.

Pesticides, cold, heat, and other abiotic stresses, such as heavy metal and ultraviolet radiation stress, all of them can result in the generation of reactive oxygen species in an organism. Low concentration of reactive oxygen species are necessary for organism. However, high concentration of reactive oxygen species will lead to oxidative damage (Finkel and Holbrook, 2000; Boileau et al., 2003; Mates et al., 2010; Ray et al., 2012). Our previous studies demonstrate that at least 80 genes may participate in the oxidative stress response in *Apis cerana cerana*. However, that is not sufficient to reveal the detailed regulatory mechanisms by which honeybees address oxidative damage caused by different environmental stress factors. Chromatin immunoprecipitation (ChIP) sequencing, ChIP-chip, RNA sequencing and proteome sequencing can be used in the future for the rapid identification of many specific genes, transcription factors, proteins regulated by the transcription factor, and signaling pathways that participate in defense oxidative damage in honeybees.

CONCLUSION AND FUTURE DIRECTIONS

Honeybees are likely to encounter various stress-related factors during their lives (**Figure 1A**). When these stress factors are present in an individual or in the whole colony, honeybees can address the problem through self-mediation by using social behavior and immune response, changing in energy metabolism, physical barriers and many genes and signaling pathway (**Figure 1B**). If honeybees cannot overcome the stressors, they may succumb to it and even die (**Figure 1C**; Potts et al., 2010; Goulson et al., 2015; Perry et al., 2015). Moreover, multiple stresses, either alone or in combination, can compromise the health of honeybees and increase colony mortality. Some abiotic stress factors repress the immune response and antioxidative ability of honeybees, render the honeybees more susceptible to parasites and viruses (**Figure 4;** Anja and Luc, 2008; Gill et al., 2012).

Although the responses of honeybee to environmental stress have been studied for a long time, many outstanding questions remain. The following research directions may be helpful in the future. First, further studies are needed to explore the process of rapid colony collapse. A detailed understanding of the colony failure process will be helpful for finding the most effective strategy to enhance the resilience of colonies. Second, more efforts should be devoted to revealing the mechanisms and rules of the interaction between different stresses. The interaction between different abiotic stresses, between different biotic stresses, and between abiotic and biotic stresses may increase the severity of their effects on the health and survival of honeybees. Third, researchers should continue to identify more possible key genes and signaling pathways that participate in the stress responses of honeybees. In this process, cytobiological, genetic, and molecular biological methods can be used. Fourth, breeding of disease-resistant honeybees is vital for apiculture. Disease-tolerant honeybees found in colonies through natural selection should be expanded. If possible, overexpression of stress resistance genes in honeybees should be performed to obtain transgenic honeybees, or knockdown of stress sensitivity genes in honeybees can be carried out to obtain mutant honeybees, both of which may exhibit improved stress resistance. However, the current challenge is that transgenesis in honeybees is prohibitively difficult. Finally, provision of sufficient nutrition to honeybees by the beekeeper is likely to play an essential role in maintaining the health of colony bees in apiculture. Good nutrition enhances the immunocompetence of honeybees, and improves the ability of the colony to defend against environmental stress (Naug, 2009; Archer et al., 2013; Schmehl et al., 2014).

AUTHOR CONTRIBUTIONS

GL, BX, and XG: designed of the work. GL, HZ, BX, and XG: drafted the work. HZ, ZL, and HW: created the figures and tables. All authors approved the final version of the manuscript.

FUNDING

ACKNOWLEDGMENTS

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GLOSSARY

ß-glucan: ß-Glucan is a heterogeneous group of glucose homopolymers found in algae, plants, fungi, and some bacteria. It can exert strong immune stimulatory activity in many invertebrate and vertebrate species (Soltanian et al., 2009).

Innate immune response: The immune response includes acquired immune response and innate immune response. Like all insects, honeybees lack an acquired immune response to defense against microorganisms. Instead, they depend on the innate immune response, which includes the humoral immune response (synthesis of specific antimicrobial peptides), the cellular immune response (wouding healing, nodulation, phagocytosis, and encapsulation) and the activation of prophenoloxidase in response to microbial infection. Prophenoloxidase can be activated by a serine protease cascade, causing transient synthesis of melanin and quinones (Richard et al., 2008; Chan et al., 2009; Masri and Cremer, 2014). Furthermore, recent study shows that due to these immune characteristics, honeybees may become a potential vivo system for screening antiviral compounds under certain experimental contexts (Fedorova et al., 2011).

Abscisic acid (ABA): ABA is a phytohormone that participates in regulation of fundamental physiological functions in plants. The presence of ABA in honey and nectar has been unambiguously demonstrated (Ferreres et al., 1996; Adie et al., 2007).