

Symbiont-mediated adaptation by planthoppers and leafhoppers to resistant rice varieties

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Abstract For over 50 years, host plant resistance has been the principal focus of public research to reduce planthopper and leafhopper damage to rice in Asia. Several resistance genes have been identified from native varieties and wild rice species, and some of these have been incorporated into high-yielding rice varieties through conventional breeding. However, adaptation by hoppers to resistant rice has been phenomenally rapid, and hopper populations with virulence against several resistance genes are now widespread. Directional genetic selection for virulent hoppers seems unlikely given the rapid pace of adaptation reported from field and laboratory studies. Among the alternative explanations for rapid hopper adaptation are changes (genetic, epigenetic, or community structure) in endosymbiont communities that become advantageous for planthoppers and leafhoppers that feed on resistant rice varieties. This review examines the nature of these symbiont communities and their functions in planthoppers and leafhoppers—focusing on their likely roles in mediating adaptation to plant resistance. Evidence from a

small number of experimental studies suggests that bacterial and eukaryotic (including yeast-like) symbionts can determine or mediate hopper virulence on rice plants and that symbiont functions could change over successive generations of selection on both resistant and susceptible plants. The review highlights the potential complexity of rice hopper–symbiont interactions and calls for a more careful choice of research materials and methods to help reduce this complexity. Finally, the consequences of symbiont-mediated virulence adaptation for future rice breeding programs are discussed.

Keywords Amino acids · Detoxification · Nutrition · Resistance breeding · Symbiotic bacteria · Yeast-like symbionts

Introduction

Since the beginning of the Green Revolution, a small group of planthoppers (Delphacidae) and leafhoppers (Cicadellidae) have continued to cause major losses to rice production throughout South and East Asia. These “hoppers” include the brown planthopper, *Nilaparvata lugens* (Stål); whitebacked planthopper, *Sogatella furcifera* (Horváth); small brown planthopper, *Laodelphax striatellus* (Fallén); green rice leafhopper, *Nephotettix cincticeps* (Uhler); and green leafhopper, *Nephotettix virescens* (Distant) (Fujita et al. 2013) (Table 1). Furthermore, in the Neotropics, the rice delphacid, *Tagosodes orizicolus* (Motschulsky), is a vector of hoja blanca virus (HBV), a damaging rice virus that causes major losses to rice yield each year (Zeigler et al. 1994). Losses due to Asian planthoppers and leafhoppers have been estimated at millions of tonnes of production in some years, particularly in China, Thailand, and

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Table 1 Leafhopper and planthopper pests of rice

Scientific name	Common name	Host plant	Resistance genes identified from rice [1]	Reports of virulence adaptation in field populations of target hoppers ^a
<i>Nilaparvata lugens</i> (Stål)	Brown planthopper	Rice	36	<i>Bph1</i> and <i>bph2</i> —widespread virulence in S and SE Asia [1]; <i>Bph3</i> and <i>bph4</i> —India [2], Thailand [3], <i>bph4</i> and <i>bph8</i> —China and Japan [4]; BPH25 and BPH26—widespread in SE Asia [5]; <i>Bph5</i> , <i>Bph6</i> , <i>Bph7</i> , <i>bph8</i> , <i>Bph9</i> , <i>Bph10</i> , <i>Bph18</i> , <i>Bph20</i> , and <i>Bph21</i> —Philippines [6]
<i>Nilaparvata</i> sp. [7, 8] ^b	Brown planthopper (from <i>Leersia</i>)	<i>Leersia hexandra</i>	None	Not a rice pest
<i>Laodelphax striatellus</i> (Fallén)	Small brown planthopper	Rice, wheat, barley, maize, oats, sugarcane, millets, and some grasses	1	Unknown
<i>Sogatella furcifera</i> (Horváth)	Whitebacked planthopper	Rice, millets, maize, and some grasses	14	<i>Wbph1</i> , <i>Wbph2</i> , <i>Wbph3</i> and <i>wbph4</i> —China and Japan [4,5,9]
<i>Tagasodes orizicolus</i> (Muir)	Rice delphacid	Rice and weeds	None (but several rice varieties are resistant)	Unknown
<i>Nephotettix virescens</i> (Distant)	Green leafhopper	Rice and weeds	14	<i>Glh1</i> —Philippines [pers. obs.]
<i>Nephotettix cincticeps</i> (Uhler)	Green rice leafhopper	Rice and weeds	7	<i>Grh1</i> and <i>Grh2</i> [10]
<i>Recilia dorsalis</i> (Motschulsky)	Zigzag leafhopper	Rice and weeds	3	Unknown

Source references: 1, Fujita et al. 2013; 2, Verma et al. 1979; 3, Thanysiriwat et al. 2009; 4, Myint et al. 2009a; 5, Myint et al. 2009b; 6, Horgan (unpublished); 7, Latif et al. 2008; 8, Latif et al. 2012; 9, Tanaka and Matsumura 2000; 10, Hiraie et al. 2007

^a Gene loci names beginning with upper case letters indicate dominant genes, lower case indicates recessive genes, BPH25 and BPH26 were named using a more recent naming convention (Fujita et al. 2013)

^b Recent molecular studies have indicated that brown planthopper populations feeding on rice and feeding on the weed *Leersia hexandra* represent distinct species (sibling species or cryptic species)

Vietnam (Fujita et al. 2013). Furthermore, sharp increases in the frequency and extent of hopper outbreaks have been noted since about 2002 (Catindig et al. 2009; Fujita et al. 2013). Damage (often called “mechanical damage” as opposed to viral symptoms) results from hopper feeding, whereby the hoppers extract nutrients directly from the rice phloem by means of a specialized stylet inserted into a salivary sheath (Wang et al. 2008b). In the process, large quantities of honeydew are produced on which fungal colonies develop, often resulting in a sooty appearance of the rice (Botrell and Schoenly 2012; Fujita et al. 2013) (Table 1). Severe infestations by leafhoppers and planthoppers can lead to “hopperburn,” a wound response that results in eventual desiccation and death of the rice plant; however, even at low densities, hoppers can cause significant yield losses when they transmit rice viruses, including tungro viruses and others that cause yellowing syndromes (Fujita et al. 2013).

For over 50 years, host plant resistance has been a major focus of research to reduce damage to rice from planthoppers and leafhoppers. Several rice varieties and wild rice species have notable resistance to hoppers, and many of these have been used in rice breeding programs as the donor parents for resistant rice varieties. A recent review has listed 79 resistance gene loci derived mainly from traditional rice varieties of South Asian origin (India, Bangladesh, and Sri Lanka) and from a range of wild rice species (Fujita et al. 2013). Most of these gene loci were discovered only in the last 20 years; however, there is now an increasing body of evidence to indicate that many of the genes have already become ineffective in large parts of Asia because of widespread hopper adaptation (Table 1) (Myint et al. 2009a, b; Peñalver Cruz et al. 2011; Fujita et al. 2013). The pace of hopper adaptation to resistant rice varieties (which we refer to as virulence adaptation) has been phenomenally rapid. For example, widespread adaptation by *N. lugens* to rice varieties with the *Bph1* and *bph2* genes occurred within 5 years from first release of the varieties (Alam and Cohen 1998; Fujita et al. 2013). This rapid adaptation suggests that virulence is unlikely to be the result of genetically based directional selection (Chen 2009; Chen et al. 2011), and points to other mechanisms that might determine resistance and contribute to virulence adaptations. Symbionts, a component of the internal flora of both planthoppers and leafhoppers (Nasu 1963; Noda 1974; Chen et al. 1981a, b; Noda et al. 1995; Xet-Mull et al. 2004; Wang et al. 2010; Tang et al. 2010; Noda et al. 2012), have recently been linked to variations in the outcome of rice–planthopper interactions (*N. lugens*: Lu et al. 2004; Chen et al. 2011). Therefore, the presence, composition, and functions of the endosymbiotic internal flora of hoppers could underlie alternative mechanisms of adaptation to resistant rice varieties.

The purpose of this review is to collate all current knowledge on the symbionts of planthoppers and leafhoppers that feed on rice, highlighting the nature and function of the symbiotic communities present in the hopper bodies and relating these to possible shifts in hopper virulence. We examine the inoculation and population cycles of symbionts (particularly the yeast-like symbionts) and relate these to hopper development, reproduction, and behavior. We also review known mechanisms of rice resistance and examine probable roles for endosymbionts during hopper virulence adaptation. In particular, we critically examine available experimental evidence that draws links between endosymbionts and virulence adaptation and discuss possible directions for future research in this area. Finally, we highlight major gaps in the current knowledge, discuss the application of novel entomological, microbiological, and molecular tools in elucidating complex interactions, and discuss some possible consequences from what is known of rice hopper–symbiont interactions for the successful breeding and deployment of resistant rice varieties.

Taxonomy and systematics of planthopper–symbiont associations

“Symbiont” is a broad term that describes the beneficial (mutualistic), parasitic, and disease-causing intra- and extracellular organisms that occur in insects and other higher organisms. Henceforth, in this review, we use the term “symbiont” to refer only to mutualistic endosymbionts (that live inside the insect body). Nasu (1963) was the first to report symbionts associated with rice planthoppers. Until the early 2000s, yeast-like symbionts (YLS) had been the only type of symbiont found in planthopper species (Noda 1974; Chen et al. 1981a; Kagayama et al. 1993; Noda et al. 1995; Xet-Mull et al. 2004). More recently, other eukaryotic and bacterial symbionts have been associated with *N. lugens* and *N. cincticeps* (Tang et al. 2010; Dong et al. 2011; Noda et al. 2012). Bacteria-like organisms have also been observed in the salivary sheaths of *N. lugens* using electron microscopy (Wang et al. 2008a; Tang et al. 2010).

Because the primary (obligate) symbionts of planthoppers and leafhoppers cannot be cultured in vitro (Noda et al. 1995), hopper symbiosis has remained relatively understudied compared to, for example, aphid symbiosis—where many of the symbionts may be secondary. Nevertheless, symbionts have been isolated from planthopper and leafhopper tissues through density-gradient ultracentrifugation (Noda and Omura 1992), which permits research into their taxonomic affiliations without the need for the pure culture isolates required with most conventional

methods of microorganism classification (Ganter 2006). In addition, sequence information of nucleic acids or proteins has been useful for the study of obligate symbionts given the difficulties in growing symbionts in vitro (Noda et al. 1995).

Several different symbiotic microorganisms occur in planthoppers and leafhoppers (Table 2). Using primer sequences designed to amplify the conserved 18S ribosomal DNA region (rDNA), Noda et al. (1995) identified YLS [GenBank accession no. AF267233.1 (*N. lugens* YLS 18S rDNA)] and located it in the class Pyrenomycetes, subphylum Ascomycotina in the fungal kingdom and suggested that the YLS from *N. lugens*, *S. furcifera*, and *L. striatellus* are monophyletic, i.e., derived from a single ancestral species (Noda et al. 1995). YLS have also been isolated from *T. orizicolus*. Surprisingly, there is also a high degree of similarity between the YLS of the three Asian rice planthoppers and those of the South American planthopper (98 % similarity), suggesting that the four YLS descended from a common Pyrenomycete ancestor (Xet-Mull et al. 2004). More recently, it has become apparent that several eukaryotic organisms occur in the fat bodies, ovaries, and eggs of *N. lugens*, although it has been difficult to separate these functionally (i.e., obligate vs. facultative and intracellular vs. extracellular) and some may represent contaminants from food or the environment (Chen et al. 2006; Zhang et al. 2007, 2009; Hou et al. 2013). Using freezing microtomy and staining, Chen et al. (2006) noted several different eukaryotic organisms associated with the fat bodies of adult *N. lugens*. A further two species, *Yarrowia lipolytica* and *Sterigmatomyces halophilus*, were identified from planthopper eggs using 26S rDNA (Zhang et al. 2007, 2009). Dong et al. (2011) also identified *Cryptococcus*-like symbionts and *Pichia*-like symbionts from the fat bodies of *N. lugens*. Isolation and identification was achieved through amplification of the 18S and ITS-5.8S rDNA sequences with universal fungal primers. Recently, using a highly sensitive new method (nested PCR-denaturing gradient gel electrophoresis [DGGE]), Hou et al. (2013) identified a range of eukaryotic symbionts associated with the fat bodies of *N. lugens* (Table 2); because of the high sensitivity of the method, some of these species are likely to represent fungal contaminants occurring at very low concentrations with no functional benefit for the hoppers.

In recent years, researchers have begun to examine the bacterial symbionts of hoppers in more detail (i.e., Tang et al. 2010; Wang et al. 2010; Noda et al. 2012). Eighteen bacterial operational taxonomic units (OTUs) that represent four phyla [Proteobacteria (13 OTUs), Firmicutes (2), Actinobacteria (2), and Bacteroides (1)] have been identified from *N. lugens*. These uncultured bacteria were detected by extracting the total genomic DNA of the

planthopper and amplifying part of the 16S rRNA gene. Comparison of the 16S rRNA sequences of these OTUs indicated a similarity between planthopper bacterial symbionts and the secondary symbionts or gut-associated microbes of other insect species, although some planthopper symbiont OTUs had not previously been found in insects (Tang et al. 2010). The study of Tang et al. (2010) indicated that bacterial OTUs differ substantially between populations of *N. lugens* reared on different rice varieties; however, many of the observed bacterial OTUs may represent secondary symbionts that are not required for hopper survival or were simple environmental contaminants.

Bacterial symbionts have also been found in *N. cincticeps*. Histological studies identified two bacteriome-associated symbionts and a rickettsial microorganism in the leafhopper (Nasu 1965; Mitsuhashi and Kono 1975). Cloning and sequencing of the 16S rRNA gene from *N. cincticeps* generated a major sequence, which was placed in the Bacteroidetes clade of *Sulcia muelleri*, a symbiont lineage associated with various hemipteran insects; another major sequence was related to a β -proteobacterial sequence from a leafhopper *Matsumuratettix hiroglyphicus* while a minor sequence was grouped in the α -proteobacterial genus *Rickettsia* (Noda et al. 2012).

Distribution, location, and transmission of symbionts in hoppers

Symbionts can live extracellularly, i.e., in the gut lumen or digestive tract of insects, or intracellularly, i.e., inside a specialized cell type (mycetocyte) in the insect (Douglas 1989; Table 2). Mycetocyte symbionts are beneficial to the insects that contain them: When these microbes are eliminated, the insects grow and develop slowly and die prematurely, often without reproducing (Douglas 1989). Mycetocyte symbiosis is characterized by the following conditions: the microorganisms are intracellular and restricted to the cytoplasm of the mycetocyte; the microorganisms are maternally inherited; and the association is required by both the insect and microbial partners (Douglas 1989). The location of the mycetocytes differs between insect groups. Mycetocytes are usually found in the cells of the digestive tract, in the abdominal hemocoel, or in the fat body of the abdomen (Douglas 1989). In *L. striatellus*, mycetocytes have been found only in the fat body cells of the abdomen and not in other parts of the insect body (Noda 1974).

Cycles in the occurrence and abundance of YLS were first described for *L. striatellus* (Noda 1974). YLS are present at every developmental stage of this hopper with the number of symbionts increasing from the egg to adult stage and attaining higher numbers in females than

Table 2 Eukaryotic and prokaryotic microorganisms associated with rice leafhoppers and planthoppers; some studies may have reported mutualistic symbionts as well as yeast-like and bacterial contaminants identified during screening

Leafhopper and planthopper species	Insect developmental stage	Symbionts identified	Symbiont location	Symbiont function
<i>N. lugens</i>	Eggs, nymphs, adults [1,2]	Yeast-like symbionts (Pyrenomycete-Ascomycotina) [3,4]; <i>Pichia guilliermondii</i> , <i>Saccharomyces</i> sp., <i>Debaryomyces hansenii</i> , other uncultured-identified fungi [4]; <i>Candida</i> , [4,5]; <i>Yarrowia lipolytica</i> , <i>Sterigmatomyces halophilus</i> [6]; <i>Cryptococcus</i> and <i>Pichia</i> -like symbionts [7]; Bacteria (<i>Proteobacteria</i> , <i>Firmicutes</i> , <i>Actinobacteria</i> , <i>Bacteroidetes</i> [8])	Abdominal fat body; ovary, eggs [1]	Nitrogen waste recycling [9,10]; supply proteins for embryonic development [11]; provision of amino acids for nymphal development; sterol provision [12]
<i>L. striatellus</i>	Egg, nymph, female adult, and male adult [13]	Yeast-like (Pyrenomycete-Ascomycotina) [3]	Abdominal fat body; ovary [13, 14]	Source of sterols for host development [12]
<i>S. furcifera</i>	Eggs, nymphs, adults [14]	Yeast-like (Pyrenomycete-Ascomycotina) [3]	Abdominal fat body; ovary [14]	Unknown
<i>T. orizicolus</i>	Eggs, nymphs, adult [15]	Yeast-like (Pyrenomycete-Ascomycotina) [15]	Abdominal fat body; ovary [15]	Unknown
<i>N. cincticeps</i>	Nymphs, adults [15]	Bacteria (<i>Salicic</i> bacterium and β -proteobacterium; <i>Rickettsia</i>) [16]	Bacteriome; ovary bacteriome, ovary, testis, midgut, Malpighian tubules, fat body [16]	Unknown

Source references: 1, Chen et al. 1981b; 2, Nasu 1963; 3, Noda et al. 1995; 4, Hou et al. 2013; 5, Pang et al. 2012; 6, Zhang et al. 2009; 7, Dong et al. 2011; 8, Tang et al. 2010; 9, Sasaki et al. 1996; 10, Hongoh and Ishikawa 1997; 11, Lee and Hou 1987; 12, Eya et al. 1989; 13, Noda 1974; 14, Noda 1977; 15, Xet-Mull et al. 2004; 16, Noda et al. 2012

in males (dropping sharply as males enter the adult stage) and in brachypterous (short-winged) females than in macropterous (long winged) females (Fig. 1). This suggests that YLS in adult females may be associated with egg production, especially since brachypterous females (adapted for reproduction) produce more eggs than macropterous females (adapted for dispersal) (Padgham 1983). Similar trends have been found for YLS in *N. lugens* (Chen et al. 1981b; Cheng and Hou 2001; Lu et al. 2004; Hou 2008).

Symbionts are passed maternally from parents to progeny through the egg. This “transovarial transmission” of YLS has been revealed by both light and electron microscopies in rice planthoppers (Noda 1977; Lee and Hou 1987; Nagamine et al. 1993; Kagayama et al. 1993; Cheng and Hou 1996; Cheng and Hou 2001). YLS transmission from the fat body to the oocyte occurs in a series of recognizable stages (Cheng and Hou 2001): (1) the YLS in the mycetocytes first move out of the syncytium (formed from a layer of fat body cells) by exocytosis and are released into the hemocoel; (2) the free YLS in the hemolymph then move to the ovarioles near the pedicel that is enclosed by follicle cells; (3) the YLS then enter the follicle cells around the primary oocyte by endocytosis at the epithelial plug of the ovariole; and (4) finally, the YLS assemble at the posterior end of the mature egg forming a symbiont ball (Cheng and Hou 2001). The mechanisms behind the transmission of bacterial symbionts from one generation to the next in planthoppers have not yet been elucidated, although some bacterial symbionts have been found in the insect ovaries (Table 2). Whether bacterial transmission is horizontal or vertical could have important consequences for planthopper and leafhopper virulence adaptation.

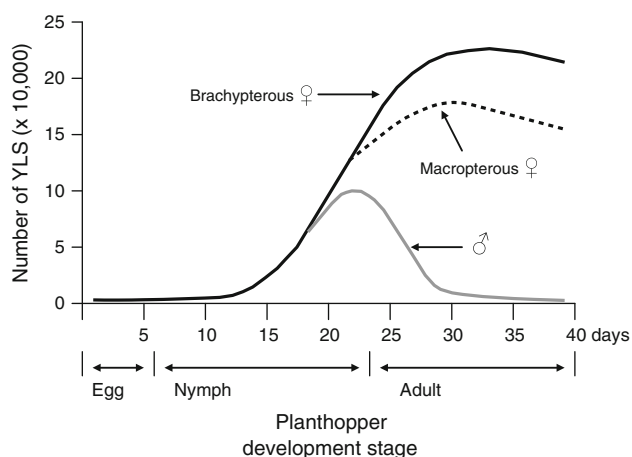


Fig. 1 Changes in the number of yeast-like symbionts (YLS) during the life cycle of the small brown planthopper, *Laodelphax striatellus* (redrawn from Noda 1974)

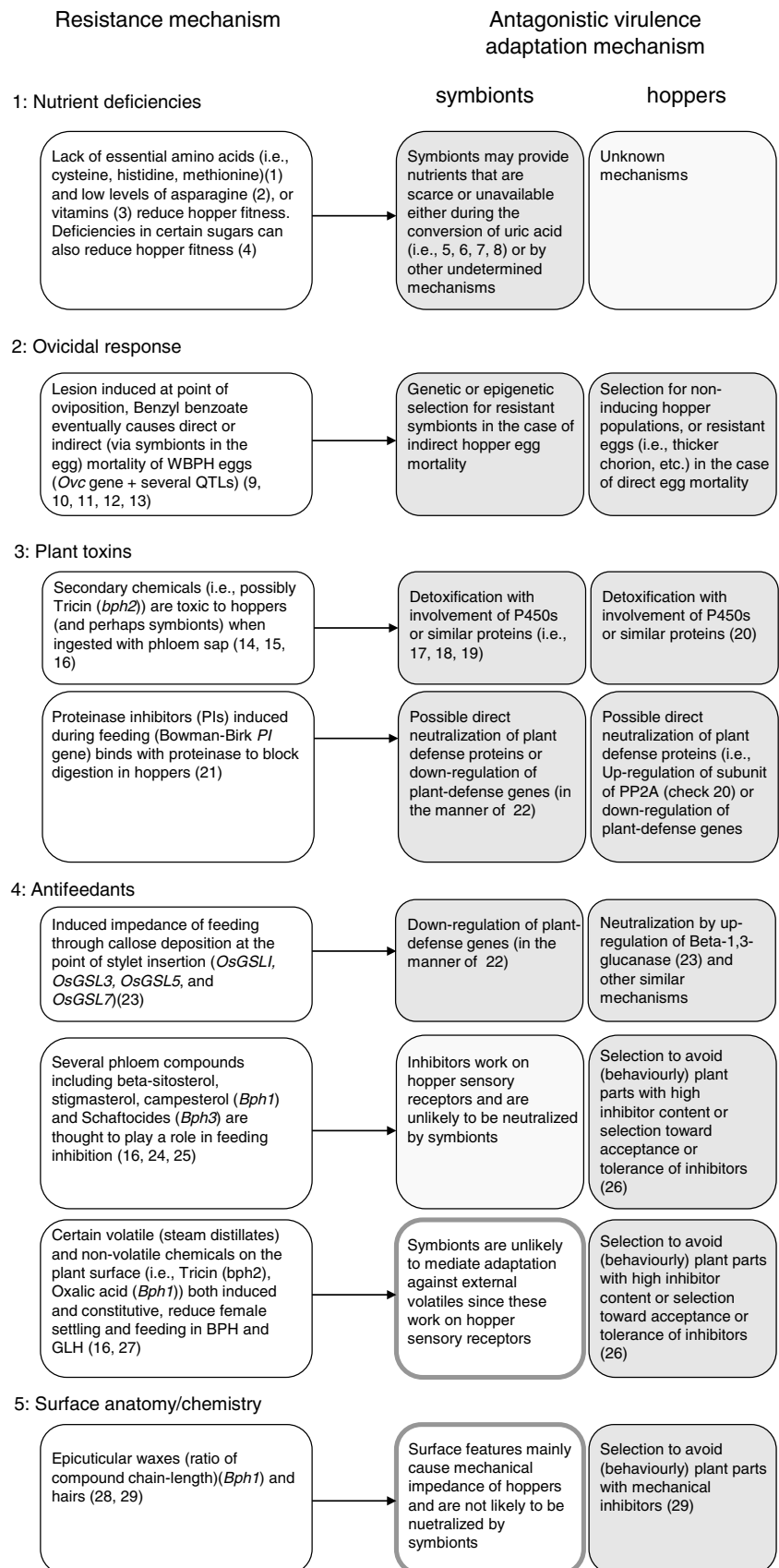
The functions of planthopper and leafhopper symbionts

Nutrition and development

Plant phloem sap is the primary diet of most hemipterans, including planthoppers and leafhoppers, and symbionts appear to provide nutrients that are scarce or unavailable in the insect diet in return for metabolites from the insect host (Chaves et al. 2009). Phloem is rich in carbohydrates but poor in essential amino acids. Hoppers feeding on resistant rice varieties are thought to be further deprived of essential nutrients, including sugars, amino acids, and possibly vitamins (Sogawa and Pathak 1970; Pathak and Kalode 1980; Koyama 1985, 1986; Jung and Im 2005; Chen et al. 2011), their bodies will also have reduced uric acid and fat (including crude fat) content (Yin et al. 2008; Hongoh and Ishikawa 1997; Sasaki et al. 1996), and reduced lipid synthesis and glycogen reserves (Padgham 1983). The role of symbionts (eukaryotic and prokaryotic) is seemingly to supply essential amino acids that are lacking in the insect diet (Wilkinson and Ishikawa 2001; Ganter 2006). In resistant rice, poor nutritional quality of the host can be directly due to deficiencies in the phloem or can arise due to the inability of hoppers to access the nutrients present in the phloem (either as a result of antidiigestive, antinutritive, and/or antiabsorbative compounds, or other plant defense mechanisms) (Fig. 2). For example, in a study by Jung and Im (2005), *N. lugens* feeding on the resistant variety Cheongcheongbyeon excreted significantly less sugars than when feeding on a susceptible variety, despite similar sugar contents in the phloem of both varieties. This suggests that unidentified phloem components disturb or alter planthopper digestive or feeding processes on resistant varieties. The amino acid composition of rice phloem is known to differ markedly between rice varieties [see Chen et al. (2011) and references therein]. Using artificial diets, the absence of sulfur-containing amino acids (cysteine, histidine, and methionine) has been shown to negatively affect *N. lugens* fitness (Koyama 1986), although the absence of any one amino acid appears not to affect the hoppers. Whether symbionts could eventually neutralize the effects of dietary deficiencies or compensate for low concentrations of key amino acids is still unknown; however, it is intuitive that dietary compensation mediated by symbiotic gut flora (particularly eukaryotic organisms) will underlie adaptation by planthoppers to certain resistant rice varieties (Fig. 2).

The nutritional role of YLS has been studied in planthoppers and is known to contribute to the nitrogen requirements of these insects (*N. lugens*: Sasaki et al. 1996). Planthoppers produce uric acid as a nitrogenous waste but do not excrete it as occurs in many other insects. For *N. lugens*, uric acid is stored in the insect tissues and

Fig. 2 Rice resistance mechanisms and possible mechanisms of virulence adaptation as mediated through leafhoppers, planthoppers and their symbionts. The order of resistance mechanisms indicates the proposed likelihood of symbiont involvement in virulence adaptation, and *dark gray*, *light gray*, and *gray-bordered boxes* indicate high, low, and zero probability of involvement by either the symbionts or hoppers in virulence adaptation. *Numbers* in parentheses indicate source references as follows: 1 Koyama 1986; 2 Sogawa and Pathak 1970; 3 Pathak and Kalode 1980; 4 Koyama 1985; 5 Sasaki et al. 1996; 6 Hongoh and Ishikawa 1997; 7 Ishikawa 2003; 8 Ganter 2006; 9 Sogawa 1991; 10 Suzuki et al. 1996; 11 Seino et al. 1996; 12 Kiyonaga et al. 1997; 13 Yamasaki et al. 2000; 14 Saxena and Okech 1985; 15 Yang et al. 2006; 16 Bing et al. 2007; 17 Karban and Agrawal 2002; 18 Jones 1984; 19 Dowd 1992; 20 Yang et al. 2006; 21 Weng et al. 2003; 22 Barr et al. 2010; 23 Hao et al. 2008; 24 Shigematsu et al. 1982; 25 Stevenson et al. 1996; 26 Fujita et al. 2013; 27 Yoshihara et al. 1980; 28 Woodhead and Padgham 1988; 29 Zhang et al. 2004



converted into compounds of nutritional value by YLS through the action of symbiont uricase when the hoppers experience nitrogen limitation (Sasaki et al. 1996; Hongoh and Ishikawa 1997; Ishikawa 2003; Ganter 2006). Interestingly, the levels of uric acid in *N. lugens* eggs are highest at oviposition and decrease significantly as the egg develops. This suggests that the egg is supplied with uric acid by the parent prior to oviposition at about the time that YLS are most abundant in the female's body (Hongoh and Ishikawa 1997).

The role of symbionts becomes most apparent when these are removed from the hopper body. For example, submitting hoppers to a high heat treatment can reduce YLS numbers significantly, and this has become a standard in studies of hopper symbionts. Aposymbiotic planthoppers produced by heat treatment display several physiological and developmental deficiencies. Vega and Dowd (2005) summarized the effects of heat-induced reduction of YLS numbers in *N. lugens* eggs and nymphs as follows: (1) in the egg, normal embryonic and postembryonic development are affected due to the absence of certain proteins synthesized by YLS (Lee and Hou 1987); (2) there is a reduction in egg hatching and (3) an increase in the duration of each nymphal stage (Bae et al. 1987; Zhongxian et al. 2001); (4) there is failure to moult resulting in the death of fifth instars during ecdysis (Chen et al. 1981a); and (5) insect weight, growth rate, and the amount of protein per unit of fresh weight are reduced (Wilkinson and Ishikawa 2001). In contrast to the severe effects on eggs and nymphs, when YLS are removed/reduced from adult planthoppers by heat treatment, there are no effects on mortality or life span, suggesting that YLS are not directly involved in adult survival (Lee and Hou 1987). However, YLS do play a role in determining the fertility of planthoppers (*N. lugens*) since heat-treated females lay fewer eggs (Ganter 2006).

Evidence of sterol synthesis by YLS has been demonstrated through comparisons of control and heat-treated individuals of *L. striatellus*: Heat treatment resulted in failure of fifth instars to moult to adults, leading Noda and Saito (1979) to conclude that YLS are involved in sterol metabolism. Furthermore, the concentration of sterols was significantly reduced in heat-treated insects where the YLS had been destroyed or significantly depleted (Noda et al. 1979). Several sterols such as cholesterol, 24-methylenecholesterol, and ergostatrienol have been isolated from *N. lugens* and *L. striatellus*. Ergostatrienol, which has also been found from purified YLS by density-gradient centrifugation, is considered to be synthesized by YLS (Wetzel et al. 1992). The ability of YLS to alter/process food inputs to provide essential nutrients for hoppers suggests that the symbionts play a key role in determining host choice and virulence adaptation. However, YLS-mediated adaptation

to resistant rice varieties should be most prominent in those varieties for which resistance is due to phloem nutrient deficiencies (i.e., poor food quality for hoppers) (Fig. 2).

Detoxification of plant allelochemicals

Some resistant rice varieties produce secondary chemicals that prevent feeding by planthoppers and leafhoppers. Some of these chemicals potentially act as antigestives, antinutritives, or antiabsorbatives (i.e., C-glycosidic flavonoids in varieties with the *Bph3* gene: Stevenson et al. 1996) but others are apparently toxic to the hoppers. For example, when planthoppers feed on the variety B5 (which contains the *Bph14* and *Bph15* genes), a P450-encoding planthopper gene is activated (Yang et al. 2006). P450s are best known for their role in the metabolism of insecticides and plant secondary chemicals, suggesting that B5 produces toxic substances that are ingested by the planthoppers. When planthoppers feed on B5 or other resistant varieties, their symbiotic gut flora are also exposed to these plant toxins (i.e., Dowd 1992). Enzymatic detoxification systems (such as P450s) are widespread among herbivores and some of these are provided as services by microbial symbionts (Karban and Agrawal 2002; Jones 1984, Dowd 1992). Insect symbionts (eukaryotic and prokaryotic) are known to play a role in the detoxification of plant toxins as well as man-made compounds such as insecticides (Barbosa et al. 1991; Kikuchi et al. 2012). Microbial-mediated detoxification and transformation of plant secondary compounds (i.e., flavonoids, tannins, and alkaloids) in the insect gut has been demonstrated in a few studies and indicates that microbial symbionts can determine the ability of phytophagous insects to overcome barriers to herbivory (*Lasioderma serricornis* (Fabricius): Douglas 1992; Dillon and Dillon 2004). For example, localized detoxification activity of defensive plant compounds occurs in the yeast (*Symbiotaphrina kochii*) mycetosomes of the cigarette beetle, *L. serricornis*, increasing beetle survival on toxic dried plant substrates or when treated with plant defense toxins (Dowd 1989; Dowd and Shen 1990).

There are no clear examples of symbiont-mediated detoxification of plant compounds in rice planthoppers or leafhoppers; however, if symbionts are mediating detoxification of rice toxins during hopper feeding, then the processes may be similar to those found in other symbiont-herbivore systems. Because several toxins and some tentative antigestives, antinutritives, or antiabsorbative compounds are known from rice (Yoshihara et al. 1980; Shigematsu et al. 1982; Stevenson et al. 1996; Bing et al. 2007), clear manipulative experiments, using aposymbiotic hoppers and known defense compounds, could help identify the role of symbionts in virulence adaptation by

planthoppers and leafhoppers. Further evidence in support of the hypothesis that symbiont-mediated detoxification of plant compounds occurs during hopper exposure to resistant rice would be welcome.

Down-regulation of plant defense genes

Under normal circumstances, plant defense signalling pathways such as the salicylic acid (SA), jasmonic acid (JA), and ethylene pathways are activated during interactions between plants and their attackers (pathogens and/or herbivores) (Pieterse and Dicke 2007). Plant defense inducers such as β -glucosidase present in the saliva of *N. lugens* have already been associated with SA, ethylene, and hydrogen peroxide production (Wang et al. 2008b, c). Studies have indicated that the JA pathway may also be activated during planthopper attack (Zhang et al. 2004; Wang et al. 2008b). The induction of these phytohormones by planthoppers regulates the synthesis of feeding inhibitory (i.e., callose: Hao et al. 2008) and digestibility-reducing compounds (i.e., proteinase inhibitors: Weng et al. 2003) as well as a variety of volatile organic compounds (i.e., linalool, (3E)-4,8-dimethyl-1,3,7-nonatriene, and indole: Xu et al. 2002). Generally, insect herbivores employ offensive strategies to counter plant-imposed challenges. For example, *N. lugens* employs β -1,3-glucanase breakdown of callose (Hao et al. 2008) and can up-regulate the β -subunit of protein phosphatase 2A (PP2A) in response to plant PP2A production (Yang et al. 2006). It is possible that planthoppers and leafhoppers possess suites of sophisticated nuclear genes that code for these counter defenses; however, it is also possible that hoppers might acquire such functional innovations through established symbiotic associations. For example, Barr et al. (2010) have shown that a symbiont, rather than the phytophagous insect itself, was involved in down-regulating several genes involved in the defense of maize (*Zea mays* L.) against the western corn rootworm, *Diabrotica virgifera virgifera* Le Conte. Analysis of microarray expression data showed genome-wide suppression of maize defense genes (i.e., cell wall defenses, production of phytoalexins, and pathogenesis-related proteins) following attack by the rootworm where *Wolbachia*, an intracellular bacteria found throughout the rootworm body, was naturally present. However, when the corn rootworms were treated with antibiotics (eliminating *Wolbachia*), these same maize defense genes were up-regulated (Barr et al. 2010). It is possible that the symbionts of planthoppers are also normally involved in the down-regulation of rice defenses (leading to negative effects in aposymbiotic planthoppers even on seemingly susceptible rice varieties), but that the symbionts also activate counter defenses when hoppers are reared continually on resistant varieties.

Implications of symbiosis for hopper adaptation to rice plant resistance

The intracellular nature of some symbionts, particularly the YLS, indicates their functional importance to planthoppers and their likely role in virulence adaptation. Conventionally, the pest status of insects has been largely attributed to their genomes. However, at least one study has ascribed pest-related traits primarily to a symbiont genotype rather than the insect genotype itself: Hosokawa et al. (2007) observed that the pest stinkbug, *Megacopta punctatissima* (Montandon), which performs well on crop legumes, has a closely related non-pest conspecific, *Megacopta cribraria* (Fabricius), which performs poorly on the same plants. In a manipulative experiment, the authors exchanged the obligate gut symbiotic bacteria between the two insect species and demonstrated a marked reversal in performance on their host plants. This clearly implies that symbionts influence the performance of herbivores on plants, to such an extent that they can ultimately determine whether a plant becomes a suitable host for an insect or not. It would be interesting to conduct similar studies on, for example, the two cryptic species that constitute the *N. lugens* complex (Latif et al. 2008, 2012: Table 1), one of which is largely monophagous on rice and the second on the grass weed *Leersia hexandra* Swartz. Similarly, because YLS are passed vertically from generation to generation through the egg, and are therefore linked to the female parent only, simple reciprocal mating experiments between selected populations on resistant and susceptible rice lines could help determine the extent to which observed virulence is determined by symbionts. For example, preliminary mating studies with *N. lugens* have indicated that whereas YLS did contribute to virulence on a resistant variety (IR62—*Bph3* gene), the male parent also influenced fitness on the resistant host, suggesting that other mechanisms (which may include bacterial symbionts) also played a role in virulence adaptation (Peñalver Cruz et al. unpublished results).

The summary of known rice defenses against planthoppers and leafhoppers presented in Fig. 2 (white boxes) indicates the diversity and complexity of resistance mechanisms. The diversity of these mechanisms, together with the observations on the frequency and extent of planthopper and leafhopper adaptations to resistant rice varieties (Table 1), suggests that virulence adaptations are likely to be similarly diverse and complex. However, symbionts are unlikely to be involved in adaptations against certain plant defense strategies, including defenses related to surface volatiles, or plant ovicidal responses (Fig. 2). Virulence adaptation against specific varieties, or the products of specific resistance genes, may involve symbionts alone (either eukaryotic, prokaryotic, or both),

involve the hoppers alone, or involve interactions between the hoppers and their symbionts. Furthermore, adaptation could be mediated through community shifts in symbionts, genetic changes (mutations), or epigenetic shifts in the symbionts or genetic changes and epigenetic shifts in the hoppers. There may also be more complex interactions that include, for example, community shifts in symbionts that are mediated through epigenetic shifts in the hoppers. Teasing the exact mechanisms apart will be a difficult task. It has also become apparent that major resistance rice genes interact with other genes located throughout the rice genome to determine the extent and durability of the rice plant's resistance to hoppers (Fujita et al. 2013). Furthermore, the same genes present in different variety backgrounds have often strikingly distinct effects on hopper populations (sometimes ranging from susceptible to resistant, i.e., Peñalver Cruz et al. 2011), and hoppers require a few generations to attain maximum fitness even on encountering seemingly susceptible varieties (Claridge and Den Hollander 1982, 1983; Alam and Cohen 1998, but see Chen et al. 2011). Indeed, movement between any two varieties with all their complex of allelochemicals and phloem components seems to constitute a barrier for planthopper and leafhopper feeding (Horgan 2012).

Given existing knowledge on the role of YLS in nutrition, it is intuitive that these symbionts in particular play a key role in mediating hopper adaptation to varieties that are resistant due to phloem nutrient deficiencies, or deficiencies caused by compounds that block the extraction or assimilation by hoppers of phloem nutrients. Lu et al. (2004) have demonstrated an initial reduction in YLS numbers after *N. lugens* were switched between rice varieties (susceptible to resistant), followed by a gradual increase in symbiont numbers over successive generations of selective rearing on the resistant varieties. The activities of alanine transaminase and aspartic transaminase were also significantly reduced in aposymbiotic planthoppers during feeding and were different between planthopper populations selectively reared on distinct host plants, suggesting that symbionts had contributed in different ways to amino acid utilization efficiency on the different plant hosts (Lu et al. 2004).

In a more detailed study, and using the same rice varieties, Chen et al. (2011) found changes in amino acid composition of *N. lugens* (all body structures) after several generations of selective rearing, with total nitrogen content and the concentration of rare amino acids increasing, but some common amino acids decreasing over successive generations. These shifts in amino acid composition seemed to be influenced more by the selection process itself, rather than the host on which the hoppers were selectively reared or the host on which they had most recently fed (where hoppers were switched between hosts

before analyses). Examination of the hopper colonies during two generations of selection (the 8th and 11th) revealed that YLS improved nymphal performance in the 8th generation, but appeared to be a drain on nymphs in the 11th generation (as shown by the higher performance of the aposymbiotic nymphs than the symbiotic nymphs in the 11th generation)(Chen et al. 2011). In the study by Lu et al. (2004), hoppers had been reared in isolation for several generations to ensure that the host plants were resistant, whereas in the study by Chen et al. (2011), the selected colonies were already virulent against the same varieties. Furthermore, the study of Lu et al. (2004) was carried out during only four generations of selection. These differences will explain some of the differences between the two studies and indicate that the role of symbionts in mediating virulence could differ depending on the strength of rice resistance and the extent of planthopper exposure to the resistant variety or to varieties with similar resistance genes: YLS may mediate virulence adaptation in early generations of selection, with the planthoppers themselves ultimately adapting to the novel resistance after several generations—the role of the YLS eventually becoming reduced and possibly representing a cost to the insect after several generations of feeding on the same host variety. Tang et al. (2010) found that the composition and abundance of bacterial symbiont OTUs differed among populations of *N. lugens* reared on the same three varieties as in the Lu et al. (2004) and Chen et al. (2011) studies. Tang et al. (2010) suggest that bacterial symbionts may also mediate virulence adaptation and this may be associated with changes in bacterial community composition and function. However, their results can only be regarded as preliminary because the bacterial functions or the nature of the association between the bacteria and the hoppers (i.e., primary, secondary, internal, or external) have not been established.

Gaps in knowledge and improving research methodologies

Research into the role of symbionts in hopper virulence adaptation is still at an early stage. It has only been in the last 10 years that the nature of the bacterial symbiont community has been examined and that experimental studies have demonstrated a possible role for symbionts (YLS and bacteria) in mediating planthopper feeding on different rice varieties (Lu et al. 2004; Tang et al. 2010; Chen et al. 2011). The slow pace of discovery has been due to a poor understanding of each of the individual components in this complex and intricate three-way interaction. For example, there is a generally poor knowledge of the mechanisms underlying rice resistance against planthoppers

and leafhoppers: Whereas gene discovery has accelerated in recent decades, and some 79 resistance genes have been discovered, there is still a paucity of available information about what these genes actually do (Horgan 2009; Fujita et al. 2013) and often by the time materials are available for research, virulent hopper populations have already developed (Myint et al. 2009b; Fujita et al. 2013). Furthermore, it has been difficult to determine whether identified symbionts (especially bacteria) have a primary or secondary role in hopper survival or whether they are intra- or extracellular. Also the symbiont communities of leafhoppers have received very little attention generally. Further research is required. We make the following suggestions on how future research may be improved:

Better choice of host plants in selection studies

During the 1980s and early 1990s, at a time when few resistance genes had been identified, a series of studies examined the responses by planthoppers and leafhoppers to resistant donor varieties and compared resistant and susceptible varieties to determine probable mechanisms underlying rice resistance (Fig. 2). Much of that research used TN1 as a susceptible variety and Mudgo (*Bph1*) and ASD7 (*bph2*) as resistant varieties. Surprisingly, even though these varieties are known to be widely susceptible to planthoppers since the 1970s and 1980s, they have been continually used in the studies of virulence adaptation (Lu et al. 2004; Tang et al. 2010; Chen et al. 2011). Although switching of planthoppers between two susceptible varieties can give useful information (Chen et al. 2011), it would be useful, at least for agriculture, to compare hopper population responses (including symbiont changes) over successive generations on highly resistant and highly susceptible varieties. To overcome problems with highly dissimilar rice genomes in different varieties, which leads to a complex of resistance mechanisms and feeding barriers, researchers can use available near-isogenic rice lines (Fujita et al. 2010, 2013). Responses by planthoppers and symbionts to selection on such lines can then be better associated with specific resistance genes and their related resistance mechanisms.

Replication of colonies in selection studies

During the 1980s, *N. lugens* colonies selected on TN1, Mudgo, and ASD7 at the International Rice Research Institute (IRRI) were central to screening and breeding for rice resistance (Fujita et al. 2013). These colonies, designated as biotype 1, biotype 2, and biotype 3, were also later employed in the studies of symbionts (Lu et al. 2004; Tang et al. 2010). Unfortunately, the biotype concept, which has

been heavily criticized (e.g., Claridge and Den Hollander 1982, 1983), cannot be adapted to field populations, and indeed for *N. lugens*, it has only been related to unreplicated laboratory populations mainly kept at IRRI. Other selection studies, with different host plants, have been conducted; however, in general, these also did not replicate selected colonies (i.e., Hirae et al. 2007; Peñalver Cruz et al. 2011; but see Alam and Cohen 1998). Without replication of colonies, it is not possible to determine whether changes in planthopper anatomy, amino acid composition, or symbiont community composition are related to the host plant or result from directional selection and inbreeding. Future experiments must replicate selected colonies, preferably using hoppers collected from different locations (to more generally test hypotheses) as conducted by Alam and Cohen (1998).

Manipulation of symbiont communities

Although the selection of hoppers for several generations on a single variety constitutes a major investment to produce materials for virulence adaptation studies, reports of changes in the hoppers or their symbionts can remain too descriptive and ultimately suffer from the problems of cause and effect that are inherent to correlative studies (i.e., Tang et al. 2010). Some studies have used such materials for later manipulative experiments with symbionts: in particular, studies have compared symbiotic and aposymbiotic planthoppers for their responses to different rice lines (Lu et al. 2004; Chen et al. 2011). Unfortunately, the obligate nature of endosymbiotic microorganisms has made it difficult to employ other types of manipulation. Aposymbiotic planthoppers are normally produced by heat treatment (described above). This reduces YLS densities in the planthoppers, but has unknown effects on the composition or abundance of bacterial symbionts. It has been suggested that YLS are part of a complex of eukaryotic species (i.e., Dong et al. 2011), and heat treatment may selectively kill one or other species, thereby affecting not only the abundance but also the composition of the eukaryotic endosymbiont community. Care should be taken in seeking a mechanistic link between symbiont abundance and developmental abnormalities in planthopper eggs and nymphs since heat treatment may affect both the symbionts and the hoppers without any direct link between the two. For example, a recent report by Piyaphongkul et al. (2012) indicates that temperatures that were originally thought to affect YLS exclusively will also directly affect *N. lugens*: These authors found that the critical maximum temperature for *N. lugens* nymphs (from a Malaysian population) was 34.9 °C. Although they did not include symbionts in their study, their claims do highlight the difficulty in distinguishing the effects of heat shock from those of symbiont

reduction. Clearly, it is necessary to expand and improve the experimental methods available to researchers to allow the effective removal from planthoppers of “native” symbionts and inoculation with “novel” symbionts. Screening of antibiotics to remove symbionts and the development of techniques for micro-extraction and insertion of symbiont balls between planthopper eggs would be helpful for future research. However, antibiotics will be selective and are likely to change symbiont community composition and not just symbiont abundance making specific symbiont functions difficult to interpret.

Further attention to bacterial symbionts

There are larger knowledge gaps associated with bacterial symbionts compared to YLS. In particular, it is still unknown how planthoppers and leafhoppers become inoculated with bacterial symbionts and whether these symbionts have primary or secondary roles in hopper survival. The occurrence of bacteria in the ovaries of planthoppers and leafhoppers (Table 2) suggests that they may be passed through the egg—either on the egg surface or within the egg itself. However, bacteria have also been associated with planthopper salivary sheaths (Wang et al. 2008a; Tang et al. 2010) suggesting that relationships between rice plants, hoppers, and bacterial symbionts may be more dynamic than for YLS. If virulence-mediating bacteria could be picked up from plants directly (perhaps after infestation by virulent planthoppers), then adaptation might be very rapid. Metagenomics can be employed to assess the complexity of the bacterial endosymbiont community in hoppers. The metagenomic approach considers the hopper as a community in which genomes belonging to other organisms, including bacteria and fungi, might be present (Vega and Dowd 2005). For this purpose, meta-“omics” approaches such as metatranscriptomics, metaproteomics, and metabolomics will be useful in profiling microbial activity (Xu 2010). These technologies can also bypass the need for culturing symbiotic microorganisms as required when studying phylogeny and taxonomy. Because of the rapid pace of development of molecular tools available for the research on bacteria, gaps in understanding the nature of bacterial symbionts will likely diminish rapidly; however, the use of novel tools should be linked with proper experimental materials, methods, and manipulations as discussed above.

Concluding remarks

Throughout this review, we have suggested that interactions between rice plants, planthoppers or leafhoppers, and their symbionts are complex. The review indicates that

both symbionts and planthoppers are involved in virulence and virulence adaptation, and that their individual roles will likely depend on the nature of host plant resistance as well as the functions required by the hopper host vis-à-vis shifting from one susceptible variety to another or adapting to a widely available but highly resistant rice variety. Several process-related hypotheses remain to be tested: These include hypotheses aimed at two distinct levels of process—those that address the proximate mechanisms of adaptation, many of which are presented in Fig. 2, and those that address the ultimate mechanisms of adaptation (changes in symbiont communities, genetic mutation, or epigenetic shifts in symbionts or planthoppers). In terms of developing rice varieties and successfully deploying the varieties to reduce the rate of planthopper adaptation, both groups of hypotheses will be useful. Agriculture will need to consider the development of resistant varieties to better manage symbiont-mediated virulence adaptation. This will involve avoidance of host resistance that relies only on antibiosis (directed mainly against nymphs) and targeting not only the planthoppers and/or leafhoppers, but their endosymbiotic microorganisms as well. In general, our knowledge of the role of symbionts in the dynamics between planthoppers or leafhoppers and their rice hosts is still quite poor. However, future research in this area has the potential to significantly change our approach to developing and deploying resistant rice varieties.

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References

- Alam SN, Cohen MB (1998) Durability of brown planthopper, *Nilaparvata lugens*, resistance in rice variety IR64 in greenhouse selection studies. *Entomol Exp Appl* 89:71–78
- Bae SD, Song YH, Park YD (1987) Effects of temperature conditions on the growth and oviposition of brown planthopper, *Nilaparvata lugens* Stål. *J Plant Prot* 26:13–23
- Barbosa P, Krischik VA, Jones CG (eds) (1991) Microbial mediation of plant-herbivore interactions. John Wiley and Sons, Inc, USA, p 530
- Barr KL, Hearne LB, Briesacher S, Clark TL, Davis GE (2010) Microbial symbionts in insects influence down-regulation of defense genes in maize. *PLoS ONE* 596:e11339
- Bing L, Hongxia D, Maoxin Z, Di X, Jingshu W (2007) Potential resistance of tricin in rice against brown planthopper, *Nilaparvata lugens* (Stål). *Acta Ecologia Sinica* 27:1300–1307
- Botrell DG, Schoenly KG (2012) Resurrecting the ghost of green revolutions past: the brown planthopper as a recurring threat to

- high-yielding rice production in tropical Asia. *J Asia-Pacific Entomol* 15:122–140
- Catindig JLA, Arida GS, Baehaki SE, Bentur JS, Cuong LQ, Norowi M, Rattanakam W, Sriratanasak W, Xia J, Lu Z (2009) Situation of planthoppers in Asia. In: Heong KL, Hardy B (eds) *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*. International Rice Research Institute, Los Baños, pp 191–220
- Chaves S, Neto M, Tenreiro R (2009) Insect-symbiont systems: from complex relationship to biotechnological applications. *Biotechnol J* 4:1753–1765
- Chen YH (2009) Variation in planthopper-rice interactions: possible interactions among three species. In: Heong KL, Hardy B (eds) *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*. International Rice Research Institute, Los Baños (Philippines), pp 315–326
- Chen CC, Cheng LL, Hou RF (1981a) Studies on the intracellular yeast-like symbiote in the brown planthopper, *Nilaparvata lugens* Stål. II. Effects of antibiotics and elevated temperature on the symbiotes and their host. *Zeitschrift für Angewandte Entomologie* 92:440–449
- Chen CC, Cheng LL, Kuan CC, Hou RF (1981b) Studies on the intracellular yeast-like symbiote in the Brown Planthopper, *Nilaparvata lugens* Stål. I. Histological observations and population changes of the symbiote. *Zeitschrift für Angewandete Entomologie* 91:321–327
- Chen FJ, Zhang JF, Xia ZE, Lu ZX (2006) Morphological observations on the yeast-like endosymbionts in brown planthopper *Nilaparvata lugens*. *Acta Zootaxonomica Sinica* 31:728–735
- Chen YH, Bernal CC, Tan J, Horgan FG, Fitzgerald MA (2011) Planthopper “adaptation” to resistant rice varieties: changes in amino acid composition over time. *J Insect Physiol* 57:1375–1384
- Cheng D, Hou RF (1996) Ultrastructure of the yeast-like endocytobiont in the brown planthopper, *Nilaparvata lugens* (Stål)(Homoptera: Delphacidae). *Endocytobiosis Cell Res* 11:107–117
- Cheng DJ, Hou RF (2001) Histological observations on transovarial transmission of a yeast-like symbiote in *Nilaparvata lugens* Stål (Homoptera, Delphacidae). *Tissue Cell* 33:273–279
- Claridge MF, Den Hollander J (1982) Virulence to rice cultivars and selection for virulence in populations of the brown planthopper *Nilaparvata lugens*. *Entomol Exp Appl* 32:213–221
- Claridge MF, Den Hollander J (1983) The biotype concept and its application to insect pests of agriculture. *Crop Prot* 2:85–95
- Dillon RJ, Dillon VM (2004) The gut bacteria of insects: nonpathogenic interactions. *Annu Rev Entomol* 49:71–92
- Dong S, Pang K, Bai X, Yu X, Hao P (2011) Identification of two species of yeast-like symbiotes in the brown planthopper, *Nilaparvata lugens*. *Curr Microbiol* 62:1133–1138
- Douglas AE (1989) Mycetocyte symbiosis in insects. *Biol Rev* 64:409–434
- Douglas AE (1992) Microbial brokers of insect-plant interactions. In: *Proceedings of the 8th international symposium on insect-plant relationships series entomologica*, vol 49. pp 329–336
- Dowd PF (1989) In situ production of hydrolytic detoxifying enzymes by symbiotic yeasts in the cigarette beetle (Coleoptera: Anobiidae). *J Econ Entomol* 82:396–400
- Dowd PF (1992) Insect fungal symbionts: a promising source of detoxifying enzymes. *J Ind Microbiol* 9:149–161
- Dowd PF, Shen SK (1990) The contribution of symbiotic yeast to toxin resistance of the cigarette beetle (*Lasioderma serricorne*). *Entomol Exp Appl* 56:241–248
- Eya BK, Kenny PTM, Tamura SY, Ohnishi M, Naya Y, Nakanishi K, Sugiura M (1989) Chemical association in symbiosis sterol donors in planthoppers. *J Chem Ecol* 15:373–380
- Fujita D, Yoshimura A, Yasui H (2010) Development of near-isogenic lines and pyramided lines carrying resistance genes to green rice leafhopper (*Nephotettix cincticeps* Uhler) with the Taichung 65 genetic background in rice (*Oryza sativa* L.). *Breed Sci* 60:18–27
- Fujita D, Kohli A, Horgan FG (2013) Rice resistance to planthoppers and leafhoppers. *Crit Rev Plant Sci* 32:162–191
- Ganter PF (2006) Yeast and invertebrate associations. In: Rosa CA, Peter G (eds) *Biodiversity and ecophysiology of yeasts (The yeast handbook)*. Springer, Heidelberg, pp 303–370
- Hao P, Liu C, Wang Y, Chen R, Tang M, Du B, Zhu L, He G (2008) Herbivore-induced callose deposition on the sieve plates of rice: an important mechanism of host resistance. *Plant Physiol* 146:1810–1820
- Hirae M, Fukuta Y, Tamura K, Oya S (2007) Artificial selection of biotypes of green rice leafhopper, *Nephotettix cincticeps* Uhler (Homoptera: Cicadellidae), and virulence to resistant rice varieties. *Appl Entomol Zool* 42:97–107
- Hongoh Y, Ishikawa H (1997) Uric acid as a nitrogen resource for the brown planthopper, *Nilaparvata lugens*: studies with synthetic diets and aposymbiotic insects. *Zoolog Sci* 14:581–586
- Horgan FG (2009) Mechanisms of resistance: a major gap in understanding planthopper-rice interactions. In: Heong KL, Hardy B (eds) *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*. International Rice Research Institute, Los Baños (Philippines), pp 281–302
- Horgan FG (2012) Diversity and defence: plant-herbivore interactions at multiple scales and trophic levels. In: Gurr GM, Wratten SD, Snyder WE, Read DMY (eds) *Biodiversity and insect pests: key issues for sustainable management*. Wiley-Blackwell, Oxford, pp 241–258
- Hosokawa T, Kikuchi Y, Fukatsu T (2007) How many symbionts are provided by mothers, acquired by offspring, and needed for successful vertical transmission in an obligate insect-bacterium mutualism? *Mol Ecol* 16:5316–5325
- Hou RF (2008) Symbiosis between planthoppers and microorganisms. In: Capinera J (ed) *Encyclopedia of entomology*, 2nd edn. Springer, Dordrecht, The Netherlands, pp 3657–3660
- Hou Y, Ma Z, Dong S, Chen YH, Xiaoping Y (2013) Analysis of yeast-like symbiote diversity in the brown planthopper (BPH), *Nilaparvata lugens* Stål, using a novel nested PCR-DGGE protocol. *Curr Microbiol* 67:263–270
- Ishikawa H (2003) Insect symbiosis: an introduction. In: Bourtzis K, Miller TA (eds) *Insect symbiosis*. CRC, Boca Raton, FL, pp 1–21
- Jones CG (1984) Microorganisms as mediators of plant resource exploitation by insect herbivores. In: Price PW, Slobodchikoff CN, Gaud WS (eds) *A new ecology. Novel approaches to interactive systems*. John Wiley and Sons, Inc, New York, pp 53–99
- Jung JK, Im DJ (2005) Feeding inhibition of the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae), on a resistant rice variety. *J Asia-Pacific Entomol* 8:301–308
- Kagayama K, Shiragami N, Nagamine T, Umehara T, Mitsui T (1993) Isolation and classification of intracellular symbiotes from the rice brown planthopper, *Nilaparvata lugens*, based on analysis of 18S-ribosomal DNA. *J Pestic Sci* 18:231–237
- Karban R, Agrawal AA (2002) Herbivore offense. *Annu Rev Ecol Syst* 33:641–664
- Kikuchi Y, Hayatsu M, Hosokawa T, Nagayama A, Tago K, Fukatsu T (2012) Symbiont-mediated insecticide resistance. *Proc Natl Acad Sci* 109:8618–8622
- Kiyonaga T, Watanabe T, Miyamoto K, Suzuki Y (1997) Varietal differences in the brown planthopper egg mortality caused by antibiotic response of rice plants. *Kyushu Agric Res* 59:75 (In Japanese.)
- Koyama K (1985) Nutritional physiology of the brown rice planthopper, *Nilaparvata lugens* Stål (Hemiptera:Delphacidae).

- II. Essential amino acids for nymphal development. *Appl Entomol Zool* 20:424–430
- Koyama K (1986) Nutritional physiology of the brown rice planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). II. Essential amino acids for nymphal development. *Appl Entomol Zool* 20:424–430
- Latif MA, Guan TS, Yusoh OM, Siraj SS (2008) Evidence of sibling species in the brown planthopper complex (*Nilaparvata lugens*) detected from short and long primer random amplified polymorphic DNA fingerprints. *Biochem Genet* 46:520–537
- Latif MA, Rafii MY, Mazid MS, Ali ME, Ahmed F, Omar MY, Tan SG (2012) Genetic dissection of sympatric populations of brown planthopper, *Nilaparvata lugens* (Stål), using DALP-PCR molecular markers. *Sci World J* 2012:586831
- Lee YH, Hou RF (1987) Physiological roles of a yeast-like symbiote in reproduction and embryonic development of the brown planthopper, *Nilaparvata lugens* Stål. *J Insect Physiol* 33:851–860
- Lu Z, Yu X, Chen J, Zheng X, Xu H, Zhang J, Chen L (2004) Dynamics of yeast-like symbiote and its relationship with the virulence of brown planthopper, *Nilaparvata lugens* Stål, to resistant rice varieties. *J Asia-Pacific Entomol* 7:317–323
- Mitsuhashi J, Kono Y (1975) Intercellular microorganisms in green rice leafhopper, *Nephotettix cincticeps* Uhler (Hemiptera; Deltocephalidae). *Appl Entomol Zool* 10:1–9
- Myint KKM, Matsumura M, Takagi M, Yasui H (2009a) Demographic parameters of long-term laboratory strains of the brown planthopper, *Nilaparvata lugens* Stål (Homoptera: Delphacidae) on resistance genes *bph20(t)* and *Bph21(t)* in rice. *J Fac Agric Kyushu Univ* 54:159–164
- Myint KKM, Yasui H, Takagi M, Matsumura M (2009b) Virulence of long-term laboratory populations of the brown planthopper, *Nilaparvata lugens* (Stål), and whitebacked planthopper, *Sogatella furcifera* (Horvath; Homoptera: Delphacidae), on rice differential varieties. *Entomol Exp Appl* 44:149–153
- Nagamine T, Miyake T, Matsumoto S, Mitsui T (1993) Intracellular yeast-like symbiotes of a brown planthopper, *Nilaparvata lugens*. *Riken Rev* 3:31–32
- Nasu S (1963) Studies on some leafhoppers and planthoppers which transmit virus disease of rice plant in Japan. *Bull Kyushu Agric Exp Stn* 8:153–349
- Nasu S (1965) Studies on some leafhoppers and planthoppers which transmit virus diseases of rice plant in Japan. *Bull Kyushu Agric Exp Stn* 3:13–349
- Noda H (1974) Preliminary histological observations and population dynamics of intracellular yeast-like symbiotes in the smaller brown planthopper, *Laodelphax striatellus* (Homoptera: Delphacidae). *Appl Entomol Zool* 9:275–277
- Noda H (1977) Histological and histochemical observation of intracellular yeastlike symbiotes in the fat body of the smaller brown planthopper, *Laodelphax striatellus* (Homoptera: Delphacidae). *Appl Entomol Zool* 12:134–141
- Noda H, Omura T (1992) Purification of yeast-like symbiotes of planthoppers. *J Invertebr Pathol* 59:104–105
- Noda H, Saito T (1979) The role of intracellular yeast-like symbiotes in the development of *Laodelphax striatellus*. *Appl Entomol Zool* 14:453–458
- Noda H, Wada K, Saito T (1979) Sterols in *Laodelphax striatellus* with special reference to the intracellular yeastlike symbiotes as a sterol source. *J Insect Physiol* 25:443–447
- Noda H, Nakashima N, Koizumi M (1995) Phylogenetic position of yeast-like symbiotes of rice planthoppers based on partial 18S rDNA sequences. *Insect Biochem Mol Biol* 25:639–646
- Noda H, Watanabe K, Kawai S, Yukuhiro F, Miyoshi T, Tomizawa M, Koizumi Y, Nikoh N, Fukatsu T (2012) Bacteriome-associated endosymbionts of the green rice leafhopper *Nephotettix cincticeps* (Hemiptera: Cicadellidae). *Appl Entomol Zool* 47:217–225
- Padgham DE (1983) The influence of the host-plant on the development of the adult brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), and its significance in migration. *Bull Entomol Res* 73:117–128
- Pang K, Dong S, Hou Y, Bian Y, Yang K, Yu X (2012) Cultivation, identification and quantification of one species of yeast-like symbiote, *Candida*, in the rice brown planthopper, *Nilaparvata lugens*. *Insect Sci* 19:477–484
- Pathak MD, Kalode MB (1980) Influence of nutrient chemicals on the feeding behaviour of the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). Mimeographed manuscript. IRRI
- Peñalver Cruz A, Arida A, Heong KL, Horgan FG (2011) Aspects of brown planthopper adaptation to resistant rice varieties with the *Bph3* gene. *Entomol Exp Appl* 141:245–257
- Pieterse CMJ, Dicke M (2007) Plant interactions with microbes and insects: from molecular mechanisms to ecology. *Trends Plant Sci* 12:564–569
- Piyaphongkul J, Pritchard J, Bale J (2012) Can tropical insects stand the heat? A case study with the brown planthopper *Nilaparvata lugens* (Stål). *PLoS ONE* 7:e29409
- Sasaki T, Kawamura M, Ishikawa H (1996) Nitrogen recycling in the brown planthopper, *Nilaparvata lugens*: involvement of yeast-like endosymbionts in uric acid metabolism. *J Insect Physiol* 42:125–129
- Saxena RC, Okech SH (1985) Role of plant volatiles in resistance of selected rice varieties to brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). *J Chem Ecol* 11:1601–1616
- Seino Y, Suzuki Y, Sogawa K (1996) An ovicidal substance produced by rice plants in response to oviposition by the whitebacked planthopper, *Sogatella furcifera* (Horvath) (Homoptera: Delphacidae). *Appl Entomol Zool* 31:467–473
- Shigematsu Y, Murofushi N, Ito K, Kaneda C, Kawabe S, Takahashi N (1982) Sterols and asparagine in the rice plant, endogenous factors related to resistance against the brown planthopper (*Nilaparvata lugens*). *Agric Biol Chem* 46:2877–2879
- Sogawa K (1991) Super-susceptibility to the white-backed planthopper in japonica-indica hybrid rice. *Kyushu Agric Res* 53:92 (In Japanese)
- Sogawa K, Pathak MD (1970) Mechanism of brown planthopper resistance in Mudgo variety of rice (Hemiptera: Delphacid). *Appl Entomol Zool* 5:145–158
- Stevenson PC, Kimmins FM, Grayer RJ, Raveendranath S (1996) Schaftosides from rice phloem as feeding inhibitors and resistance factors to brown planthopper, *Nilaparvata lugens*. *Entomol Exp Appl* 80:246–249
- Suzuki Y, Sogawa K, Seino Y (1996) Ovicidal reaction of rice plants against the whitebacked planthopper *Sogatella furcifera* Horvath (Homoptera: Delphacidae). *Appl Entomol Zool* 31:111–118
- Tanaka K, Matsumura M (2000) Development of virulence to resistant rice varieties in the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae), immigrating into Japan. *Appl Entomol Zool* 35:529–533
- Tang M, Lv L, Jing S, Zhu L, He G (2010) Bacterial symbionts of the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae). *Appl Environ Microbiol* 76:1740–1745
- Thanysiriwat T, Pattwatang P, Angeles ER (2009) New biotypes of brown planthopper in Thailand. In: Proceedings of the rice and temperate cereal crops annual conference 2009, Bangkok, Thailand, pp 386–389
- Vega FE, Dowd PF (2005) The role of yeasts as insect endosymbionts. In: Vega FE, Blackwell M (eds) *Insect-fungal associations: ecology and evolution*. Oxford University Press, New York, USA, pp 211–243

- Verma SK, Pathak PK, Singh BN, Lal MN (1979) Indian biotypes of the brown planthopper. *Int Rice Res Newsl* 4:6
- Wang X, Zhou G, Xiang CY, Du MH, Cheng JA, Luis SS, Lou YG (2008a) β -glucosidase treatment and infestation by the rice brown planthopper, *Nilaparvata lugens*, elicit similar signaling pathways in rice plants. *Chin Sci Bull* 53:53–57
- Wang Y, Tang M, Hao P, Yang Z, Zhu L, He G (2008b) Penetration into rice tissues by brown planthopper and fine structure of the salivary sheaths. *Entomol Exp Appl* 129:295–307
- Wang Y, Wang X, Yuan H, Chen R, Zhu L, He R, He G (2008c) Responses of two contrasting genotypes of rice to brown planthopper. *Mol Plant-Microbe Interact* 21:122–132
- Wang WX, Luo J, Lai FX, Fu Q (2010) Identification and phylogenetic analysis of symbiotic bacteria *Arsenophonus* from the rice brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera:Delphacidae). *Acta Entomologia Sinica* 53(6):647–654
- Weng Q, Huang Z, Wang X, Zhu L, He G (2003) In situ localization of proteinase inhibitor mRNA in rice plant challenged by brown planthopper. *Chin Sci Bull* 48:979–982
- Wetzel JM, Ohnishi M, Fujita T, Nakanishi K, Naya Y, Noda H, Sugiura M (1992) Diversity in steroidogenesis of symbiotic microorganisms from planthoppers. *J Chem Ecol* 18:2083–2094
- Wilkinson TL, Ishikawa H (2001) On the functional significance of symbiotic microorganisms in the Homoptera: a comparative study of *Acyrtosiphon pisum* and *Nilaparvata lugens*. *Physiol Entomol* 26:86–93
- Woodhead S, Padgham DE (1988) The effect of plant surface characteristics on resistance of rice to the brown planthopper, *Nilaparvata lugens*. *Entomol Exp Appl* 47:15–22
- Xet-Mull AM, Quesada T, Espinosa AM (2004) Phylogenetic position of the yeast-like symbiotes of *Tagosodes orizicolus* (Homoptera: Delphacidae) based on 18S ribosomal DNA partial sequences. *Revista de Biología Tropical* 52:777–785
- Xu J (2010) Metagenomics and ecosystems biology: conceptual frameworks, tools and methods. In: Marco D (ed) *Metagenomics: theory, methods and applications*. Caister Academic Press, Norfolk, pp 1–14
- Xu XF, Mei HW, Luo LJ, Cheng XN, Li ZK (2002) RFLP-facilitated investigation of the quantitative resistance of rice to brown planthopper (*Nilaparvata lugens*). *Theor Appl Genet* 104:248–253
- Yamasaki M, Yoshimura A, Yasui H (2000) Mapping of quantitative trait loci of ovicidal response to brown planthopper (*Nilaparvata lugens* Stål) in rice (*Oryza sativa* L.). *Breed Sci* 50:291–296
- Yang Z, Zhang F, Zhu L, He G (2006) Identification of differentially expressed genes in brown planthopper *Nilaparvata lugens* (Hemiptera: Delphacidae) responding to host plant resistance. *Bull Entomol Res* 96:53–59
- Yin JL, Xu HW, Wu JC, Hu JH, Yang GQ (2008) Cultivar and insecticide applications affect the physiological development of the brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae). *Environ Entomol* 37:206–212
- Yoshihara T, Sogawa K, Pathak MD, Juliano BO, Sakamura S (1980) Oxalic acid as sucking inhibitor of the brown planthopper in rice (Delphacidae, Homoptera). *Entomol Exp Appl* 27:149–155
- Zeigler RS, Pantoja A, Duque MC, Weber G (1994) Characteristics of resistance in rice to Hoja Blanca Virus (VHB) and its vector. *Ann Appl Biol* 124:429–440
- Zhang F, Zhu L, He G (2004) Differential gene expression in response to brown planthopper feeding in rice. *J Plant Physiol* 161:53–62
- Zhang J, Wu H, Chen J, Zheng X, Chen L, Yu X (2007) A strain isolated from brown planthopper and its molecular identification. *Chin J Rice Sci* 21:551–554
- Zhang J, Chen J, Chen F, Zheng X, Chen L, Yu X (2009) The isolation of yeast-like symbiotes in the brown planthopper and the sequences analysis of its 26S rDNA. *Scientia Agricultura Sinica* 42:2211–2216
- Zhongxian L, Xiaoping Y, Jianming C, Xusong Z, Hongxing X (2001) Effects of endosymbiote on feeding, development and reproduction of brown planthopper, *Nilaparvata lugens*. *Chin Rice Res Newsl* 9:11–12