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Biosystematics of rice brown planthopper and rice green leafhoppers

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Abstract. The Brown planthopper, *Nilaparvata lugens* (Stal) is causing serious damage to rice cultivation in tropical countries of Asia for the last 10–15 years. Green leafhoppers, *Nephotettix* spp. are also assuming serious proportions in different parts. This has been attributed to improved rice production technology, especially with the introduction of high yielding varieties. Damage is not only through loss of sap as a result of feeding but also by transmitting diseases in this process. Various reasons have been attributed like breaking of resistance, migration, insecticidal treatment etc. One such reason, which led to the biosystematic studies of these pests is the assumption that this is due to evolution of new forms referred to as biotypes. Of late biotypes have also been reported in *Nephotettix virescens.* This term is basically used with reference to differential ability of the insect to infest particular rice cultivars having resistant genes. This phenomena is attributed by some scientists, not to the breaking of resistance, but to the evolution of new forms which are different from the original population. As per the conventional taxonomy, this new form is similar to the original form, and hence biosystematists attempted various methods like chemical and honeydew analyses, cytology, acoustic behaviour, morphometrics, etc in order to help the breeders in differentiating and identifying these and also to find out the mechanism and causes evolving such biotypes.

Keywords. BPH, GLH biosystematics.

1. Rice brown planthopper

The rice brown planthopper (BPH) belongs to family Delphacidae of Homoptera which can easily be distinguished from all other families of the suborder by the presence of mobile spur at apex of post tibia. The genus *Nilaparvata* can be distinguished from other genera of the family by the presence of one or more lateral spines on the hind basitarsus. So far 14 species (Mochida and Okada 1979) are recorded throughout the world, out of which only *N. lugens* has been recorded as a pest which causes serious damages to rice cultivation both by sucking sap from the plant and also by transmitting plant diseases like grassy stunt and ragged stunt. Species is characterised by its typical male genitalia, especially styles and aedeagus and by lateral lobes of female ovipositor.

BPH is widely distributed from Pakistan to Japan and many islands of Southeast Asia (Claridges 1985). BPH existed in most of these countries since ancient times referred under different synonyms (Paik 1977). Since early part of this century frequent outbreaks of this pest have been reported from some of these countries and from early seventies situation became quite alarming. Dyck and Thomas (1979) in a conservative estimate reported US $ 300 million loss in paddy due to BPH and grassy stunt disease transmitted by it.

Intensive research on all possible aspects of BPH is in progress so that suitable pest management strategy could be evolved to combat this menace. Until recently resistant rice varieties and insecticides were satisfactory means of controlling these insects. But soon it was found that varieties resistant in one country were susceptible in another. Also the varieties resistant earlier became susceptible after sometime in the same country. This was attributed to various factors like breaking of resistance, overcoming of resistance, migration, insecticidal treatments or to the existence or evolution of different biotypes in different areas. This term biotype in planthoppers and leafhoppers though initially was used with reference to differential ability of the insect to infest particular rice cultivar having resistant genes, various other methods were attempted to find out the differences which could be used to distinguish and identify these and also to see if these could provide some clue to evolution of such biotypes. Such studies include acoustic behaviour, mate choice experiments, honeydew analysis, morphometry etc.

1.1 *Honeydew studies*

As already stated biotypes have been recognised based on the differential virulence to the resistant varieties. Virulence is taken as ability of the insect to infest and damage the plant and damage is correlated with insect feeding. Further, amount of feeding, weight gained and quantity of honeydew excreted are found to be highly correlated. This, therefore, led to honeydew studies to find out its utility in identification or confirmation of the biotypes.

Initially honeydew studies at IRRI (1978) were taken up to determine its value in measuring levels of resistance. Both quantitative and qualitative analyses were done. Former by measuring ninhydrin stained areas of honeydew on filter paper and also by measuring volume of honeydew. Latter by densitometer measurements of honeydew colour intensity and by measurements of colour intensity of amino acids separated through paper chromatography.

The filter paper technique for measuring honeydew excretion was found helpful in identifying biotypes. TN 1 which has no resistance gene and, therefore, is susceptible to all biotypes has larger areas in all the 3 biotypes and shows no significant differences. Whereas Mudgo which is resistant to biotype 3 but susceptible to biotype 2 shows significant difference between the areas of two biotypes; biotype 2 having larger area than the biotype 3. Similarly ASD 7 which is resistant to biotype 2 but susceptible to biotype 3, has larger area in case of biotype 3 and significantly smaller area in biotype 2.

Similarly, study of honeydew volume at IRRI also showed distinct differences in honeydew excretion by both biotypes feeding on susceptible and resistant varieties. Biotype 3 feeding on IR 40 excreted significantly more honeydew than those feeding on IR 42.

But according to Sogawa (1977), a biotype of BPH excretes as much honeydew even on certain resistant varieties as it does on susceptible ones and suggested that gustatory blockage of feeding is the principal cause of varietal resistance of rice to this insect. At the same time intraspecific variation in gustatory responses in BPH is considered to be a possible reason for occurrence of the biotypes which can feed on resistant varieties.

Both honeydew areas on filter paper and honeydew volume techniques are practical methods but need readily available materials and could be used by scientists anywhere for determining feeding activity which in turn can reflect biotypic nature of population, if any.

Tests with colour intensity of honeydew which was measured with densitometer after treating it with ninhydrin for amino acids or aniline hydrochloride for sugars show some promise for detecting and differentiating biotypes. The peak was higher in biotype 3 on ASD 7 to which it is susceptible whereas peak for biotype 2 is much lower to which ASD 7 is resistant. Similarly on Mudgo, peak for biotype 2 is higher to which it is susceptible while for biotype 3 is much lower to which it is resistant.

Further study of honeydew by measuring the colour intensity of amino acids of honeydew, which are separated through paper chromatography holds promise for identification of biotypes by using standard differential varieties. Works at IRRI (1978) showed that susceptible varieties have more number of amino acids with high colour intensity than the resistant varieties.

However, honeydew studies by Claridge and Hollander (1980) on 3 inbred biotype cultures of *N. lugens* from Philippines found wide range of variation within each biotype and large overlap between them. Means for each biotype on 3 test cultivars—TN 1, Mudgo and ASD 7 were significantly different from each other but means for these 3 biotypes on the same cultivar were not significantly different amongst themselves. Each biotype population had individuals which could be attributed to other biotypes. Honeydew studies conducted on randomly collected field samples showed that these are mixtures of individuals which could be attributed to biotypes 1, 2 and 3. Based on these findings showing variability and overlap of biotype populations they felt the use of biotype in this insect is misleading. Besides, their studies on morphology, cytology, biochemistry and acoustic behaviour of these Philippine biotypes did not show any significant differences. They concluded that sympatric biotypes of Philippines are simple genetic variants and therefore, suggested that use of term biotype masks the inherent variability of the population.

1.2 *Acoustic behaviour*

In forties, Ossiannilsson's (1949) pioneering work on sound production in some Auchenorrhyncha showed that besides loud and audible sounds produced by Cicadas, smaller Auchenorrhyncha like leafhoppers and planthoppers also produce species-specific sounds which are faint and inaudible. Since then, especially during the last decade extensive research was carried out to find out all aspects of these sounds such as mechanisms, structures involved in production and perception, nature of sound, its role in behaviour, speciation, etc. Use of tape recorders and electronic equipments for sound analysis have facilitated this line of research enormously. Since the most distinctive sounds are produced in behavioural context of pair formation most of the studies have been conducted on this aspect.

According to Ichikawa (1977) females of *N. lugens* have no tymbal or special sound producing organ and sound production is accompanied by obvious dorso-ventral vibration of the abdomen. In males 1st and 2nd abdominal terga are specialised as tymbal organs. He stated that the sound produced though faint was within audio-frequency range, but the planthopper never responded in mating behaviour even from 1 to 2 cm distance. Various other experiments conducted by him, indicate that the stimuli involved in the mating behaviour were neither auditory nor visual nor olfactory. It was concluded that planthoppers transmit vibratory signals directly to solid substrate on which they lived and that they perceived these signals by some vibration receptors. And therefore, he felt that physical properties of the substrate were likely to affect manner of transmission. According to Claridge (1985) abdominal vibration normally does not result in contact with the substrate. The vibrations are transferred to the plant through the legs and perhaps sometimes also through the feeding stylets which may be inserted during calling. According to him any of the variety of internal chordotonal sensilla in different parts of body might serve as vibration receptors.

According to Ichikawa (1977) and Claridge (1985) both sexes of *N. lugens* produce characteristic signals with distinctive pattern and grouping of pulses. Female calls are generally simpler than those of males of the same species and often consists of regularly repeated pulses. Sexually mature male and often also female call spontaneously on their food plants. Responsive insects reply by emitting their own signals and acoustic exchanges usually end with a male making contact with female.

Studies by Claridge *et al* (1987) on calling sounds of male and female of *N. lugens* on two different hosts in Philippines show that the female call on rice consists of sequences of regularly repeated pulses, whereas from weed grass, *Leersia hexandra* it produces similar calls but of less than a third pulse repitition frequency (PRF) of rice plant. This PRF was found very important for conspecific responses. They observed that these planthoppers associated with two different hosts i.e. rice and *L. hexandra,* both males and females responded to the recorded calls of the opposite sex of their own host population very significantly, more than they did to those of the other. However hybrids could be obtained in no choice experiment. Based on these PRF differences Claridge *et al* (1985) have taken them as two closely related sibling species with no constant morphological difference between them.

However, in view of Ichikawa's (1977) observation on the effect of physical properties of the substrate on transmission, it is not clear if calls were recorded on each other's hosts and if any differences were found.

The same team of workers (Claridge *et al* 1985) while studying the geographical variations in male calls of *N. lugens* showed that populations from Solomon Islands differed significantly in mean PRF from the populations of Australia. This difference was thought to be responsible for behavioural incompatibilities observed during hybridization experiments between these two populations. A series of crosses were made between the two populations and male calls from successful crosses were recorded. PRF of these calls were significantly nearer to male calls of the other population and lower or higher than the mean of their own populations. This again shows the importance of PRF in communication.

Role of acoustic signals of planthoppers and leafhoppers in speciation was argued by Claridge (1985) based on acoustic studies of *N. lugens.* For evolution of species, according to most of the scientists isolation mechanism is a must. Acoustic signals being species-specific and since they could be transmitted only through substrate lead in premating isolation both call-wise as well as host-wise. His work as already explained earlier showed that morphologically sibling species from the same geographical areas but having different hosts have significantly different PRF means. And the hybrids produced between these, under no choice experiment had intermediate PRF which shows the hereditory nature of the character.

Claridge *et al* (1985) also studied the PRF of *N. lugens* from widely scattered localities under standard conditions. Each was characterised by particular range of variations, some significantly different from others e.g. Australian population. Hybridization is difficult in populations that differed most in male PRFs. And therefore, calling sounds help in maintaining geographical isolations. But whenever hybrids are obtained between such divergent populations they are completely normal, fertile, produce F1 and F2 generations with no obvious anomalies. From this he concluded that though they are different in call characteristic, genetically they are very closely related and differentiation of calls occurs in advance of genetic differentiation.

1.3 *Mate choice experiments*

Mate choice experiments conducted by Claridge and Hollander (1980) on 3 biotypes of Philippines also gave the same results as honeydew tests. They were found to be variants of one freely interbreeding biological species. They were not clearly separable and different from each other. They felt that since in *N. lugens* inheritance of virulence was of polygenic nature, field populations normally varied in virulence and evolved in response to a particular rice cultivars in a particular area and therefore, same biotype occurring in widely distributed areas was not possible.

1.4 *Morphology*

Biotypes of *N. lugens* were found to be identical morphologically. However, the idea of differentiating biotypes through numerical taxonomy with morphometrical accuracy was considered possible by Okada (1977). Accordingly Sogawa (1978) studied quantitative morphological variations but found that except for the significant variations in frequency distribution of the number of spines on the hind basitarsus, Philippine biotypes show no significant differences.

Similar morphometrical studies were conducted by Bhattacharyya *et al* (1983) on macropterous forms of Pantnagar and Hyderabad populations. Little microtypic numerical variations recorded on some characters were unable to separate these two populations morphologically.

2. Rice green leafhoppers

Nephotettix virescens (Distant) and *N. nigropictus* (Stal) belong to the family Cicadellidae of the order Hemiptera, whose members can be distinguished by elongate hindlegs and tibiae having rows of spines all along the length. The genus can be distinguished from other genera by its characteristic green and black colour markings, shape of head and male genitalia. Both the species are found all over India and are widely distributed in other parts of Southeast Asia. Both nymphs and adults cause damage by sucking the sap from leaves and stems. Besides, they cause serious damage indirectly by transmitting the causal agents of various rice diseases. Out of these Tungro (a virus disease) and yellow dwarf (a mycoplasma disease) occur in India.

2.1 *Nephotettix spp.—Biotype*

Recently biotypes have been reported in *Nephotettix* species based on their differential infestation and differential tungro transmission ability to various rice cultivars at different places. Karim and Pathak (1979) have reported Bangladesh biotype and Philippines biotypes of *N. virescens* based on the resistant genes in the rice cultivars. Ling *et al* (1981) tested 10 rice varieties at 4 centres, Rajendranagar and Cuttack in India, one in Philippines and another in Indonesia for possible biotypes of *N. virescens* based on life-span and Tungro transmission ability and reported that rice varieties Ambemohar 159 at Rajendranagar and Pankhari 203 at Cuttack could be used as differentials for biotypes. Thus problem of biotypes in *Nephotettix* is at an early stage and populations from different places are likely to be taken as biotypes in future depending on their characteristics.

Ramakrishnan (1983) studied morphometrics of various populations of *N. virescens* and *N. nigropictus* collected from different agroclimatic zones to find out whether such studies can be used for differentiating these populations. These studies showed that the morphometry could be used to distinguish these populations and it was suggested that such studies should be extended to the already designated biotype populations. In biotypes, besides varying agroclimatic factors rice cultivars are also likely to play their role on the size of insect depending on susceptibility or resistance and therefore, such studies should be more useful in distinguishing and detecting the existence of biotype populations. While 16 characters were chosen for study, minimum and the most important of these had to be selected after attempting such studies on some more populations and by applying suitable statistical analysis.

Yusof (1982) studied calling sounds of *N. virescens* and *N. nigropictus* populations from different parts of Asia which showed variations. This shows the utility of these calls in biotype studies of these species.

Studies on honeydew in green leafhopper (GLH) conducted at IRRI (1978) were aimed at finding out different resistance levels in IR rice cultivars and since the results showed differences in densitometer readings of honeydew, they also show promise in biotype studies.

2.2 *Nephotettix spp.—Hybrids*

In *Nephotettix* species, besides biotypes there is another problem. Both *virescens* and *nigropictus* are widely distributed and are sympatric species. Though they are usually clearly separable on the basis of colour patterns, in fields sometimes morphologically intermediate individuals are found. Hybrids of these two species were produced in the laboratory which showed intermediate morphological characters (Ling 1968; Inoue 1983).

2.2a *Hybrids: Morphology*: Ramakrishnan and Ghauri (1978) studied male genitalic characters of spines on pygofer and aedeagus in the field collected individuals showing intermediate colour markings. None of these specimens had the combination that is usual in either of the species. This has led to the assumption that such specimens are hybrids between *virescens* and *nigropictus* resulting from crosses in nature.

Subsequently Ramakrishnan (1983) studied these spine characters in two populations of *N. virescens* and 3 populations of *N. nigropictus* collected from widely separated geographical areas. Specimens only with clearcut colour markings and other external characters typical of *virescens* and *nigropictus* were selected for the purpose. No specimen showing intermediate form or colour markings was included for the observations. In spite of such careful selection of specimens for studies, much variation was observed in the number of spines on aedeagus and pygofer. It was, therefore, concluded that variations in these characters are natural, intraspecific and not because of hybridization. Study of these characters in the pure bred material were suggested for the further verification of this observation.

Accordingly in 1986, aedeagal spines were studied in two samples of pure bred material of *N. virescens*. The spines in one sample varied from 2–3 to 5–5, while in another these varied from 3–4 to 5–5 whereas parents in both the cases had only 4–4. This confirms the earlier observations that spines variation on the aedeagus in *Nephotettix* species is intraspecific and not due to hybridization.

2.2b *Hybrids: Acoustic calls*: The above morphological observations have been further corroborated by various works on calling sounds of these species.

Acoustic sounds in GLH were first demonstrated by Ichikawa (1976, 1979) from the abdominal vibration in both male and female and he clarified that these are essential stimuli to each other before copulation.

Inoue (1983) studied acoustic signals of 4 species of *Nephotettix* which included *virescens* and *nigropictus*. Both male and female calling sounds showed that they were species-specific. He observed no communication between male and female of two different species and found that by their species-specific nature were concerned in maintenance of reproductive isolation among species. He found no identical or confusingly similar sound wave among these species studied.

Inoue also tried number of interspecific crosses and tested hybrids from successful crosses for morphological and acoustic properties. Hybrid call study was aimed at finding out the effectiveness in discriminating the mating partner. Response between the hybrid male and female derived from the same cross was clear but between the hybrid and their parental species they displayed various responses being from poor to clear.

Hybrid calls though distinct from parent calls were not distinctive of hybrids. They were irregular order of connections of elements from parents.

Yusof (1982) studied calling sounds of males and females of both the species *virescens* and *nigropictus* and their hybrids produced in the laboratory and also of the intermediate individuals collected from fields. Both male and female calls show clear differences between the two species. Calls of F1 hybrids were very variable and different from either parents though most individual hybiids showed some recognizable elements from the calls of both the parental species.

Calls of field collected intermediates were found to be either of *N. virescens* or of *N. nigropictus* type but not of hybrids. This again strengthens the view that hybridisation does not occur in nature.

Most of the works mentioned here were an attempt in identifying and differentiating biotypes as defined by breeders. Some did show promise in this direction, and showed differences in different biotypes while others gave contradictory results. This may be due to evolution of new form from the originally declared biotype populations. Because confirmatory results were obtained in 1977–78, while contradictory results came from 1980 specimens. It is likely that the so called biotype populations have undergone changes by then. Thus though these various methods like acoustic calls, honeydew analyses, mate choice experiments and morphometrics are complementary to each other and might help in differentiating the already designated biotypes or identifying the new ones, these findings will be of help only for a short time, the biotype being a changing phenomenon. However, all these various methods mentioned here can be utilized by using comparable standard techniques for finding out any difference in widely scattered geographical populations so that breeders could anticipate differential response to the varieties to be released and therefore, can adopt the screening and breeding methods accordingly.

From the perusal of literature on BPH and GLH for more than a decade one may conclude that we have landed once again on the starting point as regards definition and differentiation of the biotypes and the problem to be tackled. Despite these studies which show some differences but could not provide well defined separable characters, the biotype has to be defined on the basis of differential response of the insect to particular rice cultivar having resistant genes. Further, from the results obtained so far, it appears that the term biotype would have to be restricted to the localities i.e. local populations showing susceptibility to the rice cultivars which have been declared as resistant to BPH or GLH in other parts of the world. Reasons in such cases, for the differential virulence can only be agroecosystem in the area. But when the same resistant variety becomes susceptible in the same area in course of time it could mostly be a case of overcoming resistance and/or adaptability. And, therefore, to face this problem continuous screening and breeding of resistant varieties in well defined areas having similar agroecosystem seems to be the only answer.

The term biotype is not inappropriate as it is based on behaviour aspect. But it would be better if the biotype is referred along with the locality, period (year) and plant cultivar, instead of with numbers. For example, Biotype Kerala, 1965, Mudgo—meaning Kerala biotype population, which showed susceptibility to Mudgo in 1965.

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