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A Molecular Approach to Planthopper Systematics

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The taxonomy of four species of planthoppers from the genus *Nilaparvata* was examined. Seven populations of *N.lugens* (Stal) were collected from India, Southeast Asia, Japan or Australia. Single populations of *N.muiri* (China) from Japan, *N.bakeri* (Muir) from India and *N.maeander* (Fennah) from West Africa were studied. *N.lugens* is a major pest on rice [5] in Asia and N.lugens populations were collected from rice (*Oryza*) or from the grass, *Leersia hexandra* (Schwartz). Studies of the calling behaviour have implied that *N.lugens* from the alternative host plants are sibling species rather than separate populations of a single species [1].

Different regions of the DNA of the planthoppers were studied with the aim of constructing a phylogeny of the populations investigated and to explain further the evolutionary relationships between the populations of *N.lugens* from different host plants. Ribosomal DNA , mitochondrial DNA and single copy nuclear DNA were analysed using restriction site polymorphisms or DNA sequencing. All prokaryotes and eukaryotes have ribosomes and the DNA that codes for the ribosomal RNA has been widely used in molecular systematics. The ribosomal DNA consists of the 18S , 5.8S and 28S genes separated by spacer regions. The genes are present in hundreds of copies per genome and repeats are separated by a non-transcribed spacer [4]. Different sections of the ribosomal DNA diverge at different rates which enables a wide range of taxonomic levels to be studied [3,7].

Fig.1 A schematic diagram of the ribosomal DNA gene cluster. The external transcribed spacer (ETS), non-transcribed spacer (NTS), internal transcribed spacers (ITS) and the primers used for PCR and sequencing of the ITS are shown. DNA was extracted from individual planthoppers by lysis with SDS followed by phenol and phenol/chloroform extractions and ethanol precipitation. The polymerase chain reaction (PCR) [9] was used to amplify the 18S and ITS. Primers except for ITSA and ITSB have been published [12,13]. A mix was prepared containing 10{SYMBOL 109 \f "Symbol"}l taq polymerase buffer (supplied by the manufacturer), 1.5mM or 2mM MgCl2, 50pmol each of primers, 100{SYMBOL 109 \f "Symbol"}M of each dNTP, and was added to 10-200ng template DNA in a 0.5ml microfuge tube to a total volume of 50 or 100{SYMBOL 109 \f "Symbol"}l. *Taq* polymerase, (2 units) was added and the PCR mixes overlain with mineral oil and placed in a programmable thermocycler. Denaturation at 95{SYMBOL 176 \f "Symbol"}C for 30 s, amplification at 54{SYMBOL 176 \f "Symbol"}C for 30 s and extension at 72{SYMBOL 176 \f "Symbol"}C for 2 min was carried out for 30 cycles. A negative control containing no template DNA was run each time. Approximately 5-10% of each PCR was analysed by agarose gel electrophoresis [10]. Products generated by PCR were gel purified using Promega Magic PCR Preps for subsequent sequencing. The product was sequenced using the Promega fmol kit according to the direct incorporation method.

Initially, the 18S ribosomal DNA gene was examined by PCR amplification of the whole gene [12] and digestion with six different restriction enzymes. No variation was found except for one site in the 18S gene of *N.maeander.* As there was so little variation in the 18S gene between the species another section of the ribosomal DNA, the less conserved internal transcribed spacer, was selected for further study [8,11].

The internal transcribed spacers (ITS) were amplified by PCR using the primers ITS4 and ITS5 (Fig.1) [13]. Restriction site variations between species of *Nilaparvata* indicated the potential usefulness of the ITS region in delineating populations. In order to provide more detailed information, the spacer between the 18S and the 5.8S genes was sequenced directly from the PCR products. The spacers of two individuals from each population of *N.lugens* and *N.bakeri* were sequenced in both directions. The spacers of two individuals of *N.muiri* and *N.maeander* were partially sequenced. The lengths of the spacer for *N. muiri, N. maeander, N. bakeri* and *N. lugens* were 950, 750, 575 and 547 base pairs respectively. The sequence data were aligned using ClustalV [2] and manually optimised. In comparison to the *N. lugens* sequences, the *N. muiri* and *N. maeander* sequences had numerous substitutions, insertions and deletions. The *N. bakeri* sequence had fewer differences (over 30 substitutions and 8 insertions or deletions) but was still clearly distinguishable from the other species. The sequences from different populations of *N. lugens* varied by just 0-6 substitutions.

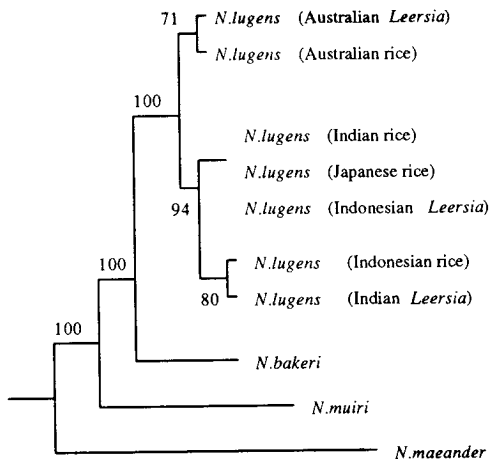

Fig.2 Phylogenetic analysis of planthopper DNA sequence data. ITS sequence data were aligned using ClustalV [2] and a phylogeny derived using parsimony analysis (DNAPARS program in PHYLIP) [6]. Bootstrap values were obtained from 100 replicates and are indicated at the branches.

Phylogenetic trees were obtained using programs in PHYLIP 3.5c [6]. The phylogeny (Fig.2) was consistent with the predicted relationships between the different species. *N. maeander* is most

distantly related to *N. lugens*. *N. muiri* and *N. bakeri* are more closely related to *N. lugens*. The hypothesis that the populations of *N.lugens* on the two host plants have diverged very recently is supported by the DNA sequence data. There is a greater divergence in the ITS sequences between populations from Asia and Australia , than between populations collected from rice and Leersia. This suggests at least two independent host shifts from Leersia (host of *N. muiri* and *N. bakeri*) to rice (in Asia and Australia) and possibly more.It would not be reasonable to split *N. lugens* into two species on host plant lines.

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