

# Specificity of acoustic signals and mate choice in the brown planthopper *Nilaparvata lugens*

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## Abstract

Mate choice experiments were made between populations of *N. lugens* from the Philippines, Solomon Is. and northern Australia. Significant barriers to mating were found between the Australian insects and the other two populations. The acoustic signals of successful males in hybridisation experiments were recorded and their pulse repetition frequencies (PRFs) were compared with the mean PRF for a random sample from their own population. In crosses between insects from Australia and the Philippines or Solomon Is. successful males were characterised by PRFs significantly different from their own populations and closer to those of the female populations in each cross than to their own. It is concluded that PRF of male calls is an important species recognition signal in *N. lugens*.

## Introduction

Many leafhoppers and planthoppers (Hemiptera, Auchenorrhyncha) communicate by means of substrate transmitted acoustic signals (Ossiannilsson, 1949; Ichikawa & Ishii, 1974; Strübing, 1977; Claridge, 1983; Inoue, 1983). Such signals are usually species specific and represent important elements of what Dobzhansky (1937) originally termed species isolating mechanisms and Paterson (1980) refers to as specific mate recognition systems.

In *N. lugens* both sexes produce signals (Ichikawa & Ishii, 1974; Ichikawa, 1979; Claridge *et al.*, 1984). Normally, sexually responsive males call spontaneously and virgin females may respond with their own calls. An exchange ensues during which the male moves actively and eventually makes contact with the generally stationary female. Claridge *et al.* (1984) studied populations of *N. lugens* from different geographically defined areas and found some to differ significantly in the pulse repetition frequencies (PRF) of male calls. The biggest

differences were found between populations from Australia and those from the Solomon Is. Also hybridisation experiments between some of the populations showed low levels of success. Lowest success rates were found in crosses involving one parent from an Australian population. It was thus suggested that PRF might be an important cue which females use to recognise and differentiate between different male calls.

Here we report experiments designed to test the importance of PRF in providing specificity to male signals.

## Materials and methods

Three geographically defined populations of *N. lugens* were selected from a series previously studied by us (Claridge *et al.*, 1984). The three were from: (1) Australia (Darwin, N. Territory) characterised by a high PRF, (2) Philippines (Liliw, Luzon) with an intermediate PRF, and (3) Solomon Is. (Honiara, Guadalcanal) with a low PRF. These

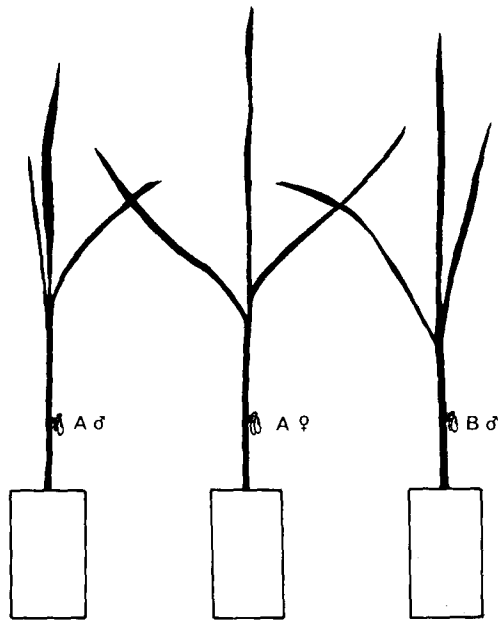


Fig. 1. Diagrammatic representation of mate choice tests showing three separate potted rice plants in acoustic contact by touching leaves. A female *N. lugens* of population A was placed on the central plant, with a male of A on one of the other plants and a male from another population, B, on the third plant.

were originally collected from rice in the field and subsequently maintained in Cardiff in glasshouses at  $25 \pm 2^\circ \text{C}$  with daylength of at least 12 h.

**Mate choice.** Final instar male and female nymphs were separated from the culture populations and isolated. Mate choice experiments were done four to five days after adult ecdysis. Three rice seedlings in separate pots were placed in series with their leaves just touching (Fig. 1). A test female was placed on the central plant and a male from her own population on one of the outside plants and a male from another population on the other, as previously described by Claridge & Den Hollander (1980). The female was thus given a choice between a male from her own population (homogametic) and one from another population (heterogametic). Such experiments do not only estimate female choice, since males also have the opportunity either to respond and move towards the female or not. Each individual experiment was continued for up to 30 min, after which, if no mating had occurred, the insects were removed and a new experiment was

begun with new insects. At least 25 replicates were done for each of the six possible combinations from the three test populations.

Positive matings were only recorded when they resulted in successful sperm transfer. This was determined by dissecting the spermatheca of each female at the end of the experiment and examining microscopically for the presence of living sperm. Isolation indices were calculated for each set of experiments, after Merrell (1950).

**Pulse repetition frequency and mating success.** Virgin males and females resulting from isolated nymphs, as described above, were used. Single pair crosses were set up on rice seedlings in separate glass tubes ( $20 \times 2.5 \text{ cm}$ ). Each pair was kept together for 24 h after which the females were removed for spermatheca dissection. For those crosses where sperm transfer had occurred, the male courtship call was recorded, using a crystal cartridge transducer and tape recorder (see Claridge, 1983; Claridge *et al.*, 1984). A random sample of males from each of the three parental populations was also recorded. Pulse repetition frequencies were calculated from oscillograms, also as previously described by Claridge *et al.* (1984).

## Results

Mating behaviour in *N. lugens* consists of a sequence of events which terminate in copulation. Typically the sequence develops as follows: after virgin insects have been placed on a plant, generally males call spontaneously, as also occasionally do females. However the calling starts, it will normally lead to an exchange between male and female during which the male will approach and attempt to mate with the female. The calls are described by Claridge *et al.* (1984). Typically a male, after locating a female orients parallel with her, but slightly to her rear. He then raises his mid leg and taps the female beneath her abdomen. After this, if she is receptive, she lifts her abdomen while the male raises his wings vertically above his body. At the same time the male turns so as to make genitalic contact with her and, if successful, copulation ensues. In our experiments copulation varied in duration between 1 and 2 min, after which it was terminated by the female kicking and walking away,

Table 1. Results of mate choice experiments between populations of *N. lugens* from Australia, Solomon Is. and Philippines. Isolation indices significantly different from 1 ( $P < 0.05$ ) using  $\chi^2$  test are indicated.\*

♀	♂	♂	No. trials	Successful Matings		Merrell's Isolation Index
				Homogametic	Heterogametic	
Aus.	Aus.	Sol. Is.	37	19	1	0.05*
Sol. Is.	Aus.	Sol. Is.	39	19	1	
Aus.	Aus.	Phil.	59	17	3	0.15*
Phil.	Aus.	Phil.	25	22	3	
Sol. Is.	Sol. Is.	Phil.	31	11	9	1.36
Phil.	Sol. Is.	Phil.	35	8	17	

often dragging the male for a time before release. Acoustic exchange between male and female continues until copulation is achieved. No obvious post copulatory behaviour or calls were recorded.

During a mating sequence, the female may prevent copulation either by not responding to the male calls, or by walking away when the male approaches, or by walking away or moving her abdomen from side to side when the male attempts to tap her abdomen, or by failing to raise her abdomen, or by kicking at the male prior to establishing genitalic contact. The male does not usually refuse copulation after contact is made with a female, but he may not respond to a female call and may not move towards her when she calls.

*Mate choice.* In most choice experiments where two different males were in acoustic contact with one female, the males responded to the female calls and often approached and attempted to copulate. In many, the female consistently rejected a particular

male. Results of all experiments are summarised in Table 1, from which it is clear that some mating preferences occurred.

Heterogametic matings were very rare between insects from the Australian populations and those from either the Philippines or Solomon Is. ones. Discrimination was marked even when the heterogametic male responded to the female: he was then vigorously rejected. Occasionally a female repeatedly rejected the heterogametic male only to mate immediately with the homogametic male when he approached. Merrell's isolation indices of significantly less than one for Australia  $\times$  Philippines and Australia  $\times$  Solomon Is. crosses confirmed the existence of definite breeding barriers between them.

By contrast, hybridisation between Solomon Is. and Philippines insects seemed to be essentially at random. Indeed, Merrell's index showed a slight tendency towards heterogametic matings between them.

In all experiments, when successful genitalic engagement occurred it resulted in sperm transfer. However, duration of copulation varied between the populations (Table 2). In some Australia  $\times$  Philippines crosses sperm transfer was achieved in as short a time as 25 s.

Table 2. Mean duration (s) of copulation in crosses between populations of *N. lugens* from Australia, Philippines and Solomon Is.

	Aus. ♂	Phil. ♂	Sol. Is. ♂
Aus. n	38	3	1
♀ mean	123	86	154
S.D.	32	4	
Phil. n	3	30	16
♀ mean	40	65	60
S.D.	12	27	26
Sol. Is. n	1	9	30
♀ mean	64	88	81
S.D.	-	21	21

*Pulse repetition frequency (PRF) and mating success.* Previously reported hybridisation experiments demonstrated significant behavioural barriers to interbreeding between Australian populations and most others of *N. lugens* (Claridge *et al.*, 1984). The present experiments confirmed these results (Table 3).

The PRFs of successful males in each cross were calculated and compared with random samples of males taken from each population (Fig. 2). For

Table 3. Number of replicates and number of successful matings in hybridisation experiments between populations of *N. lugens* from Australia, Philippines and Solomon Is. Numbers of successful males recorded acoustically for PRF determination shown in parentheses. Means and standard deviations for PRFs of successful male calls and/or random samples from male populations are given and those values which differ significantly ( $P < 0.05$ ) from PRF of the parental male population are indicated by.\*

Crosses		No. replicates	No successful matings	PRF/s of random sample from ♂ popul.		PRF/s successful ♂♂	
♀♀	♂♂			mean	S.D.	mean	S.D.
Aus.	× Phil.	111	36 (31)	72.5	4.9	*74.7	2.8
Phil.	× Aus.	64	9 (4)	99.1	8.5	95.9	8.3
Sol. Is.	× Phil.	43	19 (12)	72.5	4.9	71.41	4.3
Phil.	× Sol. Is.	34	34 (12)	62.6	3.3	61.5	3.9
Aus.	× Sol. Is.	180	7 (3)	62.6	3.3	*72.2	3.4
Sol. Is.	× Aus.	194	13 (10)	99.1	8.5	*91.5	6.0

three crosses (Australian male × Solomon Is. female, Solomon Is. male × Australian female, and Philippine male × Australian female) there were significant differences between the PRFs of the successful males and the mean PRF of a random sam-

ple of the population from which the males came. In each the successful males departed from the mean of their own populations in the direction of the mean male PRF of the population from which the female derived. For the other cross involving the

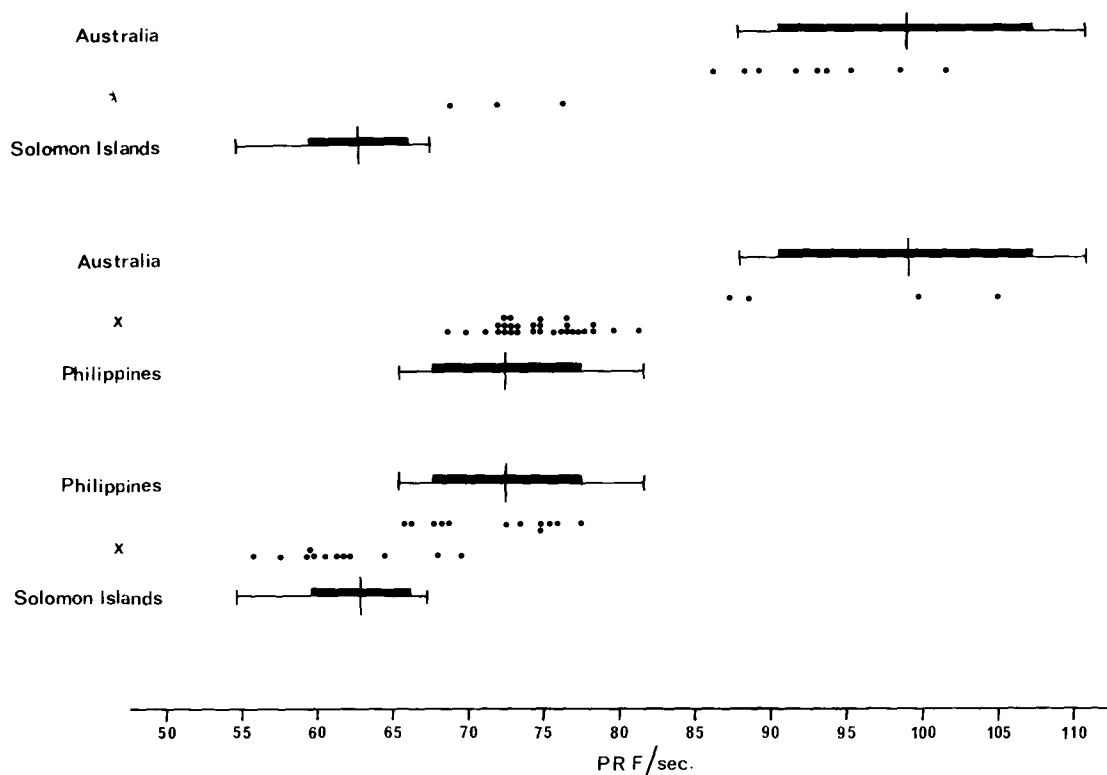


Fig. 2. PRFs of calls of successful individuals male *N. lugens* from experimental crosses between Australia × Solomon Is., Australia × Philippines, and Philippines × Solomon Is. indicated by .. For comparison the mean (vertical line), one standard deviation on either side of the mean (horizontal heavy bar), and total range of PRFs of male calls for a random sample of each population are shown for each cross.

Australian population (Australia male  $\times$  Philippines female), there was no significant difference between PRF of successful males and those of the parental population. However, we were only able to record four out of the nine successful males and there seemed to be a tendency for them to have a PRF biased towards Philippines males. Results are summarised in Table 3.

In both crosses between Philippines and Solomon Is. insects there was little evidence for selective mating and no significant differences in PRF between successful males and the random sample from the parental population.

Thus, it may be concluded that there is clear evidence to suggest that individual females show a preference for mating with males, the mating call PRFs of which most resemble the mean PRF of males from their own populations.

## Discussion

Paterson (1980, 1981, 1982) has stressed that for sexually reproducing species during courtship the two sexes exchange precise signals: the specific mate recognition system. This is essentially the same as the premating isolating mechanisms of Mayr (1942, 1963) and others. Paterson argued that the receptors of a particular species will be closely tuned to the signals of that species and will not respond to the different signals of related species. Thus he suggested that stabilising selection should maintain a very narrow range of variation in such a system.

In *N. lugens* the specific mate recognition system is primarily acoustic and consists of an exchange of substrate transmitted amplitude modulated signals (Ichikawa *et al.*, 1975). We have previously suggested that PRF might be an important element endowing specificity to male calls in this species (Claridge *et al.*, 1984). The correlation between greatest difference in this aspect of male signals and greatest difficulties in hybridisation between different populations supported this. In the present study we have been able to show that when successful hybridisation occurs between two such populations, successful males tend to be extreme individuals for PRF within their own populations. They are closest in call PRF to the population from which the female came. Thus, in crosses between Australian males and Solomon Is. females, successful males had

PRFs significantly lower, and therefore closer to those of Solomon Is. males, than to typical Australian males. On the other hand in reciprocal crosses, successful Solomon Is. males had significantly higher PRFs than the mean for their own population. This clearly supports our argument that PRF in this species is an important element of the mutual signalling system.

In addition, it is clear that in at least some of the populations we have studied, there is sufficient variation in PRF for selection to change mean values in spatially isolated populations. The precise reason for such differential selection pressures in different populations is not clear, but it is apparent that there may be more variation usually present in specific mate recognition systems than Paterson has suggested. For example, within any of the geographically defined populations tested here, control hybridisation experiments showed 100% mating success within each population, despite the variation in PRF known to occur. If it is true that specific mate recognition systems generally show such variation, then it is possible to understand how divergences may occur when populations are spatially isolated in the early stages of speciation.

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## Résumé

*Spécificité des signaux acoustiques et sélection sexuelle chez Nilaparvata lugens (Stål)*

La communication sexuelle de *N. lugens* s'effectue par signaux acoustiques transmis par le substrat. Quelques populations géographiquement définissables diffèrent par la fréquence des répétitions

des impulsions (PRF) produits par les appels des mâles. Des populations des Philippines, des Iles Salomon et de l'Australie du Nord ont été étudiées.

Des expériences de sélection sexuelle ont montré que des obstacles au transfert de spermatozoïdes apparaissaient dans les croisements qui impliquent un parent originaire de la population australienne. Des expériences d'hybridation ont été répétées entre individus mâles et femelles de chacune des 3 populations étudiées. Le taux de succès était faible quand l'un des parents était originaire d'Australie. Le PRF des mâles de chaque croisement réussi a été déterminé et comparé au PRF moyen d'un échantillon constitué au hasard de mâles provenant de la même population. Pour les croisements impliquant un parent australien, le PRF des mâles victorieux différait de la moyenne et se rapprochait de celui des mâles de la population dont dérivait la femelle.

On en a conclu que le PRF des appels mâles est important dans la reconnaissance spécifique de *N. lugens*, mais que chaque population montre un polymorphisme important pour ce caractère.

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