

Evolutionary aspects of acoustic communication  
in *Ribautodelphax* planthoppers  
(Homoptera, Delphacidae)

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**Evolutionary aspects of acoustic  
communication in *Ribautodelphax*  
planthoppers  
(Homoptera, Delphacidae)**

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**Proefschrift**

ter verkrijging van de graad van  
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*Aan mijn moeder en de nagedachtenis van mijn vader  
Voor Josine en George*

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## STELLINGEN

- 1 Mate recognition-kenmerken zijn minder stabiel dan veelal wordt verondersteld.  
Dit proefschrift, *contra* Paterson, H.E.H. (1985), Transvaal Museum Monogr. 4: 21-29.
- 2 Bij het meten van reacties van insecten op communicatie-signalen dient rekening gehouden te worden met de mogelijkheid van pre-imaginaal 'leer'gedrag.  
Dit proefschrift.
- 3 In experimenten waarbij de responsiviteit van soorten t.o.v. elkaars communicatie-signalen wordt vergeleken, dient rekening gehouden te worden met de mogelijkheid dat ook nauwverwante soorten op verschillende leeftijden hun optimale respons vertonen.  
Dit proefschrift.
- 4 Patronen van 'non-random' paring tussen organismen kunnen het resultaat zijn van complexe interacties tussen de uitgezonden signalen en de 'ontvangers'. Processen als soortherkenning en seksuele selectie kunnen samen verantwoordelijk zijn voor de waargenomen 'reproductieve isolatie' en sluiten elkaar niet persé uit.  
Ryan, M.J. & Rand, A.S. (1993). *Evolution* 47: 647-657.  
Dit proefschrift.
- 5 In theoretische beschouwingen over de evolutie van communicatiesystemen wordt vaak vergeten dat bij veel insecten ook (soms zelfs alleen) de vrouwtjes soort-specifieke communicatie-signalen produceren. Ten onrechte veronderstelt men dat alleen vrouwtjes selectief zijn in hun preferentie voor een partner.  
Dit proefschrift, *contra* Lande, R. (1981), *Proc. Natl. Acad. Sci. U.S.A.* 78: 3721-3725; Kirkpatrick, M. (1987), *Annu. Rev. Ecol. Syst.* 18: 43-70.
- 6 Ook bij delphaciden-genera waarvan de soorten op verschillende waardplanten leven, lijkt het waarschijnlijk dat de acoustische signalen een belangrijke rol hebben gespeeld in het soortvormingsproces.  
Dit proefschrift.
- 7 Als maat voor de responsiviteit van mannelijke delphaciden op akoestische signalen van vrouwtjes is het zoekgedrag een beter criterium dan de akoestische respons.  
Dit proefschrift.

- 8 De taxonomische status van niet-cultuurvolgende landslakkensoorten met een grote verspreiding is in principe verdacht en verdient nader onderzoek.

Solem, A. (1984). pp. 6-22 in Solem, A. & Van Bruggen, A.C. (Eds.), *World-wide snails, biogeographic studies on non-marine Mollusca*. Brill, Leiden.

- 9 Gezien de opkomst van het phylogenetisch soortconcept moet geconcludeerd worden dat de 'Nouvelle Ecole' van Jules René Bourguignat c.s. zijn tijd ver vooruit was.

Cracraft, J. (1989). pp. 28-59 in: Otte, D. & Endler, J.A. (Eds.), *Speciation and its consequences*. Sinauer Ass., Sunderland, Mass.

Bourguignat, J.R. (1882). *Lettres malacologiques à Mm. Brusina d'Agram et Kobelt de Francfort*. Paris.

- 10 Het gebruik van niet-inheemse en weinig specifieke parasieten of predatoren voor biologische bestrijding van plagen in het vrije veld is slechts toelaatbaar indien uitgesloten kan worden dat niet-schadelijke organismen schade ondervinden. Daar dit veelal niet mogelijk is, dient het gebruik van dergelijke organismen te worden vermeden.

Howard, F., 1987. The ugly side of introductions. *IUCN Bulletin* 18: 18-19.

Tillier, S. & Clarke, B.C., 1983. Lutte biologique et destruction de patrimoine génétique: le cas des mollusques gastéropodes pulmonés dans le territoire français du Pacifique. *Génét. Sél. Evol.* 15: 559-566.

- 11 Gezien de angstaanjagende toename van de wereldbevolking zou ook in Nederland een actieve politiek ter ontmoediging van het krijgen van meer dan twee kinderen gevoerd moeten worden. Als eerste stap zou het huidige stelsel van kinderbijslag vervangen kunnen worden door een progressieve kinderbelasting na het tweede kind.

- 12 Publiek gefinancierde 'bijzondere' onderwijsinstellingen kunnen beter omgevormd worden tot bijzonder goede algemene.

Stellingen behorend bij het proefschrift:

**Evolutionary aspects of acoustic communication in *Ribautodelphax* planthoppers (Homoptera, Delphacidae)**

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## INTRODUCTION

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### Introducing Delphacidae

#### *Systematic position and ecology*

Delphacidae, commonly referred to as planthoppers, are insects belonging to the Order Hemiptera, Suborder Homoptera, Infraorder Auchenorrhyncha. The name 'planthopper' refers to the members of all twenty families which comprise the superfamily of Fulgoroidea, of which the Delphacidae are the largest (> 1800 species described), and by far the best studied group (O'Brien and Wilson, 1985). Most delphacids are small (< 5 mm), and the taxonomy is generally difficult, especially that of females. In several studies cryptic species have recently been discovered from non-morphological traits, like acoustic signals and hostplant preferences.

All Delphacidae are herbivores, which predominantly feed on monocots, especially grasses, by sucking fluids. Although some are polyphagous, there is a tendency towards oligophagy and even monophagy. Some planthoppers are major pests on agricultural crops, like rice, corn, sorghum and sugar cane. Damage is brought about either directly by feeding, or by transmitting plant viruses; in addition, damage has been reported by females cutting slits in order to oviposit, allowing pathogens to enter, and by abundant honeydew production (O'Brien and Wilson, 1985). The pest species have received the greatest attention by scientists. Probably the largest body of planthopper literature concerns the brown planthopper *Nilaparvata lugens*, which is a major pest of rice cultures, especially in Asia. In this species a number of 'biotypes' are known, which differ in their virulence to different rice cultivars (Wilson and Claridge, 1985).

Planthoppers reproductive cycles obviously have to be adapted to the life cycles of their hostplants, and consequently most species are probably short-lived, at least in temperate regions. The number of generations per year varies, both among and within species, according to the climate of the geographical area they live in. In northern regions of Europe there are usually only one or two generations per year. Most species appear to hibernate as small nymphs, of which further development is triggered by increasing day length, but a few are known to hibernate as eggs or as adults (Ossiannilsson, 1978).

In the field the majority of female planthoppers probably mate only once, although multiple matings have been observed in some species in the laboratory (e.g. Drosopoulos, 1985). In contrast, males can mate several times per day, but their insemination capacity appears to vary among species. As a result, there is usually a great excess of sexually active males compared to females in the field.

Planthoppers vary in wing length within and among populations and species, and thus in their ability to fly. The variation takes the form of a dimorphism, with short-winged (brachypterous) and long-winged (macropterous) animals. Apart from a genetic

component, the character is greatly influenced by environmental factors as well, because in some species the frequency of brachypterous forms drops with increasing population densities (Denno and Roderick, 1990). Wing shape appears to be correlated with important life-history characters. For example, brachypterous animals produce more eggs, and in some groups (e.g. *Prokelisia*) their sexual maturation appears to be more rapid (oogenesis-flight syndrome, Denno and Roderick, 1990).

### *Breeding systems*

As far as known, most planthoppers reproduce bisexually. However, in two genera, *Muellerianella* (Drosopoulos, 1976), and *Ribautodelphax* (Den Bieman, 1988a), triploid females have been found living in association with diploid males and females, which reproduce by sperm-dependent parthenogenesis or pseudogamy; they need to mate with a male, but the sperm does not contribute genetic material, and only serves to trigger the development of the eggs. Because the pseudogamous females only produce females, they possess a two-fold reproductive advantage over diploid females, thus theoretically their frequency will increase at the expense of that of the diploids. Eventually this will lead to their own demise, as no male will be left to mate with. However, in the field the ratio diploid:triploid females was found to vary among populations, but to be stable over time within populations (Booy and Guldemon, 1984, Den Bieman, 1987c). Several mechanisms have been proposed explaining this stable coexistence (Kirkendall and Stenseth, 1990), but none seems to be really satisfactory. The origin of this peculiar reproductive system appears to be autoploid in *Ribautodelphax* (Den Bieman, 1988b), rather than allopolyploid, as was held by Drosopoulos (1978) for the genus *Muellerianella*.

Recently, a case of true parthenogenesis was reported in the genus *Delphacodes* (Den Bieman and De Vrijer, 1987).

### *The genus Ribautodelphax*

The genus *Ribautodelphax* comprises about 19 species, including a complex of closely related taxa around *R. collinus*. The European members of the genus, especially the *collinus*-complex, have recently been the subject of a biosystematic study by Den Bieman (1987a). By studying hostplant relations, crossability, cytology, isozymes, acoustic signals, and morphology, he was able to redefine a number of already described species, while in addition a number of cryptic species was discovered and formally described. Morphological characters proved to be rather variable, and diagnostically difficult to apply, even in males. Females still cannot be identified morphologically. However, the species can be reliably identified by the hostplant they live on, and by the acoustic signals produced by both males and females.

All *Ribautodelphax* species feed on grasses. Each species was found to be confined to a single species of hostplant, although in one instance the same species utilized different species of plants of the same genus in different geographical areas. Most species can only survive and reproduce on their respective hostplants. Although the

ranges of the species used in this study at least potentially overlap, they are rarely found syntopically, because of their hostplant specificity (Den Bieman, 1987).

*Ribautodelphax* species possess a XO sex determination system, and a large number of holocentric chromosomes ( $2n=30$  in females, 29 in males). Triploid females living in association with *R. pungens* and *R. imitantoides* are rather variable in chromosome numbers ( $3n=40-46$ , Den Bieman, 1988a).

Especially within the *collinus*-complex, it turned out to be possible to obtain viable and fertile offspring from no-choice interspecific crossings, but hybridization success was found to differ according to the species involved and their gender. However, hybridization did not occur when species were confined with both conspecific and heterospecific partners (Den Bieman, 1988b). Thus, the integrity of species appeared to be maintained by behavioural rather than mechanic or genetic causes, but the behavioural factors responsible for assortative mating were not identified. However, in the absence of other obvious factors, it would seem that acoustic communication is likely to be involved.

### Acoustic communication

From the wealth of recent literature on this topic it appears that in the majority of insect groups some kind of acoustic communication occurs, even in groups which use other ways of communication as well, like vision and chemical signalling. Acoustic signals are most often used in connection with sexual behaviour; other uses mainly occur in social insects, like bees and ants (Ewing, 1989). In most cases there is one signaller, usually the male, and one receiver, usually the female.

### Physical properties

Acoustic signals can be classified according to various criteria, like the distance covered (near-field, far-field), the frequencies of the signals used (low-high frequency, ultrasound), the mode of sound transmission (substrate-borne, air-borne), and the mechanism of sound production (vibration of body parts by direct muscle action, stridulation by friction of two body parts moving across one another) (Ewing, 1989).

The different kinds of signals used appear to be adaptations to the biotic and physical properties of the signallers and the environment they live in. For small animals it is difficult to use air-borne sounds, except at close range, because they cannot produce enough sound energy to overcome sound attenuation; theoretically sound pressures in open space are halved with each doubling of the distance. Small insects have the option to signal at close range, or to use ultrasonic signals. However, ultrasounds are not suited for penetrating environments dominated by plants. Therefore, many small insects use a third option, namely the production of low-frequency sounds with a solid substrate rather than the air as transmission medium. Within the Homoptera Auchenorrhyncha different strategies have been used: large cicadas (Cicadidae) produce loud, high frequency air-borne sounds, whereas the much smaller planthoppers and leafhoppers communicate by low-frequency substrate-borne vibrations (For treatments of the complicated physical

background see Ewing, 1989; Michelsen et al., 1982, and references therein). Planthoppers also produce air-borne sounds as a by-product of substrate-borne vibrations, but these range only for a few cm's, and the animals appear not to react to them (Ichikawa, 1976). Different plants vary greatly in their mechanical properties, but the filtering of frequencies appears to be rather similar, although it seems to be impossible to predict which frequencies will be attenuated or amplified (Michelsen et al., 1982). Planthopper communication appears to be adapted to these filtering properties of the plants, by producing signals that cover broad frequency ranges, some of which always get through. In *Javesella* planthoppers frequencies of calling signals range between 100 and 2000 Hz, with the main energy concentrated between 100 and 500 Hz (De Vrijer, 1984). Thus, this adaptation is not specific to the properties of specific plants (Michelsen et al., 1982). Consequently, most information appears to be contained in the temporal patterning of impulses produced by the animals. Apart from the other messages contained in the signal, it appears theoretically to be possible that receivers obtain information about the direction and distance of the singing animal from the distortions of the signal brought about by the plant (Michelsen et al., 1982). However, Claridge (1985b) suggested that planthopper males search at random for calling females. Planthopper signals are known to range up to at least 0.8 m in the planthopper *Nilaparvata lugens* (Ichikawa et al., 1975), but for similar signals of other insects ranges up to 2 m are known (Michelson et al., 1982).

### *Morphology, production and perception of calls*

In planthoppers, both sexes produce acoustic signals. In all genera studied so far, at least the male call was found to be species-specific, and to be of great value in solving taxonomic problems (Claridge, 1985a,b; Claridge and De Vrijer, 1993).

In the genus *Ribautodelphax* both male and female calls proved to be species-specific (Den Bieman, 1986, 1987c). The morphology of the male calls is more complicated than those of females, and can be divided in at least structurally different components. Male calls of all species are basically similarly structured, consisting of a number of 'chirps', followed by a 'buzz' (see chapter 5, Fig. 1). Species differ in the duration of these sections, in the number of chirps, as well as in the temporal patterning of chirps. Apart from these components, calls may be preceded and/or followed by various types of pulses, the presence or absence of which varies greatly among individuals, and thus seems to be less important, although their effects have not been explicitly studied.

In all planthoppers, female response calls consist of series of simple pulses (see e.g. chapter 4, Fig. 1). *Ribautodelphax* species differ in the duration of the calls, and in temporal patterning of the pulses, i.e. the pulse repetition frequency (here usually measured as the interpulse interval) and the change of pulse rate within the signal. In most *Ribautodelphax* species the pulse rate is initially high, but becomes lower as the signal proceeds. In one species (*R. imitans*), the pulse rate accelerates towards the end of the signal. In another (*R. albostrigatus*), the pulse rate first increases, and then decreases again. These characters are remarkably constant within individuals. The pattern of the

female signal and the hostplant they live on appear to be the only reliable ways to identify females of the different species (Den Bieman, 1986). However, the situation is more complicated in those populations of two species (*R. pungens* and *R. imitantoides*) with associated pseudogamous triploid females. Here, a great variety of female call types occur among such populations. Only a limited number of call types are found within each population, and the sexual and asexual females tend to differ in the calls they produce (Den Bieman, 1987c).

Male planthoppers produce calls by an organ situated in the posterior region of the metathorax, of which the structure and functioning resembles that of the external tymbals and associated muscles found in male Cicadidae. The largest muscle in this region is a paired dorsal longitudinal muscle which runs from the metaphragma to the second tergite. According to Ossianilsson (1949), vibration of this tymbal muscle homologue would produce the energy for sound production; the associated two pairs of large dorsoventral muscles in the first abdominal segment are probably involved in the modification of the basic sound produced. Quite unlike in the males, female calls are brought about by dorsoventral vibrations of the entire abdomen, without touching the substrate. The functional morphology of the sound producing organs are described by Ossianilsson (1949) and Mitomi et al. (1984).

The call perception mechanism of planthoppers appears to be unknown. They lack the tympanum-like receptor organs present in female and male Cicadidae, and other vibration receptors have as yet not been identified (Claridge, 1985b).

### 'Functions' of calls

In planthoppers, like in many acoustic insects, different kinds of acoustic signals can be distinguished: calling (attraction) signals, rivalry (aggression) calls, and courtship signals.

The calling signals, also termed 'attraction calls' mainly serve to bring receptive mating partners together, and are the most commonly produced, and best studied. According to Ewing (1989), three kinds of information may be coded within calling songs: range, position within space, and species identity. In addition, signals may also provide cues used for assessment of the attractiveness of the mating partner (sexual selection). Attraction calls produced by male insects tend to be species-specific, and this appears to hold for all planthopper species studied so far. However, examples are known of closely related insect species with virtually identical calls. Mating confusion in such cases might be avoided in different ways, e.g. by occupying different habitats, calling at different periods of the day (examples in Ewing, 1989; Bailey, 1991), or, in species with both sexes calling, by the possession of species-specific response-delay times (time-windows) (e.g. Heller and Von Helversen, 1986).

Calling songs may also play a role in male spacing, establishment of social status, territory maintenance, and aggression. Many insects have developed distinct rivalry signals, but the absence or presence often varies, even among related taxa. Most rivalry signals seem to be derived from calling signals (Ewing, 1989). In planthoppers, distinct rivalry calls are produced by males of some genera, like *Muellerianella* (Booy, 1982)

and *Nilaparvata* (Ichikawa, 1982), but are completely unknown in *Ribautodelphax* (Den Bieman, 1986; Chapter 6).

### *Roles of males and females in sexual behaviour*

Acoustic signalling can be performed by both sexes in insects, but in the majority of groups studied, usually only the male calls, and the female approaches the male. Female acoustic signalling has been reported in several groups, like Lacewings (Henry, 1985), planthoppers and leafhoppers (Claridge, 1985), and stoneflies (Szczytko and Stewart, 1979). However, in most textbooks and theoretical models on the evolution of mate recognition systems, female signalling (both acoustic and non-acoustic), and male searching behaviour is still largely neglected.

In most theoretical models (e.g. Lande, 1981; Kirkpatrick, 1985), it is assumed that females approach calling males. This is in agreement with the theory that the sex with the highest parental investment, which is usually the female, should be more selective (Trivers, 1972). However, a number of examples are known where male investment is considerable, and sex role reversals occur (Gwynne, 1991). In many insects males approach signalling females, and at least in some groups male choice seems to be an important factor in species recognition, even when male parental investment is relatively low. At close range females often become selective as well, but at that stage already a lot of time and energy have been invested in male searching behaviour, which is a risky and costly enterprise (Bell, 1990). Only one theoretical model of evolution of mate recognition systems appears to deal with systems where females produce signals, to which males are attracted (De Jong, 1988; De Jong and Sabelis, 1991).

In *Ribautodelphax*, males usually call first. Only receptive, virgin females respond, and while alternate calling (duetting) takes place, the male actively searches for the female, which remains stationary once acoustic contact between the sexes is established. *Ribautodelphax* females appear to mate only once during their lifetime. This basic pattern appears to be similar in all planthopper taxa studied so far, although in most genera studied spontaneous female calling seems to be more frequent than in *Ribautodelphax* (Claridge and De Vrijer, 1993).

As soon as a receptive pair is in close proximity, the courtship phase in the strict sense starts (Alexander, 1967). Recognition is usually no longer the main priority, and often both the shape and the effects of signals change. Some planthopper males (e.g. *Muellerianella*, Booy, 1982) produce distinct courtship signals in addition to the signals produced during the attraction phase. In others, this appears not to be the case, which led Claridge (1985a) to conclude that in small Auchenorrhyncha there is probably no sharp division between calling and courtship signals. In *Ribautodelphax* the male call basically remains unchanged during courtship, but the female response call is greatly reduced in length (Chapter 6).

## Origin and evolution of species-specific signals

### *Are species-specific signals adaptive?*

Species-specific sexual signals have long been considered to be adaptive devices (isolating mechanisms) preventing interspecific matings (Dobzhansky, 1940). These signals were thought to have arisen by natural selection, favouring homogamic mating by selection against hybrids in the area of overlap, after two previously isolated populations came in secondary contact (speciation by reinforcement). Most examples supposedly supporting the reinforcement theory turned out to be flawed (Paterson, 1978, 1982; Butlin, 1989), and a series of convincing arguments against this view have been put forward (review in West-Eberhard, 1983). There is an alternative view that species-specific sexual characters arise as by-products of other processes. Paterson (1985) also suggested a change in wording, by preferring to speak about the *effects* of signals in species *recognition*, instead of speaking about their *function* as *mechanisms* for *reproductive isolation*, but the old terms are still commonly used.

Two different non-adaptive views on the origin of species-specific signals prevail. Paterson (1985, and earlier references therein) suggested that species-specific signals are shaped by natural selection to ensure the most effective recognition and location of conspecific partners. The 'isolating' effects of species-specific signals are thought to arise as by-products of the demands for effective conspecific recognition. Each species possesses a specific mate recognition system, consisting of a specific coadapted signal response chain; inappropriate responses to any of the signals will lead to ineffective recognition, preventing successful mating.

A somewhat different view came about with the renewed interest in the sexual selection theory (see below). Specificity of signals may arise from social competition for mates within species (West-Eberhard, 1983). Driven by active or passive preferences by females for certain traits in males, these traits and the preferences for them may evolve to the extent that they become different in related taxa, and may become involved in species recognition.

### *Genetics and evolution of mate recognition systems*

In order to be able to understand and predict how characters evolve, it is very useful to know something about their genetic control. The pattern and rate of response to selection depends on the mode of inheritance, the presence of genetic variation, genetic correlations with other characters, and the population size (Falconer, 1981). Most quantitative models of sexual selection have been developed without actual knowledge of the genetic architecture of the characters involved.

To the extent that sexual signals have influence on mating success, they are to be considered as fitness components (Boake, 1986). Characters related to fitness are often considered to possess little or no heritable genetic variation, because they are supposedly under strong selection, and consequently their evolution is thought to be highly constrained (Fisher, 1958). In fact, characters possessing high heritabilities were

considered to be unimportant for the species' fitness (Falconer, 1981). A similar reasoning follows from Paterson's view on mate recognition characters. Because such characters are part of a highly coadapted signal-response chain, individual components are thought to be under strong stabilizing selection, and consequently to have very little genetic variation (Paterson, 1978). However, recent studies have reported significant heritabilities for various characters related to fitness. Several theories have tried to explain the evolution of such characters, by presenting ways of origin and maintenance of their additive genetic variation, including 'mutation-selection balance' (Lande, 1981), 'antagonistic pleiotropy' (Rose, 1982), and 'environmental fluctuations' theories (Felsenstein, 1976; Cade, 1984). There is now both empirical and theoretical evidence that at least components of fitness can possess significant amounts of heritable variation. However, their pace of evolutionary divergence still seems to be a matter of dispute.

### *Sexual selection*

The sexual selection theory was originally proposed by Darwin (1871), in order to explain the origin of spectacular morphological attributes involved in sexual behaviour, like the plumage of male peacocks, or the enormous horns of many mammals. Darwin found these difficult to explain by natural selection, because many such structures are clearly maladaptive. He recognized two potential mechanisms of sexual selection, intermale competition and female choice, but was unable to explain the preference of females for males possessing extreme characters, which otherwise seemed to be useless or even deleterious for survival.

Fisher (1930, 1958) was the first to propose a solution for Darwin's problem. He described a genetic mechanism for the joint evolution of male sexual characters and female preferences. If females possess a preference for males exhibiting a strong expression of a sexual attribute, then this preference will become stronger, because their sons will be better signallers, that is, when this preference will not be counteracted by other selection pressures at the onset. The strength of the selection on the signalling ability of males will increase, and a genetic correlation between the male character and the female preference can develop, which will further increase the development of both. Because of this positive feedback, the evolution of the male character can be very rapid ('runaway selection'), until stopped by natural selection, e.g. because the signalling becomes energetically too costly, or attracts too many predators. Originally designed for morphological characters, the theory has been applied to other kinds of characters as well, including acoustic signals (West-Eberhard, 1983). Although theoretically plausible (Lande, 1981; Kirkpatrick, 1985, 1987, and references therein), direct experimental data supporting the runaway theory do not seem to be available. One problem seems to be how the female preference originates in the first place. Several mechanisms for the establishment of preferences have been proposed. The simplest explanation would be that females possess preexisting biases inherent in their sensory systems. The sexual selection will initiate by changes arising in mating systems, ecological conditions, or male traits, which will allow the expression of the preference (Kirkpatrick, 1987). Other proposed mechanisms explaining the origin of female preferences include selection for species



recognition, and pleiotropy (Kirkpatrick, 1987).

Although 'runaway' sexual selection has received the largest attention from theoreticians, it is by no means the only way in which sexual selection can take place. A variety of other mechanisms may bring about a more slowly change in both character and preference (termed 'walkaway' selection by Kirkpatrick, 1987). These include direct selection on preferences, when these affect any component of the fitness of females, or indirect selection through pleiotropic effects of selection on other traits (see Kirkpatrick, 1987).

It is usually assumed that signals and preferences are inherited genetically, but signals may also evolve from cultural inheritance ('learning'), as is the case in many species of birds. Theoretically, learning can affect genetic character divergence in different ways (West-Eberhard, 1983). In species which easily mimic local dialects or even songs of other species, song evolution is likely to be retarded. However, in species where learning leads to narrowing of the range of the signal, signal evolution, and even speciation may be accelerated. West-Eberhard (1983) mentioned examples of such birds, which never hybridize in the wild, but freely mate with members of other genera of even subfamilies, and produce viable offspring under forced conditions. Most discussions on the effects of learning concern the signal itself, and not the preference for it, which could likewise be influenced by learning.

Two schools of sexual selection theory exist, the 'nonadaptive' school, which holds that female preferences cause changes in male traits, which are not (necessarily) adaptive with respect to their ecology, and the 'good genes' school, according to which females use male displays as an indicator of their genetic quality (Kirkpatrick, 1987; Boake, 1986). Although most members of the latter school acknowledge that female preferences can cause disadvantages for males expressing the preferred traits, they believe that by mating with such males, females receive 'better' genes, which are genetically correlated with the preferred trait, thus improving the genetic quality of their offspring. The evolution of male traits is considered to be a byproduct of the adaptive evolution of female preferences. Thus, natural and sexual selection essentially work in the same direction, in which case the distinction between the two selective forces largely disappears. The debate between the two schools is still continuing.

#### *Joint evolution of signal and signal recognition*

Somewhat independently from the sexual selection theory, two conflicting views exist with respect to the evolution of the signal and its recognition (Butlin and Ritchie, 1989). One theory holds that both the signal and its recognition are determined by the same genes ('genetic coupling'). The other theory does not necessarily assume the existence of a common genetic basis, but instead assumes that the tuning of the receptor to the signal is brought about by the coevolution of both, the signal being tuned to the receptor by selection, and vice versa. Butlin and Ritchie (1989) studied nine cases claiming either the genetic coupling, or the coevolutionary scenario. In most cases it turned out to be impossible to rule out the alternative theory. These authors suggested that the distinction is only useful in systems, which are controlled by a few major loci, rather than in

polygenic systems.

## **Speciation and the evolution of mate recognition characters**

### *Species concepts and speciation*

Many different modes of speciation have been proposed (for an overview see e.g. White, 1978; Futuyma, 1986). The following account concentrates on those ideas, that involve the evolution of mate recognition characters.

Species have long been viewed as groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups, the so-called 'biological species concept' or 'isolation concept' (Mayr, 1963). The process of speciation was considered to be more or less equivalent with the acquirement of isolation mechanisms, intrinsic barriers to gene exchange, working either before or after insemination has taken place (premating and postmating isolation mechanisms). The origin of species-specific mate recognition characters within this theory is thought to have arisen in a reinforcement scenario (see above). The biological species concept has been severely criticized, and several alternative evolutionary (genetic) species concepts have been introduced (review in Templeton, 1989).

Paterson (1985, and earlier papers cited therein) introduced the 'recognition concept', where species are viewed as entities that possess a common specific mate recognition (fertilization) system (see above). Paterson emphasized the recognition and effective fertilization of conspecifics rather than isolation of heterospecifics. According to this view, new species arise, when their specific mate recognition system has changed. Such changes occur by natural selection adapting the mate recognition system to the population's new habitat in small peripheral populations after allopatric separation.

Since in certain organisms hybridization more or less regularly occurs, and many taxa purely or partially reproduce asexually, the applicability of the former two species concepts is limited. Templeton (1989) instead introduced the 'cohesion' concept, stressing the coherence of groups of phenotypes over evolutionary time, despite occasional hybridization or asexual reproduction.

Although sexual selection is typically a within species phenomenon, some people have extended the theory to explain the origin of species-specific traits involved in species recognition (sexual isolation). Lande's (1981) polygenic mathematical model showed that, theoretically, evolution of male sexual characters and associated female preferences in a Fisherian way can result in rapid divergence of both the character and the preference, especially in small, unstable populations, leading to increased assortative mating, and thus speciation. Also West-Eberhard (1983) explicitly suggested that rapid divergence in socially selected characters may accelerate speciation, because populations with diverged signals are preadapted for species recognition by the acquisition of species-specific markers.

Paterson's views on speciation differ from the one given above, in that divergence of signals is brought about by natural rather than sexual selection, and that they are optimally adapted to the species' preferred habitat, whereas the outcomes of sexual

selection are unpredictable.

### *Speciation in planthoppers*

In planthoppers two factors are thought to be primarily responsible for maintaining the integrity of different species: the hostplant, and the acoustic communication system (Claridge and De Vrijer, 1993). Because closely related planthoppers species tend to feed on different hostplants with often different ecological requirements, it is difficult to determine the relative importance of the acoustic signals, as many species probably rarely meet in the field.

Some species exhibit geographical variation for acoustic characters. The best example in this respect comes from studies by Claridge et al. (1985a), who found a significant variability in a male call character (pulse repetition frequency, PRF) among widely separate Asian, Australian and Indopacific populations of *Nilaparvata lugens*. Hybridization success between these populations in the laboratory was negatively correlated with differences in PRF in the male calls. They also found two sympatric, morphologically inseparable species of *Nilaparvata*, to be completely sexually isolated, and to differ greatly in both male and female call characters (Claridge et al., 1988). From these observations it was tentatively concluded that isolation among species is maintained by their species-specific acoustic signals (Claridge et al., 1985a,b, 1988, 1990). However, in the former example populations were allopatric, and in the latter the two species lived on different hostplants. Thus, although the examples show that the acoustic signals *can* contribute to sexual isolation, they do not prove that they *actually* have that effect in the field.

Claridge et al. (1988) used the presence of the huge variability in acoustic characters among *Nilaparvata lugens* populations as an argument against Paterson's (1985) view that components of the mate recognition system should be stable within species. Instead they were inclined to the view that signal differentiation was brought about by sexual selection, without providing the exact mechanisms involved.

Differentiation of acoustic signals in allopatry could potentially be the first step to speciation (Claridge and De Vrijer, 1993). The example of *Nilaparvata lugens* shows that acoustic differentiation and the development of sexual isolation is possible without a shift in hostplant. Also various species of *Chloriona*, all exclusively feeding on reed, are readily recognizable by their acoustic signals (Gillham et al., 1992). However, in more hostplant-specific species like *Ribautodelphax*, an alternative scenario seems equally likely. A shift to a new hostplant would isolate a population effectively from the original population; different selection pressures may produce further genetic differentiation, including changes in acoustic properties, which could e.g. arise by sexual selection. In the latter scenario speciation is mainly brought about by a hostplant shift, but the acoustic recognition system could have an effect in protecting the derived population, when such populations come to live syntopically. If the speciation process is triggered by a change in hostplant, spatial separation of the populations may not be strictly necessary, in which case the speciation process may be either sympatric or allopatric.

### Aims and outline of the thesis

This study was originally initiated to assess the importance of acoustic signalling in reproductive isolation between planthopper species, and its impact on speciation in this group. The genus *Ribautodelphax* has recently been the subject of a multidisciplinary taxonomic study by Den Bieman (1987a). One of the interesting results from Den Bieman's study was that both male and female acoustic calls are species-specific. Therefore it was decided to concentrate on species belonging to the *Ribautodelphax collinus*-complex, in addition to which a distantly related species (*R. albostrigatus*) was used as an outgroup.

In addition to experiments aimed at elucidating the effects of male and female calls and detailed observations on the whole sexual behaviour, it was felt necessary to study the genetic control of the acoustic characters, especially in order to be able to make inferences about the evolution of this acoustic communication system and its contribution to speciation.

In Chapter 2 the importance of species-specific male and female acoustic signals in species recognition is investigated by means of measuring responses of animals to both conspecific and heterospecific playback signals of the opposite sex. It appeared that species recognition during distant calling is mainly brought about by male preference for conspecific female calls, rather than by female preference for conspecific male calls.

Chapter 3 examines whether male preference for conspecific female calls is affected by previous experience of these calls, in other words, whether the male searching response is completely genetic, or partly 'learned'. Males are continuously exposed to playback calls of either conspecific or heterospecific calls during their development from egg to adult, and then tested for their preference for conspecific and heterospecific calls. In addition, completely naive males are tested in this way.

Chapter 4 investigates the genetic control of female acoustic signalling. An important character of the female call, interpulse interval (IPI) is subjected to artificial bidirectional selection. In this way heritability estimates for the character are obtained, and the presence of correlated responses of other female call characters is examined. An estimate is made of the minimum number of genetic factors determining the character selected for. Sexual isolation tests between animals from oppositely selected lines are performed by confining males with females from both their own and from oppositely selected lines. Such males are also tested separately for their preference for female playback calls differing greatly in IPI.

Chapter 5 is aimed at collecting data on the genetic control of several characters which make up the male signal. Heritability estimates for these characters are obtained by means of parent offspring regression. Phenotypic, genetic and environmental correlations among the male call characters are calculated. Also, data on correlated responses of male call characters to artificial selection for female IPI described in the previous chapter are considered. The data obtained in this and previous chapters are used to discuss the evolution of the acoustic mate recognition system of *Ribautodelphax*.

In Chapter 6 the sexual behaviour of various *Ribautodelphax* species as observed

from video recordings is studied, and the importance of acoustic communication in different phases of sexual behaviour is assessed. The sequence of non-acoustic behavioural elements during courtship is described and compared among the different species. In addition, the sexual behaviour of a number of interspecific pairs is examined. Attention is paid to the existence of potential other mate recognition cues than acoustic ones.

**Chapter 7** is a general discussion on the topics raised in the previous chapters.

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# THE IMPORTANCE OF MALE AND FEMALE ACOUSTIC BEHAVIOUR FOR REPRODUCTIVE ISOLATION IN *RIBAUTODELPHAX* PLANTHOPPERS (HOMOPTERA, DELPHACIDAE) <sup>1</sup>

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## Abstract

The importance for reproductive isolation of species-specific acoustic signals between closely related *Ribautodelphax* planthopper species is tested by measuring responses to playbacks of both conspecific and heterospecific signals. Females respond to heterospecific male calls at about 80% of the conspecific response level, irrespective of the degree of cross-insemination of the combination involved. In a combination involving *R. albostrigatus*, a taxonomically more distantly related species, female response levels are only 15-33% of the conspecific level. Study of the development of both female responsiveness and mating receptiveness shows that response levels correspond fairly well with insemination levels. Female heterospecific response is far too high to explain isolation between the species. Female calls in response to heterospecific males do not have deviating response delay-times and durations.

When offered a two-way choice between female playback calls, males significantly more often approach the conspecific call in almost all combinations tested. Offering only a heterospecific female signal induces the male to call, but not to search. Males are capable of maintaining at least part of the sexual isolation by distinguishing between different female calls. This seems in conflict with the theory that the sex with the greater parental investment, here the female, should be exerting the choice.

## Introduction

In recent times it has become increasingly clear that in many groups of arthropods one or the other type of acoustic communication occurs. Apart from the well known taxa which produce sounds audible to the unprepared human ear, many others are now known to communicate by means of low frequency substrate-borne vibrations, e.g. wolf spiders (Stratton & Uetz, 1981), lacewings (Henry, 1986), gerrid water striders (Wilcox, 1972), stoneflies (Zeigler & Stewart, 1986), cydnid bugs (Gogala et al., 1974), leafhoppers, and planthoppers (Claridge, 1985a,b). In many groups these calls are known to be species-specific, and are traditionally viewed as adaptive devices preventing interspecific

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hybridization (Dobzhansky, 1940). Recently, several authors have argued that signalling systems have not been selected to function as premating isolation mechanisms, but have evolved within species as the result of sexual selection, and have received a function in species isolation secondarily (West-Eberhard, 1983, 1984). Or, as viewed by Paterson (1985), they have arisen by adaptation to the species' 'preferred habitat', i.e. by natural selection, ensuring recognition by the conspecific partner. However, the extent to which species-specific acoustic signals alone are capable of preventing interspecific matings has rarely been tested.

In planthoppers (Delphacidae) vibrational signals are produced by both sexes, and apparently serve to bring receptive males and females together (Claridge, 1985a,b). So far as known, male calls are species-specific and have a more complicated structure than female calls. Receptive, virgin females answer male calls by a signal which consists of a series of pulses. Males approach the calling female, which hardly moves once the exchange of signals has commenced. Female planthoppers probably only mate once, in contrast to males.

In *Ribautodelphax* planthoppers, calls of both sexes are species-specific (Den Bieman, 1986). Forced hybridization experiments between species resulted in different insemination levels for different combinations of species, ranging from none to almost complete cross-insemination. Once inseminated, females produce about normal numbers of fertile offspring (Den Bieman, 1988). When offered a choice, interspecific insemination has never been observed, thus the isolation is largely maintained by pre-mating barriers (Den Bieman, 1988; unpublished data).

The European members of the planthopper genus *Ribautodelphax* have recently been subject of a biosystematic study by Den Bieman (1987c). The genus constitutes at least 14 species, 12 of which are closely related and have been referred to by Den Bieman (1987c) as the '*R. collinus*-complex'. Of the two more distantly related species only *R. albostrigatus* was used in this study. This species deviates from the *R. collinus*-complex in morphological, electrophoretic, and acoustic properties, and was never found to hybridize with any of the members of the *R. collinus*-complex (Den Bieman, 1986, 1987c, 1988, Den Bieman & Eggers-Schumacher, 1987). All feed on grasses, but each species is confined to a single species or genus of host plants (Den Bieman, 1987a). Because of this strong ecological differentiation the species probably rarely meet in nature. However, the ranges of many species overlap, and some examples of sympatric occurrence are known (Den Bieman, 1987c).

In the present paper we report on experiments measuring responses of both sexes in a number of *Ribautodelphax* species to playbacks of pre-recorded conspecific and heterospecific calls, in order to exclude possible influences of other recognition cues, like vision and chemical sense. We especially compared combinations of species which either hybridize easily or not at all. The main aim of the present study is to elucidate whether acoustic differences among *Ribautodelphax* species can explain the degree of sexual isolation observed, and what part of the isolation, if any, can be attributed to the different sexes. Results are discussed in the context of current views on sexual selection and sexual isolation, especially the prevailing view that the choosing sex should be the

one with the greatest parental investment, which is usually the female.

Table 2-1. List of *Ribautodelphax* species, host plants, and collection sites of populations used.

Species	Host plant	Population (country, province, locality)
<i>R. albostratus</i>	<i>Poa pratensis</i>	YU, Srbija, Trstenik
<i>R. angulosus</i>	<i>Anthoxanthum odoratum</i>	NL, Utrecht, Leersum
<i>R. collinus</i>	<i>Agrostis capillaris</i>	NL, Limburg, Plasmolen
<i>R. imitans</i>	<i>Festuca arudinacea fenas</i>	FR, Pyr. Or., St. Cyprien
<i>R. imitantoides</i>	<i>Brachypodium phoenicoides</i> (rearing) and <i>F. a. fenas</i> (experiment)	FR, Vaucluse, St. Estève
<i>R. pungens</i>	<i>Brachypodium pinnatum</i>	NL, Limburg, Bemelen
<i>R. vinealis</i>	<i>Agrostis vinealis</i>	NL, Gelderland, Hoge Veluwe

## Materials & methods

### *Populations and rearing*

The species used in the experiments as well as their origins and host plants are given in Table 1. Data on the acoustic properties of these populations are given by Den Bieman (1986, 1987b). All species were reared on their specific host plants in a greenhouse under long-day conditions (18 hours light) at  $20 \pm 2$  °C. Females to be tested against male playbacks were isolated as fifth instars, and were collected within 24 hours after final ecdysis to ensure virginity, and kept separate until they reached the required age. Newly hatched, virgin males were collected from rearing cages within 24 hours after removal of all adults, and were allowed to mature for about 7 days in the absence of females.

### *Female response experiments*

Responsiveness of females to both conspecific and heterospecific male song was first tested for the species *R. albostratus* and *R. collinus*. Independent groups of 7-9 day old females were exposed to pre-recorded signals of either a conspecific or a heterospecific male, with gaps of 5 seconds rest after each playback call or answer. Of each female the number of responses to a series of 10 playback signals was scored. Male calls were played back from a Revox B710 cassette tape deck. Other technical details were as described by De Vrijer (1984). Experiments were carried out in a thermostatic cabinet at  $20 \pm 1$  °C. The male calls used for playback were recorded at the same temperature.

Heterospecific response calling was further studied in *R. imitans* and *R. angulosus* females. Because we did not obtain a 100 % response level of *R. albostratus* females to their conspecific male call, we studied the development of female response to both conspecific and heterospecific male calls. In order to examine the possibility that response calling of isolated, virgin females is not indicative of mate recognition and preference, but an artefact caused by sexual deprivation (Butlin and Hewitt, 1987), we also studied the increase with age of female receptiveness in these species.

The development of receptiveness (readiness to mate) was studied in both species by confining 200 freshly emerged virgin females with a surplus of 5-7-day-old males, divided over eight cages containing the species' host plant. Each successive day a sample of 20 females from one of the cages was examined for the presence of motile sperm in their spermathecae, until an insemination level of 100 % was reached.

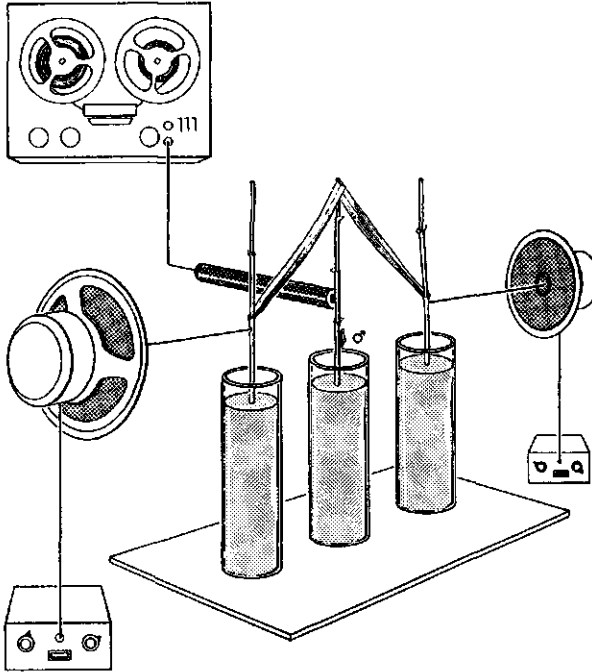
The development of responsiveness in both species was examined by daily testing of independent series of 20 females for response to either conspecific or heterospecific male calls over a period of 0-7 days after final ecdysis. Females of *R. imitans* were tested for calls of male *R. imitantoides*, while *R. angulosus* females received calls of male *R. pungens*. The former combination is known to result in almost 100 % insemination under no-choice conditions, while in the latter combination insemination has never been observed (Den Bieman, 1988). Again, for each female the number of answers to 10 playback signals was measured, with intervals of 5 seconds rest after each playback call or response.

Response-delay times and duration of female responses to both conspecific and heterospecific male playback signals were measured from oscillograms of recordings made during the *R. collinus/R. albostratus* experiments. The male call and the female response were recorded separately on different tape tracks on a Revox B77 MKII tape recorder, and the oscillograms were displayed simultaneously by separate channels of a Siemens Oscillomink. Response-delay times were measured from the start of the buzz-section of the male playback call in both species. Mean call durations calculated per female were given equal weight, even if based on different number of calls, because the within individual variance for this character was significantly smaller than the among individual variance (Den Bieman, 1986).

All *Ribautodelphax* populations used in these experiments were of allopatric origin. As *R. imitans* and *R. imitantoides* can live on the same host plant (Den Bieman, 1987a), these species were reared together for 10 generations, in order to see if forced sympatry would affect heterospecific response levels. Ten pairs of each species were confined in a cage with a *Festuca arundinacea fenas* plant. Each generation a random sample of 20 pairs were taken as parents for the next generation. After this period, 5-6-day-old *R. imitans* females were tested for response to *R. imitantoides* male calls in the same way as described above.

### *Male preference experiments*

Males normally initiate the exchange of acoustic signals between the sexes, but in



**Fig. 2-1.** Set-up of male choice tests, showing three interconnected grass stems. Playback signals of females are played to the outer stems via two small speakers with needles attached to the speakers's coil pressed into the stems. A recording device is attached to the central stem. See text for further details.

addition to calling after a female answer they show searching behaviour, in contrast to females, which hardly move once acoustic contact is established. Therefore a different approach was used in which males were offered a two-way choice between different female playback signals.

Three stems of the hostplant of the species to be tested were connected by their leaves. For each trial a male was placed on the central stem (Fig. 1). Female calls of two species of about equal amplitude were played each to one of the outer stems. Both the playback calls and the male call were monitored and recorded by means of a recently developed electrodynamic transducer (Strübing & Rollenhagen, 1988), attached about halfway up the central stem. Immediately after each call of the male, the female calls were released simultaneously from two digital storage devices with analogous recording and playback functions via two small modified speakers, similar to the ones used in the female playback tests. Usually the male started running up and down, and eventually went to one of the outer stems. When the male remained there for 10 seconds, this was arbitrarily considered to be a choice. The stem from which the conspecific call was broadcasted was altered between trials, in order to exclude a possible preference for one side. When no choice was made after 25 minutes, the trial was aborted, and was not used in the analysis. Each male was tested only once.

In an additional experiment *R. imitans* males were provided with only one playback

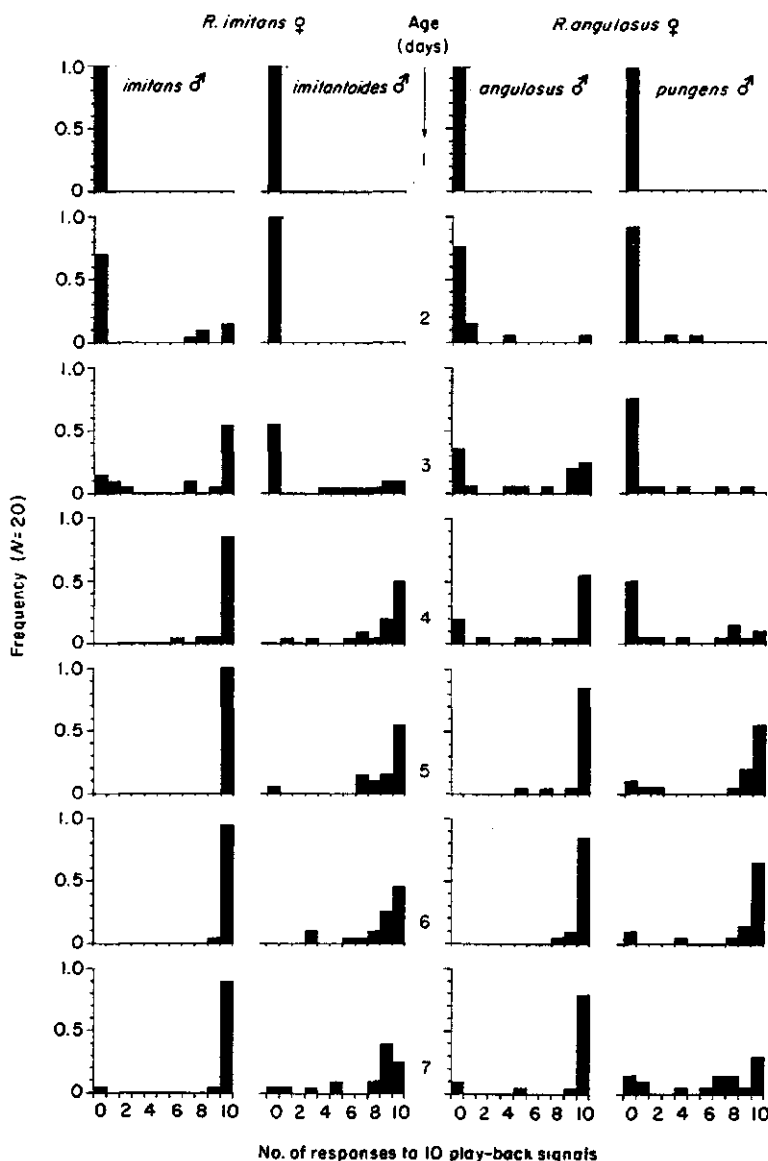


Fig. 2-2. Frequency distributions of female response to playback calls of males. The first two columns show the responses of *R. imitans* females to the calls of resp. *R. imitans* and *R. imitantoides* males. The third and fourth column show the responses of *R. angulosus* females to calls of resp. *R. angulosus* and *R. pungens* males. Each row represents the female response at certain age, from 1 to 7 days after final ecdysis.

signal, either that of *R. imitans* or *R. imitantoides*.

## Results

### Female responsiveness and receptiveness

The increase of female receptiveness with age as measured by insemination levels is shown in Figs. 2 and 3. Insemination of *R. imitans* females started at an age of 2 days. At 5 days old all females were mated. In *R. angulosus* insemination started from 3 days onwards, and 100 % insemination was reached at an age of 6 days.

Development of responsiveness to the conspecific male for *R. imitans* and *R. angulosus* is illustrated in the first and third columns of Fig. 2 respectively. In both species all individuals turned out to be mated at the age at which the maximum response level was reached. When the maximum response level obtained is taken as a measure of responsiveness, we can express the responsiveness at any age as the proportion of individuals responding 8 or more times to a series of 10 playback signals. For both species, the increase of response with age according to this measure corresponded fairly

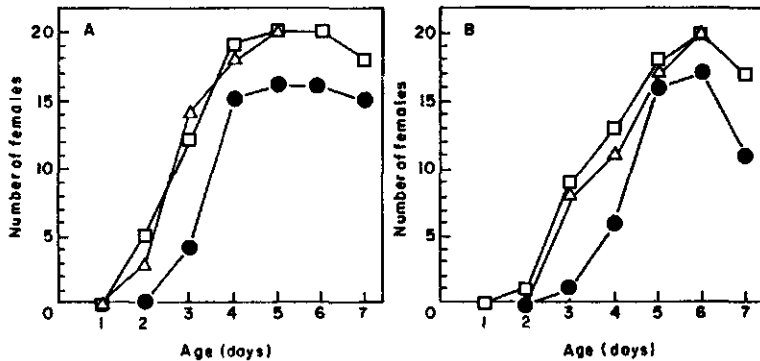


Fig. 2-3. Increase with age of insemination levels and female response calling in *R. imitans* (A) and *R. angulosus* (B). Response is expressed as the number of females responding 8 to 10 times to a series of 10 playback calls of males. Triangles, number of females inseminated; squares, response to conspecific males; circles, response to heterospecific males, *R. imitantoides* (A) and *R. pungens* (B).

well with the increase of receptiveness with age (Fig. 3).

### Female response to heterospecific males

Response data for *R. imitans* and *R. angulosus* females to conspecific and heterospecific male playback calls are given in the second and fourth columns of Fig. 2 respectively. In both species the increase of response with age to the heterospecific male was slower than in the conspecific situation, but the maximum response was attained at the same age. In both heterospecific combinations the response was rather high, 80 and 85 % respectively of that in the conspecific combinations (Fig. 3). After the maximum response level was reached, there was a certain decline in response in all combinations

with further increase of age. This drop was most pronounced in *R. angulosus*.

The response of 7-9-day-old *R. albostratus* and *R. collinus* females to each others males is displayed in Fig. 4. The responsiveness of *R. collinus* females to *R. albostratus* males was only 0.15, while that of *R. albostratus* females to *R. collinus* males was 0.33.

Frequency distributions of response-delay times for *R. albostratus* and *R. collinus* females are given in fig. 5. Response-delays of *R. albostratus* females to *R. albostratus* and *R. collinus* male calls were not significantly different ( $t=1.318$ , 47 df, ns), whereas *R. collinus* females answered significantly faster to playback signals of their own male

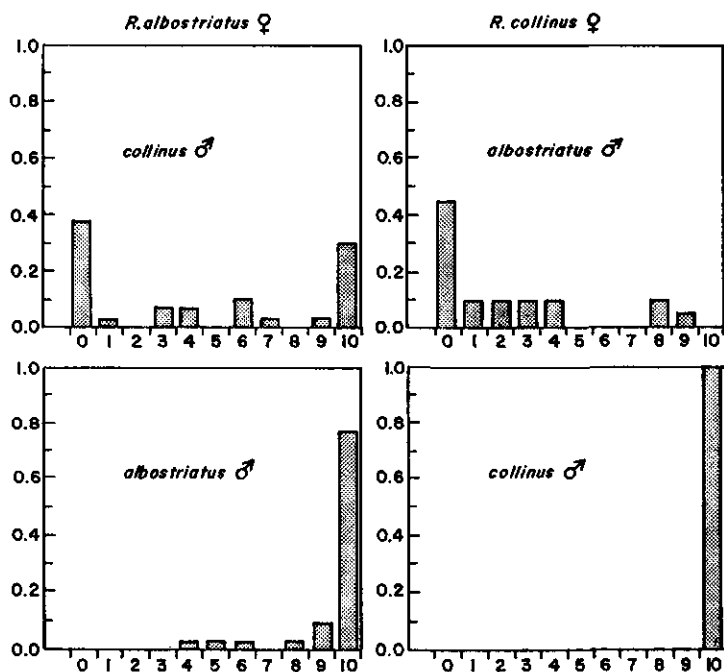


Fig. 2-4. Frequency distributions of response of 7-9-day-old *R. collinus* ( $N=20$ ) and *R. albostratus* ( $N=30$ ) females to playback calls of conspecific and heterospecific males.

than to those of *R. albostratus* ( $t=2.381$ , 31 df,  $p<0.05$ ). Because female call duration in *R. albostratus* is considerably longer than in *R. collinus*, call durations were only measured for *R. albostratus* females. Call durations in response to *R. albostratus* and *R. collinus* male signals were not significantly different ( $t=0.547$ , 44 df, ns).

#### Male choice tests

The combinations of species tested and the results obtained are shown in Table 2. In



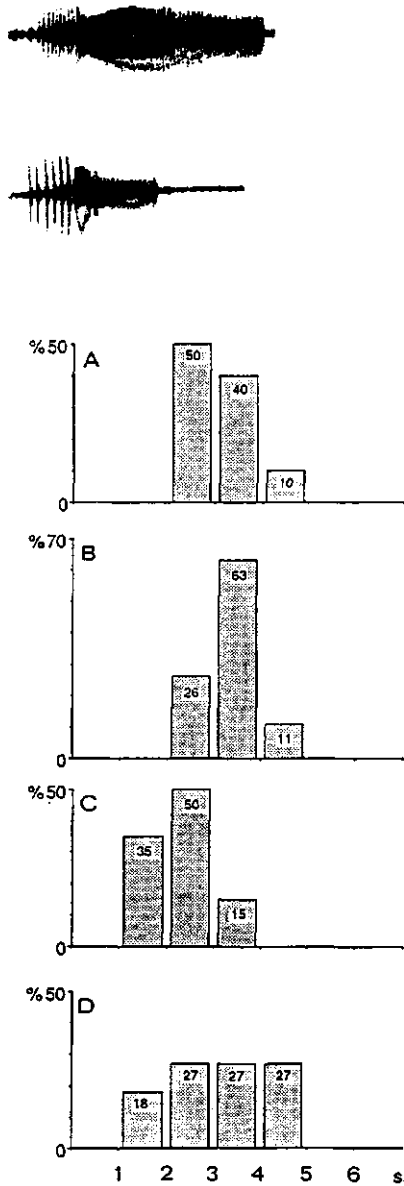


Fig. 2-5. Frequency distributions of mean response-delay times of *R. albostratus* and *R. collinus* females to conspecific and heterospecific male playback calls. A, *R. albostratus* conspecific response (N=29); B, *R. albostratus* response to *R. collinus* male call (N=18); C, *R. collinus* conspecific response (N=20); D, *R. collinus* response to *R. albostratus* male call (N=11). Oscillograms of male *R. albostratus* (upper) and *R. collinus* (lower) calls are displayed at the same time scale.

all but one combinations, the males chose significantly for the conspecific female call. *R. imitantoides* males showed a slight, but non significant preference for the conspecific call when tested against *R. imitans* signals.

Table 2-2. Phonotactic responses by males in choice experiments in which males were simultaneously offered the playback calls of a conspecific and a heterospecific female. The last column gives the significance value of a two-tailed binomial test.

Male/female 1	Female 2	Number of choices made for		P
		female 1	female 2	
<i>R. angulosus</i>	<i>R. pungens</i>	34	2	0.01
<i>R. pungens</i>	<i>R. angulosus</i>	11	1	0.02
<i>R. albostratus</i>	<i>R. vinealis</i>	17	4	0.02
<i>R. imitans</i>	<i>R. vinealis</i>	22	1	0.01
<i>R. vinealis</i>	<i>R. imitans</i>	31	3	0.01
<i>R. imitans</i>	<i>R. collinus</i>	24	6	0.01
<i>R. imitans</i>	<i>R. imitantoides</i>	32	1	0.01
<i>R. imitantoides</i>	<i>R. imitans</i>	12	6	N.S.
<i>R. vinealis</i>	<i>R. angulosus</i>	13	2	0.01

The experiments in which *R. imitans* males were offered only either the conspecific or heterospecific female call revealed a drastic difference in male behaviour in response to these calls. Males (n=20) provided with *R. imitans* calls searched very actively, and all went to the correct side. Males provided with only the *R. imitantoides* call (n=10) answered consistently, but hardly moved. None of these made any choice within 25 minutes. After this period they were provided with their own female signal, in response to which they all started searching very actively, and went to the side from which the call was broadcasted within 7 minutes.

Males which did not make a choice within 25 minutes could not be reactivated by providing them with only the conspecific female call after the trial, in contrast to normally receptive males. The numbers of such unreceptive males were subject to periodical, sometimes daily fluctuations, for reasons unknown. They were therefore not included in the analysis.

## Discussion

### *Female discriminatory ability*

The experimental setup of the female preference experiments reported here differs from most other playback studies in that different male calls were not played to the same

female. This was done to exclude a possible influence of prior experience with either a conspecific or heterospecific call. Females have been reported to be less selective to a heterospecific call after prior exposure to a conspecific signal in e.g. crickets (Zaretsky, 1972) and stoneflies (Zeigler & Stewart, 1986). Both inhibition by heterospecific calls and excitation by conspecific calls were observed in some (but not all) *Ribautodelphax* females during preliminary experiments.

The good correspondence between the increase with age of responsiveness and receptiveness in both *R. angulosus* and *R. imitans* females can in our opinion be taken as confirmation that acoustic response is a measure of female readiness to mate, and that responses of isolated females are not artefacts caused by sexual deprivation.

The increase of response and insemination levels with age in *R. angulosus* is consistently one day delayed compared to *R. imitans*. These observations suggest that comparing such data from females of different, closely related, species at the same age, as is common practice, is not without risk.

After the maximum response level has been reached, there was a drop in response to both the conspecific and heterospecific male calls. Decrease of conspecific response after a certain age was also reported by Kumar and Saxena (1985) for the leafhopper *Amrasca devastans*. It seems unlikely that females remain unmated long enough in nature for such a decline to occur. However, the non-100 % response of the 7-9-day-old *R. albostratus* females to conspecific male playback calls may be explained by assuming that these were not at their optimal age.

Of the four combinations of species studied here three are known never to result in heterospecific insemination. Only the combination *R. imitans*/*R. imitantoides* resulted in almost complete insemination when offered no choice. When offered a choice, interspecific insemination never occurred, however (Den Bieman, 1988; unpublished results). Taxonomically *R. albostratus* stands somewhat apart from the so-called *R. collinus*-complex (Den Bieman, 1987c), to which all other species studied here belong, and has a somewhat different male call with obscure, not clearly separated chirps, as well as a much longer buzz-section (Den Bieman, 1986). Female responses in combinations involving *R. albostratus* females or males were much lower than in any of the other combinations, where female responses reached about 80 % of the normal response level, regardless of the potential crossability of the combination involved. The female response levels to playback calls of alien males are far too high to be consistent with the isolation observed, and consequently, the species-specific acoustic traits of *Ribautodelphax* males appear to contribute little to species isolation, at least during distant calling.

During the whole development of heterospecific response there was always a large fraction of individuals either virtually non-responding (0-2 responses) or all-responding (8-10 responses). The fraction of females responding between 3 and 7 times, representing 45 % of the response classes, constituted at most 20 % of all females at any age. At about the maximum response level there were still 5-25 % weakly or non-responding females. These were tested against their own male call, to which all responded maximally. Thus, there always appeared to be a small fraction of selective

females present in the population.

*Ribautodelphax* species rarely live syntopically (Den Bieman, 1987c), and all populations used in these experiments were of allopatric origin. Therefore, one could argue that they never have been exposed, or are no longer exposed, to selection for precise recognition of species-specific acoustic characters. It should be kept in mind that in all species *Ribautodelphax* male calls have a similar basic pattern. One can envisage that under sympatric conditions the selective females are at an advantage and quickly outcompete the non-selective ones, either by producing superior non-hybrid offspring, or just by being more efficient with time and energy. However, rearing *R. imitans* and *R. imitantoides* together for 10 generations failed to show any increase in the fraction of selective *R. imitans* females. From the signals of males and females of the mixed culture there was no reason for suspecting hybridization to have taken place. After 10 generations *R. imitans* individuals were about twice as numerous as *R. imitantoides*. This again shows the strength of the pre-mating barriers.

A number of non-selective females were exposed to the calls of species of the delphacid genera *Javesella* and *Delphacodes*, but never responded once to a series of 10 calls. Even highly excited, spontaneously calling females immediately ceased calling after hearing non-congeneric calls, thus again showing that females were selective to some extent.

### *Response-delay times and call durations*

It has been argued that female answers to heterospecific male signals, if occurring at all, differ from those to conspecific calls in such a way as to prevent males from continuing courtship. Species-specific response-delay times have been reported in e.g. some phanopterid bushcrickets (Heller and von Helversen, 1986), fireflies (Lloyd, 1968), and Neuroptera (Rupprecht, 1975; Henry, 1986). Claridge et al. (1985b) reported in the planthopper genus *Nilaparvata* that females answer males of other taxa with delayed, less vigorous, and shorter calls.

It is difficult to interpret the response-delay data of *R. albostrigatus* and *R. collinus* females. Response-delay times of *R. albostrigatus* females to both own and alien male calls were about the same, in contrast to those of *R. collinus* females. However, *R. collinus* females joined *R. albostrigatus* male calls at about the correct place, whereas *R. albostrigatus* females on average answered *R. collinus* male calls later than did *R. collinus* females. All heterospecific response delays were within the range of the conspecific ones, and cannot be considered as atypical.

Several authors (e.g. Claridge et al., 1985b; Henry, 1985) have reported that call durations of females in response to alien calls were shorter than to the conspecific call. An attempt to confirm this quantitatively for *R. albostrigatus* females failed. This might be caused by the use of different experimental designs: in the studies mentioned the same female was exposed to both conspecific and heterospecific calls, whereas in the present study different calls were played to different females.

### Male discriminatory ability

The male choice experiments show that males can distinguish between female calls of different species, and that males do make a choice based on species-specific acoustic characters only. Ichikawa et al. (1975) also reported that in three species belonging to three different genera of rice attacking planthoppers, males showed only behavioural responses to calling females of their own species. However, as their experiments were carried out with live females, non-acoustical cues cannot be ruled out completely. Claridge et al. (1985b) found males of the related planthopper genus *Nilaparvata* to respond much better to playback calls of females of their own population.

The fact that *Ribautodelphax* males do call after a heterospecific female signal, but do not search, suggests that change in locomotor behaviour may be a better measure of male responsiveness than response calling only, at least in this genus, but probably also in other planthoppers.

Playback of two different calls simultaneously did not seem to affect seriously the discriminatory ability of the males. Confusion of phonotaxis by masking sounds has been reported in air-borne sound producing animals like frogs and bushcrickets (e.g. Dyson & Passmore, 1988; Bailey & Morris, 1986). In these examples phonotaxis is performed by females towards the calling males. We have no explanation for this difference in liability to confusion. It may have to do with the substrate-borne mode of transmitting sound waves, or with the simple structure of the female planthopper signal, which consists basically of a series of regularly repeated pulses.

Species-specific differences in female signals are produced by different combinations of few parameters, like pulse rate, strophe duration and modulations of pulse rates within a strophe (Den Bieman, 1986). Wave pattern of each separate pulse has been proposed by Ichikawa et al. (1975) as an additional species-specific cue, but this character has been shown to be subject to modification by non-biological parts of the system (Michelsen et al. 1982; De Vrijer, 1984, 1986). One wonders if so few, sometimes quite variable characters can constitute unequivocal recognition cues, in view of the large number of delphacid species existing, which probably all produce female songs of somewhat similar series of pulses. We speculate that female signals are only functional within a group of closely related species, say a genus. *Ribautodelphax* females were found to respond only to congeneric male calls, thus adequately preventing the attraction of males of unrelated species with similar female calls.

### Importance and origin of species-specific calls

The present study shows that species-specific differences in calling songs can have a certain role in species isolation. The question arises whether they still have that role in *Ribautodelphax*. As mentioned earlier, *Ribautodelphax* species are strongly isolated by their different ecological requirements, as the result of which they probably rarely meet. However, the ranges of many species largely overlap, and the sympatric occurrence of two or three species has been documented, and was probably more common in the past (Den Bieman, 1987c, personal communication). In addition, there is good evidence that

post-copulatory isolation is at best poorly developed (Den Bieman, 1988), so in certain situations acoustic cues may have an isolating effect in nature.

We are not implying that acoustic cues are the one and only cause of the complete premating isolation between *Ribautodelphax* species. Distant calling between the sexes is the first of several steps in a sequence of events leading to copulation in planthoppers (Booy, 1982). At close range other cues like chemical signalling are likely to be important as well, as suggested from video-observations of courtship (unpublished data). Nevertheless, from an economic point of view early recognition of a conspecific partner is likely to be, or has been, an important force shaping and/or maintaining the species-specific acoustic features, that is, when potential errors were frequent enough.

The lively debate on the possibility of speciation by reinforcement has not yet been settled, but most authors now seem inclined to believe that differences in recognition cues were not selected for the specific purpose of species isolation (e.g. Paterson, 1978, 1985; Butlin, 1985). Rather, they are thought to have that effect secondarily after character divergence arose by social competition for mates within the species (sexual selection), possibly enhanced by runaway selection (West-Eberhard, 1983, 1984), or, by selection adapting the signal to the specific habitat occupied by the species, ensuring effective recognition by the conspecific partner (Paterson, 1985). Unfortunately, few recent papers on sexual selection have extended this theory in order to explain the origin of species-specific characters used in species isolation, and almost all have focused on the evolution of male characters by female choice (e.g. Lande, 1981; Kirkpatrick, 1982, 1987), neglecting the existence of species-specific female features. The reason for this may be that the traditionally best studied animals in this respect are those in which females approach signalling males, as, for example, many Orthoptera, Cicadidae, Anura and birds. The behaviour of these animals is in agreement with the established idea, that the sex with the higher parental investment, usually the female, should be more choosy (Trivers, 1972).

We feel that the importance of male choice in species isolation is strongly underestimated. In many insects males are the active sex, and female signalling is a common feature in many groups (Thornhill & Alcock, 1983). Examples which suggest that sexual isolation is (partly) accomplished by male choice include diverse groups like drosophilid flies (von Schilcher & Dow, 1977; Wood & Ringo, 1980; Hoikkala, 1986), *Ips* beetles (Barr, 1969), *Photinus* fireflies (Lloyd, 1968), *Calopteryx* dragonflies (Waage, 1979), some phanopterid bushcrickets (Heller and von Helversen, 1986), and Lepidoptera (Roelofs & Cardé, 1977).

In *Ribautodelphax* there is no obvious reason for assuming a relatively greater parental investment by males than by females. Females mate only once, while males can inseminate several females during their lives. Observations of courtship suggested that females exert a choice among competing conspecific males (unpublished results). Den Bieman (1988) tentatively explained the differentiation in female calls by the relatively low insemination capacity of *Ribautodelphax* males, compared to that in some other planthopper genera. However, there too males are reported to be selective (Ichikawa et al. 1975; Claridge et al. 1985b).

Von Schilcher & Dow (1977) described a simple qualitative model explaining the

origin of male choice in species isolation in groups where males are the initiating sex in courtship. According to this model, after divergence in signalling systems has arisen in both sexes, the importance of male choice in sexual isolation will gradually increase at the expense of female choice, because it is advantageous to recognize conspecific mates at an early stage of courtship. Eventually we arrive at the situation where both sexes produce species-specific calls, but the sex with the lower parental investment actually exerts the interspecific choice. The model predicts that in evolutionary younger groups the importance of female choice will be greater, which may be correct for the situation in *Nilaparvata*, as described by Claridge et al (1984, 1985a,b). If the premises of the model prove to be correct, it may serve to explain the situation occurring in *Ribautodelphax*, where male choice seems more important in sexual isolation, while female choice may be more important in sexual selection.

Perhaps we should conclude from the conflict between the findings in *Ribautodelphax* and the assumptions of the sexual selection theory, that female acoustic differentiation has not arisen by sexual selection at all. Alternatively, it may be considered as support for Paterson's (1985) view, that mate recognition systems arose by natural selection, and that female acoustic signalling and male searching behaviour are adaptations to the habitat occupied by planthoppers, i.e. grasslands and its plant species composition.

Another explanation may be provided by De Jong's (1988) reasoning for systems, in which females mate only once in contrast to males. In contrast, for example, to Lande's (1981) model, this model allows for sexual selection on both sexes. However, sexual selection will be more intense on males, because males can perform many more matings during their lives. As females mate only once, the more attractive females first disappear from the population of available mating partners, which causes males to encounter relatively more unattractive females. This leads to a situation in which variances for mate recognition characters will be far greater in females than in males. If we apply this to the response of both sexes to each other signals, we expect the female response to be less specific than that of males. As interspecific interactions are expected to be rare in nature, this might provide an explanation for the relatively aspecific response of females, as well as for the more specific response of males in *Ribautodelphax*. Data on variation of the acoustic characters themselves will be provided in subsequent papers.

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# DIFFERENCES IN PREFERENCE FOR SPECIES-SPECIFIC FEMALE CALLS BETWEEN ACOUSTICALLY EXPERIENCED AND ACOUSTICALLY NAIVE MALE *RIBAUTODELPHAX* PLANTHOPPERS (HOMOPTERA, DELPHACIDAE) <sup>1</sup>

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## Abstract

Males of the planthopper *Ribautodelphax imitans* were exposed to playbacks of either conspecific or heterospecific (*R. imitantoides*) female calls during their development from egg to adult, and thereafter these, as well as naive males, were offered a two-way choice between these calls. Males of all treatments approached the conspecific call significantly more often. However, males primed by the conspecific call chose the heterospecific call almost four times less often than did males primed by heterospecific calls or naive males, thus showing that the preference for conspecific calls can be partly 'learned'. Males primed by heterospecific calls performed very similarly to completely naive males, suggesting that the signal recognition mechanism is much less sensitive to heterospecific calls than to conspecific calls. Males with experience of the conspecific female call tended to take more time to reach the call source in the trials than both other types of males. The evolutionary implications of these findings are discussed.

## Introduction

In recent years there has been an increasing interest in the acoustic behaviour of small cicadas (Auchenorrhyncha), especially planthoppers. A number of studies have been devoted to the importance of species-specific planthopper calls in assortative mating (review in Claridge, 1985; Claridge et al., 1985b; De Winter and Rollenhagen, 1990; Heady and Denno, 1991). These studies did not address the possibility that planthoppers respond differently to calls depending on past experience. Consequently, the planthoppers to be tested for their call preferences are usually isolated from the rearings only as last instars or freshly emerged adults. However, although these animals are 'virgin', it remains possible that experience of conspecific calls prior to the actual experiments could have had an influence on their mate preference. Such might especially be expected for animals from rearing cages, which are often inhabited by some hundreds of animals in

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different developmental stages, including sexually receptive, calling ones. In several other insect groups 'learning' has been reported to affect various aspects of behaviour (see e.g., Papaj and Prokopy, 1989). More specifically, studies in crickets (Shuvalov et al., 1990, and references therein), and in some *Drosophila* species (O'Hara et al., 1976; Sene, 1977; Pruzan et al., 1979) appeared to show that mate preferences or mating success can be influenced by prior experience of sexual signals or matings. Therefore, it seemed worthwhile to explore this possibility in planthoppers.

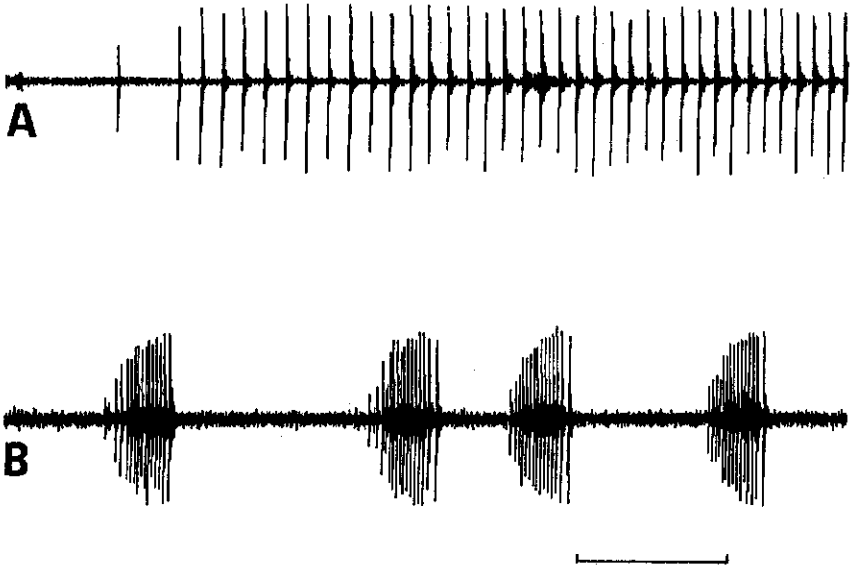


Fig. 3-1. Oscillograms of female calls of *Ribautodelphax imitans* (A, in part) and *R. imitantoides* (B) used for priming and in the call preference experiments. Scale 1 s.

Planthoppers communicate by means of substrate-borne acoustic signals (Claridge, 1985). In all species investigated so far, both sexes have been shown to produce calls, each of a different structure. Males usually call first, whereupon receptive, virgin females respond. After acoustic contact is established, the male exhibits an active searching behaviour for the female, while the female remains stationary during the exchange of calls (Claridge, 1985 and references therein).

In the planthopper genus *Ribautodelphax* the calls of both sexes are species-specific (Den Bieman, 1986). In a previous study De Winter and Rollenhagen (1990) showed that in a congeneric choice situation a large majority of males approaches only the conspecific female playback call, whereas most females respond by calling equally well to both conspecific and heterospecific male calls. Thus in this genus assortative mating seems to be at least partly brought about by the preference of males for conspecific female calls. Consequently, both the call and the call preference are to be considered as part of the specific mate recognition system (Paterson, 1985) of these species. Although

in the field the species of the genus *Ribautodelphax* are monophagous on different plant species, two closely related species used in this study, *R. imitans* and *R. imitantoides*, can be reared in the laboratory successfully on the same hostplant (Den Bieman, 1987). These species exhibit a considerable incidence of hybridization under no-choice conditions (Den Bieman, 1988), but appear to mate completely assortatively in choice situations (Den Bieman, 1988, De Winter and Rollenhagen, 1990). Their female calls differ profoundly in strophe duration, interpulse interval, and pattern of change in pulse rate within the strophe (Fig. 1). Usually, *R. imitantoides* females produce several short strophes in response to a male call, in contrast to *R. imitans* females, which normally respond by one much longer strophe. As far as known, females mate only once during their lives, whereas males can mate several times (Den Bieman, 1988). A mated female no longer responds to the calls of males.

In the present study data are provided which indicate that previous experience of a conspecific call does influence the behaviour of males when given a choice between both conspecific and heterospecific female playback calls.

## Methods

The origin and rearing conditions of the populations of the planthoppers used are explained elsewhere (De Winter and Rollenhagen, 1990).

In three cages containing the species' hostplant, *Festuca arudinacea fenas*, ten pairs of sexually mature *R. imitans* were allowed to mate and deposit eggs. After 10 days all adults were removed, that is, before the first eggs hatched. The plants in each of two of the cages were connected to a digital storage device, which has analog recording and playback functions as well as a timer. From these devices a prerecorded call of *R. imitans* (Fig. 1A) was broadcast to one cage, and a call of *R. imitantoides* (Fig. 1B) to the other cage. These signals were transmitted to the plants via a small modified speaker, in which the paper cone had been removed, and a needle with one end attached to the speakers' coil and the other end pressed into the plant (Claridge et al., 1985a). The planthoppers were continuously exposed to the calls during the entire development from egg to adult at 2-min. intervals. The third cage received no calls, and the naive male offspring served as a control. Care was taken to remove any emerging adult females within 24 hours, because some females can be induced to call when only 2 days old (De Winter and Rollenhagen, 1990). Adult males were isolated from the cages within 24 hours after final ecdysis, and were kept separate for 6 to 8 days until being tested for their preference. During this period the acoustic treatment was continued in the same manner. Like most planthoppers, *R. imitans* possess a wing length polymorphism. In principle males were chosen at random with respect to wing form, but in practice more than 98 % of all males tested were brachypterous.

Male preference tests were conducted as outlined in De Winter and Rollenhagen (1990). Individual males were placed on the central stem of three interconnected grass stems of the hostplant. After each call of the male, the female playback calls of the two species were broadcast simultaneously to each of the outer stems respectively, from two devices similar to that used for priming. Calls were monitored by a recording device

attached to the central stem. Most males reacted typically, sooner or later, by running up and down and, eventually, moved to one of the outer stems. Preliminary experiments revealed that many males visited both outer stems for just a few seconds after which they moved on. On the other hand, males often left even the stem broadcasting the conspecific call after a period of more than 10 s, apparently after having ascertained that no female was present on that stem. Therefore staying for at least ten seconds at one of the outer stems was considered somewhat arbitrarily as a 'choice'. If after 15 minutes no choice was made, the trial was aborted. Each male was tested only once.

## Results

The results of the preference tests are given in Table I. In all three treatments males significantly more often approached the conspecific call (two-tailed binomial test,  $P < 0.01$  in all cases). However, males primed by their conspecific female call chose significantly more often for the *imitans* call than did males primed by the *imitantoides* call ( $X^2$  test for two independent samples,  $X^2 = 7.23$ ,  $P < 0.005$ ) or completely naive males ( $X^2 = 6.85$ ,  $P < 0.005$ ), the fraction of males choosing the heterospecific call being about four times smaller in males with experience of the conspecific female call than in both other treatments. The choice behaviour of males primed by the *imitantoides* call did not differ from that of the naive males ( $X^2 = 0.24$ , NS). Less than 7 % of the males tested did not make a choice within 15 minutes for unknown reasons, but as this

**Table 3-1.** Preference tests of *Ribautodelphax imitans* males, with different call experiences before trials, for either conspecific or heterospecific (*R. imitantoides*) female calls.

	Percentage of choices for			N
	<i>imitans</i>	<i>imitantoides</i>	no choice	
Experience:				
<i>imitans</i>	84.4	8.9	6.7	45
<i>imitantoides</i>	64.5	32.9	2.5	79
naive	63.1	32.3	4.6	65

occurred about equally in all three groups, these were not considered in the analysis.

The durations of the different trials are summarized in Fig. 2. *R. imitans* males

previously exposed to the conspecific female call took a significantly longer time to reach the call source than those exposed to *imitantoides* (one-tailed Mann-Whitney test,  $Z = 3.32$ ,  $P = 0.0004$ ), and took nearly significantly more time than naive males ( $Z = 1.43$ ,  $P = 0.076$ ). The latter two groups also differed somewhat (two-tailed test  $Z = 2.03$ ,  $P = 0.043$ ). The time taken by the males choosing the conspecific call (Fig. 2, black bars) did not differ from the time taken by those approaching the heterospecific call (shaded bars) in any of the treatments; the largest difference occurred in the group exposed to *imitantoides* ( $Z = 1.19$ ,  $P = 0.23$ ).

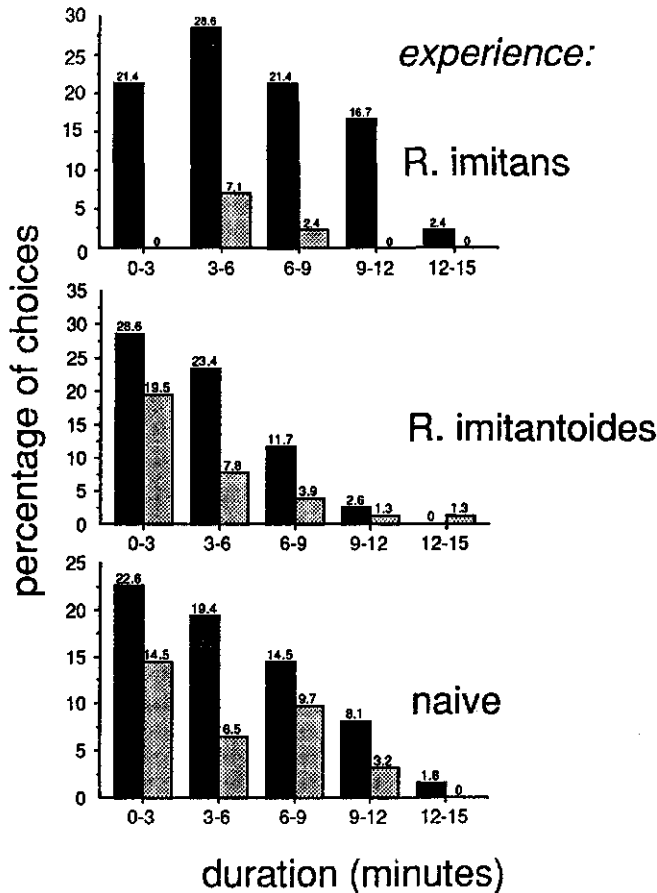


Fig. 3-2. Frequency distributions of durations of call preference experiments of *Ribautodelphax imitans* males with different acoustic experience. Black bars: conspecific choices. Shaded bars: heterospecific choices.

### Discussion

From the above results it appears that previous contact with the conspecific female call considerably improves the choice behaviour of naive males. In other words, the preference of males for conspecific female calls is largely genetically determined but can, to a certain extent, be modified by previous experience. The results of the males primed with *R. imitans* female calls did not differ significantly from those of *R. imitans* males in a previous experiment (De Winter and Rollenhagen 1990), where 31 *R. imitans* males (reared in continuous unispecific culture) were found to approach the conspecific female call but only 1 that of *R. imitantoides* ( $X^2 = 0.43$ , NS). This suggests that these males had experienced calls of conspecific females prior to the trials. As these had been isolated from the cultures within 24 hours after final ecdysis, it follows that the priming must have affected their choice behaviour either during their nymphal stages, or on the very first day of their adult lives. De Winter and Rollenhagen (1990) found a less specific response for *R. imitantoides* males confronted with the same two female calls compared to other species combinations tested (6 'wrong', 12 'good' choices), which resembles the results of inexperienced *R. imitans* males reported here. There is some reason to assume that those *R. imitantoides* males could have been acoustically naive indeed: at the time these experiments were carried out, the *R. imitantoides* rearings were not running quite as well as those of the other species and were much less crowded, and thus there might have been few, if any, virgin calling females present when the males to be tested emerged. This illustrates that the results of signal preference experiments with planthoppers of unknown experience should be treated with some caution.

Another, perhaps more surprising, finding is that the choice behaviour of male planthoppers seems not to be influenced by prior experience with heterospecific calls. The very similar results of males exposed to the heterospecific call to those that were totally inexperienced appear to indicate that the call recognition mechanism of this species is rather insensitive to heterospecific calls. This is in agreement with the earlier observation that, after offering only playbacks of heterospecific female calls, males do call, but rarely start searching (De Winter and Rollenhagen, 1990). It might explain the finding that males are not confused by the complex pattern of pulses that arises when two different calls are played simultaneously through the same substrate (De Winter and Rollenhagen, 1990).

Exposing males to a conspecific female call prior to the trial did not generally lead to a decrease in the duration of the time taken to reach the call source, which might have been expected if this represents a simple case of sensitization (in the sense of McGuire, 1984). On the contrary, males primed by a conspecific female call tended to take more time than other males, which generally appeared to be due to their less 'fanatic' searching behaviour. Shuvalov et al. (1990) found female crickets with experience of conspecific calls to exhibit a greater selectivity and a lower locomotor activity level towards deviating calls compared to inexperienced ones. Their experiments may not be directly comparable, as the female crickets were not given a simultaneous choice between different calls. However, these results suggest the possibility that the presence of an alien call may have a stronger inhibitory effect on animals having previously



experienced a conspecific signal than on naive animals. In other words, experienced animals may be more 'cautious' in the presence of heterospecific calls than inexperienced ones. A second explanation for this observation is that in the males exposed to the conspecific female call, habituation to the signal has occurred, especially as potential attempts to find the sound source during the treatment never were rewarded; in both other treatments males heard the conspecific call for the first time at the actual trial. Several other explanations are possible, but at present there are no data supporting them.

Active searching behaviour for calling receptive females is a highly characteristic feature of male planthoppers. As mates constitute an all-important resource, and as searching potentially incurs costs in terms of risk of predation and energy, mate searching behaviour is likely to be shaped by selection for efficiency and accuracy (Bell, 1990). Therefore this partial plasticity in the male preference for female acoustic signals seems to be somewhat surprising. It also appears to be in conflict with Paterson's (1978, 1985) expectation that the recognition of a species-specific signal is to be highly stereotyped. In Paterson's view signal and signal recognition are very much coadapted parts of the specific mate recognition system, and as such should be under strong stabilizing selection.

It is difficult to see the importance of this 'learning' process in stable field populations. It can be considered as being part of the normal maturation process of males, except at the very start of the season, when no calling females are present when the first adult males emerge. The partial plasticity in call preference is probably maintained in this species as a selectively neutral character, because in the field the chances of mating a heterospecific female are small; not only are *Ribautodelphax* males apparently less sensitive to heterospecific signals, but also the different species of this genus probably rarely meet because of their hostplant specificity (Den Bieman, 1987). In this situation the 'learning' ability appears to be of importance mainly for the experimenter, as illustrated above.

However, the phenomenon could be potentially advantageous during the process of allopatric splitting of populations. Variation in female call characters in *R. imitans* have been shown to be highly heritable (De Winter, 1992), and can thus be expected to deviate rapidly under changing selection pressures and/or by chance after a founder event. The considerable geographic variation in call characters in the related planthopper *Nilaparvata lugens* (Claridge et al, 1985a) could be an example of this. While considering founder event speciation theory, Templeton (1980) listed a set of population attributes favouring what he calls 'genetic transience'. He argued that speciation after a founder event can be more likely if a mate recognition system is partly learned instead of completely genetic. This would allow the naive offspring of a single founding female to cope with aberrant mate recognition signals arising either by environmental or genetic causes in the new habitat. Such a reasoning could well apply to *Ribautodelphax*, especially because other characteristics of these planthoppers also appear to support a founder event speciation model for this group (De Winter, in preparation).

It is a matter of debate whether the improvement of choice behaviour after acoustic experience is to be viewed as learning. Many definitions of learning exist. Papaj and Prokopy (1989) considered processes such as imprinting not to be learning, because of

their irreversible nature. However, too little is as yet known about the phenomenon described here to be able to classify it with any of the numerous types of noninherited behaviour that are currently recognized in the literature.

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# THE GENETIC BASIS AND EVOLUTION OF ACOUSTIC MATE RECOGNITION SIGNALS IN A *RIBAUTODELPHAX* PLANTHOPPER (HOMOPTERA, DELPHACIDAE)

## 1. THE FEMALE CALL <sup>1</sup>

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### Abstract

Both sexes of the planthopper *Ribautodelphax imitans* produce species specific acoustic signals. Earlier experiments have shown that isolation between *Ribautodelphax* species in captivity is at least partly due to male preference for calls of conspecific females. The genetic basis of the female call is studied by bi-directional artificial selection for large and small interpulse intervals (*IPI*). This resulted in non-overlapping distributions of *IPI* after only five generations. The mean of eight realized heritability estimates over five generations was above 80 %; estimates over ten generations were generally well above 50 %. The character is shown to be of a polygenic nature, determined by at least 6 segregating genetic factors. The other features of the female call, strophe duration, and modulation of pulse repetition frequency within the strophe, showed significantly correlated responses. Sexual isolation tests after 10 generations of selection revealed significant symmetrical assortative mating, but coselected males did not exhibit a significant preference for playback calls of females from their own selection line. In view of the high heritability for the call character, and the considerable ecological isolation among *Ribautodelphax* species, it seems unlikely that the female call differentiated as an adaptation to prevent hybridization (reinforcement). More likely, call and call preference were shaped by e.g. sexual selection during allopatry, and may have (had) incidentally an effect in species isolation.

### Introduction

Species-specific sexual signals were traditionally believed to function as adaptive pre-mating isolation mechanisms (Dobzhansky, 1940). More recently the alternative view has received much support, which assumes that isolating effects of such signals are incidental results of other processes, such as social competition for mates (sexual selection) (reviewed by West-Eberhard, 1983, 1984), or adaptations to the preferred habitat to ensure effective

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recognition of conspecific partners (Paterson, 1985, and earlier papers cited therein).

The process of sexual selection has been studied by genetic models (e.g. O'Donald, 1980; Kirkpatrick, 1982, 1985, 1987; Arnold, 1985), of which some (Lande, 1981a; De Jong and Sabelis, 1991) explicitly examined the consequences for speciation. None of these models (except De Jong and Sabelis, 1991) allowed for sexual selection on female traits, which is considered equivalent to the preference for male secondary sexual characters. In the model of De Jong and Sabelis both male and female traits are subject to sexual selection, no pertinent distinction being made between preference or the preferred character; in fact, their model relates to a lepidopteran mating system in which the female produces a signal. Although females produce recognition signals in many insects (Thornhill and Alcock, 1983), female signalling behaviour has been largely neglected in theoretical studies.

The genus *Ribautodelphax*, a group of morphologically poorly differentiated planthoppers, provides an interesting system for studying the evolution of acoustic signals. Here both sexes produce species-specific acoustic signals by means of low frequency, substrate-borne vibrations (Den Bieman, 1986). Reproductive isolation in this genus is of a premating type (Den Bieman, 1988a; De Winter and Rollenhagen, 1990), and each species feeds on a single species or genus of graminaceous host plants (Den Bieman, 1987a). As in other planthoppers, the male usually initiates calling. Only receptive, virgin females respond, where upon the male approaches the calling female, which remains stationary during the signal exchange (Claridge, 1985; De Winter and Rollenhagen, 1990). Playback experiments revealed that *Ribautodelphax* males generally only approach calls of conspecific females, whereas most females respond just as well to calls of either conspecific or heterospecific males (De Winter and Rollenhagen, 1990). Clearly, both the female call and the male preference for the female call are part of the mate recognition system of *Ribautodelphax* species in the sense of Paterson (1985).

Progress in understanding the evolution of mate recognition systems is hampered by a lack of experimental data, especially on the genetic variation of the components. Paterson (1978) assumed that mate recognition systems have very little heritable variation due to strong stabilizing selection on such characters. Other authors (e.g. Falconer, 1981; Cade, 1984; Hedrick, 1988) expressed a similar expectation, based on Fisher's (1958) fundamental theorem of natural selection, which considers fitness to have very little genetic variation. However, this only holds for net fitness, and not necessarily for its components (Rose, 1982; Charlesworth, 1987), and several studies have indeed revealed the existence of heritable variation for characters related to sexual behaviour (reviews in Cade, 1984; Löfstedt, 1990).

The aim of the present study is to provide information on the genetic control of acoustic signalling in *Ribautodelphax* planthoppers, in order to reach a better understanding of the importance and evolution of such mate recognition systems. This paper mainly concerns the female signal, which is analysed by applying artificial bi-directional selection to one of the signal characters. This procedure has the advantage that it provides data on the amount of heritable variation of the character selected for, as well as information on associated changes in other parts of the system, thus giving insight into the genetic architecture of the system as a whole. Aspects of the male signal will be treated in a subsequent paper.

## Materials and methods

### *Animals and rearing*

A population of *R. imitans* (Ribaut), collected from St. Cyprien, Département Pyrénées Orientales, France was used in this study. The laboratory population was established from at least 20 wild-caught gravid females, and had been cultured for 14 generations (nearly two years) before the start of the experiment. The culture consisted of two parallel rearings of ten pairs each, with exchange of males between the rearings at each generation (Den Bieman, pers. comm.). The species was reared on its natural host plant, *Festuca arundinacea* fens in a greenhouse under long-day conditions (18 hours light) at  $20 \pm 2^\circ\text{C}$  and 60-70 % r.h. For further details see Den Bieman (1987a).

### *Recording and analysis of calls*

In planthoppers, female calls are simpler than those of males. In the genus *Ribautodelphax* female calls consist of series of pulses, and differ among species in strophe duration, interpulse interval (*IPI*), and changes in pulse repetition frequency within a strophe (Den Bieman, 1986, 1987b). Virgin, receptive females rarely call spontaneously, but readily respond to the signal of a conspecific male. Females were separated from the cultures as fifth (final) instars, and were collected within 24 hours after final ecdysis. When 5-7 days old, at maximum responsiveness (De Winter and Rollenhagen, 1990), they were stimulated individually with a pre-recorded call of a male from the base population, played from a Revox B710 cassette tape deck. Recordings were made in a thermostatic cabinet at  $20 \pm 1^\circ\text{C}$ . Measurements were made from oscillograms displayed by a Siemens Oscillomink. Other technical details were as described by De Vrijer (1984).

Compared to those of other species, female calls of *R. imitans* characteristically have relatively long interpulse intervals, which become gradually shorter after the onset of the strophe, but remain more or less constant after an elapse of about 40-50 pulses (Fig. 1). Before the start of the selection experiment repeatabilities for call features were obtained by recording 10 calls per female for 9 females from the base population. Each call was divided into sets of 10 interpulse intervals (*IPI*), denoted from *IPI*-1, being the duration of 10 *IPI*'s between the first and 11th pulse in the strophe, to *IPI*-5, the duration of 10 *IPI*'s between the 41st and 51st pulse. Only the first five sets were considered, because all animals produced at least 51 pulses. The repeatability, as the ratio of the among individual variance to total variance (Falconer, 1981), was calculated for the duration of each subsequent set of 10 *IPI*'s, and for total strophe duration. Variation among individuals for all characters was significant ( $p < 0.001$ ). The repeatabilities ranged from 0.56 (total strophe duration) to 0.99 (*IPI*-5). In view of the relatively large within-individual variation of the total strophe duration (see also Den Bieman, 1986), and the increase of the pulse frequency in the initial part of the strophe, *IPI*-5 was chosen as the selection measure.

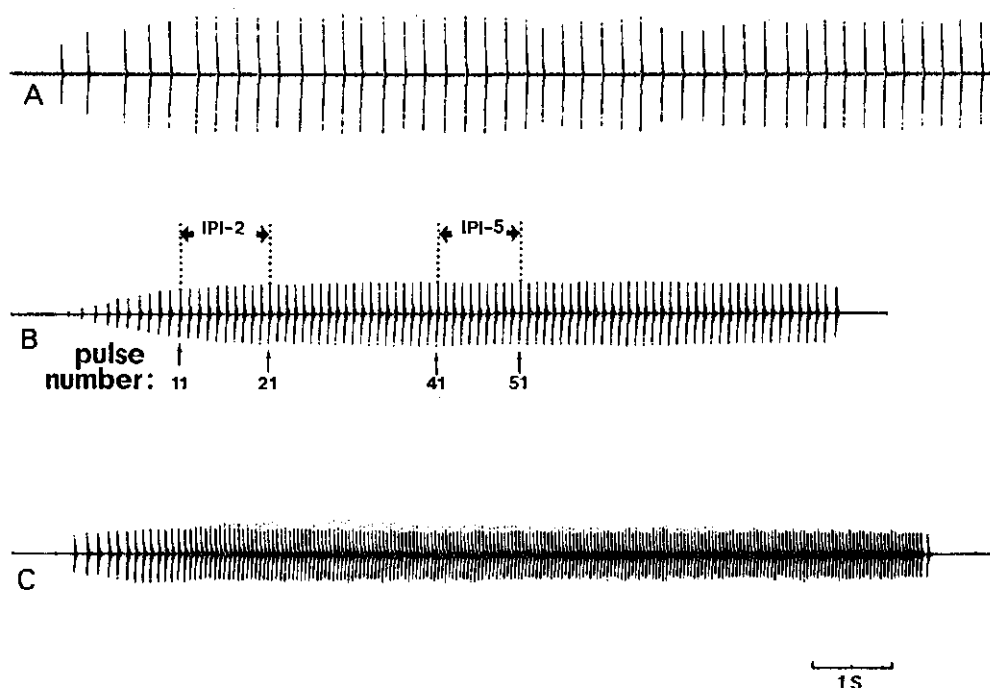


Fig. 4-1. Examples of oscillograms of female calling strophes from the unselected population (B), and extremes from lines after six generation of selection for long (A, only first part shown) and short *IPI*-5 (C).

### *Selection procedure*

Four independent samples (A, B, C, D) of 40 virgin females each were taken from the base population. From these samples four selection lines for long *IPI* (HLA, HLB, HLC, HLD), and four for short *IPI* (LLA, LLB, LLC, LLD) were established by selecting as parents the 10 individuals with the longest and shortest *IPI*-5. This selection measure was calculated as the mean of five calls per female. At each generation 40 females were recorded, of which the 10 extreme females were allowed to contribute to the next generation. About 20 males taken at random from the same line were added. As after the 11th generation some lines produced barely enough animals to proceed, selection was relaxed during the 12th generation, and the procedure was continued up to the 14th generation with HLB, LLA and LLB only. After 10 generations, selection was suspended in two LL (LLC, LLD) and two HL (HLA, HLB) lines, which were followed for 4 generations at irregular intervals. Because selection in each direction was made on four independent lines, no control line was kept. The base population was examined for the character at 5 and 14 generations after the start of the experiment.

### *Sexual isolation tests*

Mating preferences after selection were examined by 'male-choice' tests using animals from the 11th and 12th generation selection lines. Two males from one of the selection lines were confined with two females of their own line and two females of an oppositely selected line, in a cage containing the species' natural host plant. After three hours the males were removed, and the females were dissected for the presence of sperm in their spermathecae. Females were marked by using either naturally occurring brachypterous or macropterous females. Earlier experiments failed to detect any preference for either wing morph. Nevertheless, the same number of macropterous and brachypterous female pairs were used for each selection line.

In addition, individual males from different LL and HL lines from the 13th generation of selection, as well as from the unselected base population, were offered a two-way choice between the playback calls of a LLA11 and a HLD11 female, for which the *IPI*-values were close to the average scores in these lines. The setup consisted of three inter-connected grass stems of the host plant. The male to be tested was placed on the central stem. After each call of the male, the pre-recorded female calls were simultaneously played from two digital storage devices to the outer stems via two small modified speakers, with needles attached to the speakers' coil and pressed into the stems. The same two playback calls were used throughout the experiment. The calls were monitored by means of a recording device attached to the central stem. Males usually reacted by calling and running up and down the stem, and eventually moved towards one of the outer stems. If a male stayed there for ten seconds this was arbitrarily considered a choice. The method is described in greater detail elsewhere (De Winter and Rollenhagen, 1990).

### *Correlated responses*

The effects of selection for long and short *IPI*-5 on the two other features characterizing the female call, i.e. strophe duration, and modulation of *IPI* in the course of the call, were monitored during the selection experiment in the LLA and HLB lines. The duration of 10 *IPF*s between the 11th and 21st pulse (*IPI*-2) relative to *IPI*-5 was used as a measure of change in *IPI* within a strophe. In *R. imitans* *IPI*-2 is normally greater than *IPI*-5 (Fig. 1), but in other species within the genus this is the other way around (Den Bieman, 1986). Correlated responses in the other six selection lines were only examined after 11 generations of selection.

Phenotypic correlations between *IPI*-5 and both *IPI*-2 and strophe duration were also examined for each generation in LLA and HLB. Data for these characters were taken from the same calls from which the selection measure was derived.

## **Results**

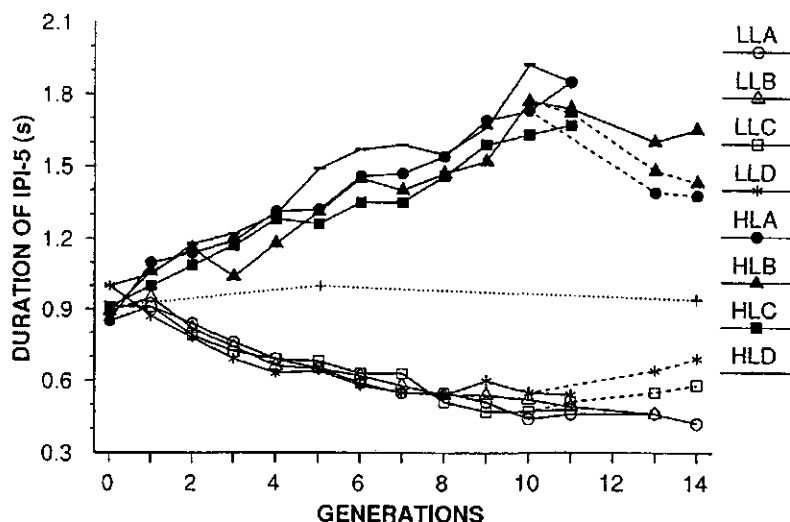
### *Response to selection*

Selection for both long and short *IPI*-5 was very successful. Responses to selection in



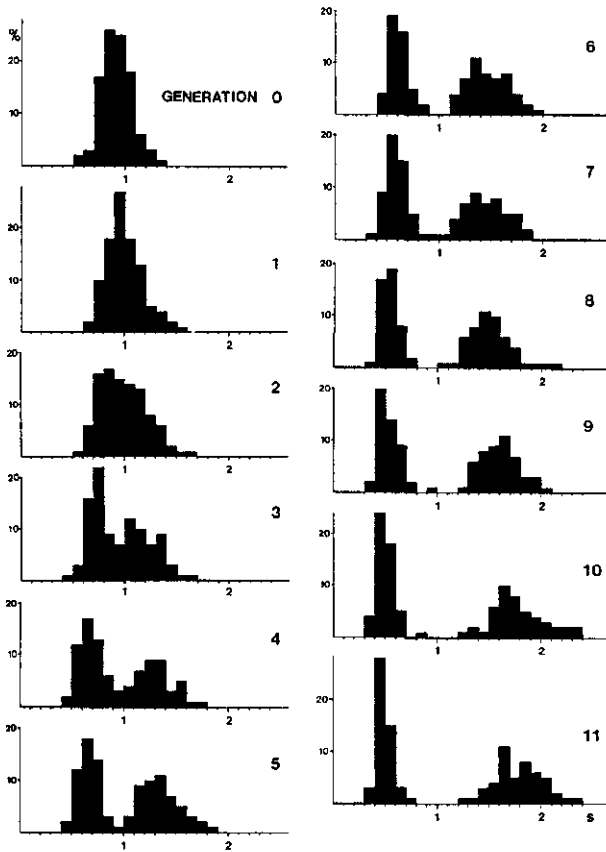
**Table 4-1.** Means, coefficients of variations (CV), and ranges of *IPI-5* in replicate lines (A, B, C, D) before and after 10 generations of selection for short (L) and long (H) *IPI*.

Line		unselected	gen. 10 L	gen. 10 H
A	Mean	0.85	0.42	1.73
	CV	0.14	0.12	0.13
	Range	0.59-1.13	0.32-0.56	1.55-2.40
B	Mean	0.89	0.52	1.77
	CV	0.13	0.13	0.13
	Range	0.69-1.18	0.38-0.66	1.36-2.33
C	Mean	0.91	0.47	1.63
	CV	0.13	0.15	0.15
	Range	0.59-1.21	0.33-0.63	1.18-2.28
D	Mean	1.00	0.55	1.92
	CV	0.15	0.22	0.12
	Range	0.71-1.34	0.38-0.92	1.46-2.30



**Fig. 4-2.** Mean *IPI-5* duration (s) plotted against generation of selection. Solid lines: response to selection. Dashed lines: selection relaxed. Dotted lines: unselected base population.

replicate lines were remarkably similar (Fig. 2). After only 5 generations of selection the



**Fig. 4-3.** Frequency distributions of *IPI-5* duration in the unselected population ( $N=160$ ), and in 11 successive generations of bi-directional selection, with data of all lines combined per generation ( $N=320$ ).

ranges of the LL and HL lines became non-overlapping (Fig. 3). After 10 generations of selection the mean *IPI-5* values in LL and HL lines differed 7 to 10 'average' standard deviations (coefficients of variation) (Table 1). These values are at or beyond the *IPI* values reported for the entire genus (Den Bieman, 1986).

Realized heritabilities (Table 2) were calculated over 0-5 and 0-10 generations of selection, as the slope of cumulative selection response on cumulative selection differential. Standard errors of the realized heritabilities are given as the standard error of the slope for each replicate line (which, however, underestimates the true standard error (Falconer, 1981)), and as the standard error of the heritability estimates in replicates. Selection

**Table 4-2.** Realized heritability ( $h^2$ ) estimates and standard errors (SE) of *IPI-5* calculated over 0-5 and 0-10 generations of selection for short (LL) and long (HL) *IPI-5* in four replicate lines (A, B, C, D).

Line	$h^2$ (0-5)	SE	$h^2$ (0-10)	SE
HLA	0.51	0.07	0.53	0.02
HLB	0.59	0.07	0.47	0.23
HLC	0.58	0.11	0.52	0.03
HLD	0.88	0.09	0.71	0.06
Mean	0.64	0.08	0.56	0.05
LLA	0.98	0.07	0.85	0.04
LLB	1.14	0.20	0.77	0.08
LLC	0.91	0.15	0.85	0.06
LLD	1.03	0.23	0.64	0.11
Mean	1.02	0.05	0.78	0.05

differentials were halved because selection was applied to females only (Falconer, 1981). Heritability estimates over 0-5 generations in the LL lines were found to be close to unity, indicating that almost all variation is heritable. Heritability values over 0-10 generations were lower, but still considerable, thus showing that additive genetic variance was not yet exhausted. The heritability estimates obtained for the HL-lines were consistently lower than those for the LL-lines (Table 2), although the response to selection in the HL-lines was more rapid (Figs. 2, 3). The asymmetry in response is likely to be caused by physiological and physical constraints of the neuromuscular system affecting the character in the LL-lines. By transforming the *IPI-5* data to a log scale the response to selection becomes more symmetrical, and the standard errors become about equal. The higher realized heritability estimates for the LL-lines may be due to genotype-environment interaction (Falconer, 1981); the constraints imposed by the neuromuscular system cause a truncation at the left side of the environmental variation distribution, which cause animals with small *IPI-5* values to reveal a higher heritability than the ones with higher values.

After 10 generations, progress in the desired direction became less in most lines, possibly because of opposing natural selection. Suspending selection in two LL and two HL-lines after the 10th generation resulted in a return in the direction of the unselected population, compared to the lines in which selection was continued (Fig. 2).

#### *Minimum number of segregating genetic factors*

After 10 generations of selection crossings were made between two combinations of LL and HL-lines (LLA10 x HLD10, and LLC10 x HLB10) and their reciprocals. From means

**Table 4-3.** Means, variances and sample sizes (N) of log transformed *IPI-5* values of parental selection lines (P), F1 and F2 progeny, and estimates of the effective number of segregating factors ( $n_E$ ) with their standard errors ( $Sn_E$ ).

	P♀	P♂	F1	F2	$n_E$	$Sn_E$
	HLD10	LLA10				
mean	0.2794	-0.3583	0.0245	0.0480		
var.	0.0028	0.0029	0.0025	0.0111		
N	40	40	43	77	5.9	1.30
	LLA10	HLD10				
mean	-0.3583	0.2794	0.0024	-0.0499		
var.	0.0029	0.0028	0.0030	0.0127		
N	40	40	45	69	5.2	1.23
	HLB10	LLC10				
mean	0.2444	-0.3311	-0.0227	-0.0146		
var.	0.0028	0.0040	0.0040	0.0093		
N	40	40	27	75	7.8	2.78
	LLC10	HLB10				
mean	-0.3311	0.2444	-0.0149	-0.0485		
var.	0.0040	0.0028	0.0036	0.0090		
N	40	40	28	52	7.7	2.88

and variances of log transformed *IPI-5* data of the parental lines, and the F1 and F2 generations, 4 estimates of the minimum number of segregating genetic factors determining the selected character were obtained using the formulae given by Lande (1981b) (Table 3). The frequency distributions for the character in the F1 and F2 progeny were continuous. The estimates range from 5.2 to 7.8 (mean 6.65), which is well under the haploid chromosome number of 15 occurring in this species (Den Bieman, 1988b). Although these figures tell us little about the actual number of loci affecting the character, they at least show that the character is truly polygenic. This was also to be expected from the almost linear course of the selection response observed over generations.

#### *Sexual isolation tests*

Due to the fact that insufficient numbers of females could be obtained to allow the

**Table 4-4.** Results of sexual isolation tests, in which two LL males (A) or two HL males (B) were given a choice between two HL and two LL females. The numbers of trials with different combinations of numbers of HL and LL females inseminated are tabulated.

(A) LL♂♂		Inseminated LL ♀♀:		
		0	1	2
Inseminated	0	4	7	5
HL ♀♀:	1	3	6	6
	2	1	1	5
Total 38 trials (152 females offered, 75 inseminated)				
(B) HL♂♂		Inseminated LL ♀♀:		
		0	1	2
Inseminated	0	13	3	0
HL ♀♀:	1	19	3	1
	2	2	1	0
Total 42 trials (168 females offered, 38 inseminated)				

analysis for each selection line separately, results of trials with similar animals but from different replicate LL and HL lines were combined. In view of the rather similar response to selection of the replicate lines, this procedure is regarded as acceptable. The numbers of LL and HL females inseminated by LL males and HL males are summarized in Tables 4A and 4B, respectively. LL males inseminated 75 of 152 available females, whereas only 38 of 168 females were inseminated by HL males. LL males managed to inseminate all (four) females in 5 out of 38 trials, whereas this never occurred in 42 trials involving HL males. These data seem to indicate a greater mating propensity or insemination capacity of the LL males. As trials in which all available females become inseminated provide little further information with regard to assortment, these were excluded from the analysis. Thus HL males inseminated 29 HL and 9 LL females, whereas 19 HL and 36 LL females were inseminated by LL males, indicating a significant deviation from random mating (Joint Isolation Index (Malogolowkin-Cohen et al., 1965)  $I = 0.40 \pm 0.095$ ,  $p < 0.001$ ). Omitting data from trials in which 3 females were inseminated leads to a further increase of the isolation index to 0.48.

Of 59 males from the unselected population offered a two-way choice between playback calls of a LLA11 and a HLD11 female, 31 went to the side from which the LL call was broadcast, while 28 went to the opposite side. Of 27 HL males tested in this way, 16 went

to the HL side, whereas of 44 LL males, 25 approached the call of the LL female. However, the slight preference of both LL and HL males for the calls of respectively LL and HL females is not significant (Joint Isolation Index  $I = 0.15 \pm 0.10$ ,  $0.10 < P < 0.20$ ). As found in other, similar playback tests (De Winter and Rollenhagen, 1990), about 10 % of all males tested failed to show a preference. These males were rather sluggish, and were probably not receptive for any call for other reasons. Such trials were therefore not included in the analysis.

### Correlated responses

The effects of selection for *IPI-5* on strophe duration and *IPI-2* in LLA and HLB can be seen in Fig. 4.

*IPI-2* showed a correlated response, closely following the course of *IPI-5* over all generations of selection. The divergence of *IPI-2* in LLA and HLB over 0-14 generations is highly correlated with that of *IPI-5* ( $R=0.997$ ,  $P < 0.001$ ).

Within generations, highly significant phenotypic correlations were found between *IPI-2* and *IPI-5*, correlation coefficients ranging from 0.84 to 0.94 in HLB, and from 0.65-0.93 in LLA. These correlations did not change consistently over successive generations of selection.

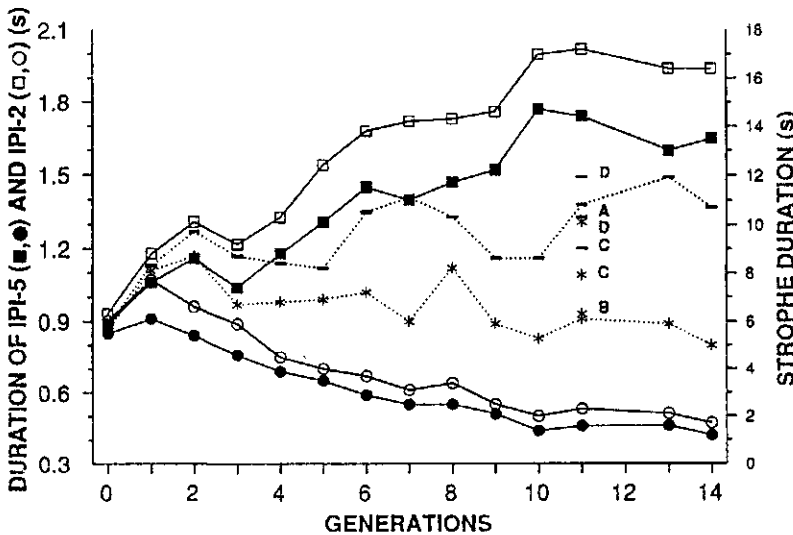


Fig. 4-4. Mean *IPI-5* duration and correlated changes in mean *IPI-2* (solid lines) and strophe duration (dotted line) in LLA (circles, asterisk's) and HLB (squares, dashes) plotted against generations of selection. Strophe durations in other replicate lines are only given for generation 11 (LL: asterik's, HL: dashes). Individual strophe durations were log transformed, and the antilog of the generation means are shown.

The correlated response of log strophe duration, expressed as the regression of the divergence between HLB and LLA on generation number, was highly significant ( $b=0.39$ ,  $P < 0.001$ ). In HLB there was a significant regression of log strophe duration over 0-14

generations ( $b=0.25$ ,  $P=0.006$ ). In LLA this regression was negative and just significant ( $b=-0.14$ ,  $P=0.04$ ), although there was hardly any change in strophe duration before and after the selection experiment. In the other six selection lines only 11th generation animals were examined for strophe duration. The HL lines, with the exception of HLC, had a longer mean strophe duration than the LL lines (Fig. 4).

Within generations, phenotypic correlations between *IP1-5* and log strophe duration were quite different in LLA and HLB. In HLB the correlations within successive generations were positive (mean 0.32, range 0.08-0.51), and at or near significance. In contrast, significant phenotypic correlations between *IP1-5* and strophe duration in LLA never occurred; here correlations even tended to be negative (mean -0.01, range -0.24-0.22). In samples from the unselected population correlations between these characters were also absent. In none of the selection lines these correlations showed a consistent trend over successive generations of selection.

## Discussion

### *Genetic determination of female acoustic behaviour*

Earlier observations have indicated that the female call is part of the mate recognition system of *Ribautodelphax* species. *Ribautodelphax* males exhibit a strong preference for the calls of conspecific females, whereas in contrast most females appear to respond equally well to calls of both conspecific and heterospecific males (De Winter and Rollenhagen, 1990). As *IP1* is a major parameter of the female call (Den Bieman, 1986), it seems unlikely to be a selectively neutral character. It is therefore a surprising result that the female call can be changed drastically within a few generations by using a relatively low selection coefficient (37.5 %). Other studies have also revealed substantial heritability values for acoustic characters in insects, but usually smaller values than the ones reported here (e.g. McDonald, 1979; Ikeda and Maruo, 1982; Butlin and Hewitt, 1986). Hedrick's (1988) study on calling-bout lengths in the cricket *Gryllus integer* provided heritability estimates (0.69-0.76) comparable to those found in *Ribautodelphax*.

The presence of a large additive genetic variance for characters involved in mate recognition seems in conflict with theoretical considerations. Paterson (1978) stressed that components of mate recognition systems are expected to have little genetic variation, because they are under strong stabilizing selection. Thus in Paterson's view changes in the mate recognition system will be necessarily slow. This also follows because each selective change requires selection on the other partner to restore coadaptation of the mate recognition components (Paterson, 1978). Similar expectations follow from Fisher's (1958) fundamental theorem of natural selection. Mate recognition traits are expected to become fixed in the population as the result of strong selection by one of the partners for preferred traits in the other (Maynard Smith, 1978, Ch. 10; Cade, 1984).

Cade (1984) and Hedrick (1988) considered a number of possible mechanisms through which significant additive genetic variation for characters related to sexual behaviour can be maintained. It is difficult to make a well based decision in this particular case among the theoretical possibilities. Even a combination of mechanisms may be responsible for the very

high heritability estimates found. Two of these appear to be supported by some data.

One explanation is that the abundant additive genetic variance is maintained through a negative genetic correlation ('trade-off') between *IPI* and another character related to fitness, such as to resemble the effects of stabilizing selection (Rose, 1982, 1984). The rapid return in the direction of the original *IPI*-5 values after relaxation of the selection, the diminished response and the noticeable reduction in numbers of offspring in most lines already after 10 generations of selection, may be taken as support for this view. However, these antagonistic response patterns could also have been the result of inbreeding depression due to the fixation of deleterious alleles (Rose, 1982, 1984), but the rather similar behaviour of replicate selection lines does not seem to support this explanation.

Another possibility is that genetic variability is maintained in the field by environmental fluctuations. Heritability estimates under controlled laboratory conditions are likely to be higher than under natural conditions, because of the inevitable reduction of the environmental variance (Falconer, 1981). Temperature is known to affect the *IPI* phenotype in the related planthopper genus *Javesella* (De Vrijer, 1984). Thus the genetic variation for the character may be buffered against selection through phenotypic variation caused by temperature changes in the field.

On the other hand, selection on female call characters may be less severe than assumed above, because of the difference in parental investment between the sexes. Female planthoppers mate only once, in contrast to males. Therefore sexual selection on female characters will be much less than on those of males. In most sexual selection models (e.g. Lande, 1981a) the possibility of sexual selection on a female character (usually the preference for a male trait) is excluded, because of the assumption that all females become inseminated. Only the model by De Jong and Sabelis (1991) allows for asymmetrical sexual selection on both sexes, accounting for the risk that females remain unmated. Assuming that females mate only once, males encounter an increasing proportion of less attractive females as the season progresses, which causes some sexual selection on females, but less severe than on males, resulting in a larger variance for the female trait than for the male trait. This model is inspired by a lepidopteran pheromone signalling system, in which the female produces the signal, which in a way is comparable to the situation in *Ribautodelphax*.

In addition, reproductive isolation among *Ribautodelphax* species in the field is mainly accomplished by their different host plants, and therefore there is probably less stabilizing selection on the call than in the case it would have a species-isolating effect in sympatry.

The correlated increase of strophe duration in HL, but its relative constancy in LL lines might be explained by assuming a genetically fixed threshold for the minimum number of pulses, as well as the minimum call duration, that will evoke a phonotactic response from a male. In the unselected population, *IPI* is the ratio of a sufficiently long strophe duration and a sufficient number of pulses. Long *IPI*'s brought about by (artificial) selection then need to be compensated by longer strophe durations in order to arrive at a sufficient number of pulses produced; selection for short *IPI* may lead to an increase of the numbers of pulses to provide a minimum strophe length, but no correlated change in strophe duration is necessary. The hypothesis that males ignore short female calls with few pulses was not rigorously tested, but incidental observations of reactions of males to unintentionally aborted female playback calls were in accordance with this explanation (unpublished results).



Similarly, a genetically fixed range within which the ratio *IPI-2:IPI-5* can vary may be assumed in order to explain the highly correlated response of *IPI-2*. Thus the correlations between *IPI-5* and other characters, which constitute the female call, might be due to fixed functional associations rather than to genetic correlations in the strict sense.

### *Assortative mating*

It is questionable whether the female call character selected for was entirely responsible for the assortment among the oppositely selected lines. Mating tests of co-selected males with females from both their own and oppositely selected lines revealed significant symmetrical assortative mating, suggesting that the forced mating conditions during the selection experiment have resulted in a genetic covariance between the female call and the male preference. However, when provided with playback calls of such females, co-selected males showed only a slight, not significant preference, whereas unselected males responded equally well to both call types. The cause for these apparently conflicting results is not clear. The two experimental setups are quite different. In the one with live males and females a possible effect of other recognition cues than just the female call cannot be ruled out. For example, females may tend to mate with males from their own population on account of correlated acoustic or non-acoustic characters in males (sexual selection). In some male song features slight, but statistically detectable correlated changes indeed occurred, but these were well within the range found in the unselected population (De Winter, in preparation). However, the assortment observed was also not spectacular considering the extent of divergence in female *IPI-5* relative to the variation occurring among different species.

We are also faced with the problem that males discriminate between female calls of different species (De Winter and Rollenhagen, 1990), but not significantly between calls differing only in *IPI*. As suggested from the correlated responses of other parameters making up the female call, selection for one feature, *IPI*, did not succeed in disturbing the association between each of the call characters. A drastic change in *IPI* only, without breaking up this balance between the call parameters, may not be sufficient to affect the male preference significantly.

Due to insufficient numbers of females available, assortment among similar selection lines was not examined. Therefore a possible role for genetic drift in causing assortment between HL and LL lines (Carson, 1975; Ringo et al., 1985) cannot be ruled out. However, because of the symmetry of the assortment, drift seems an unlikely explanation.

Theoretically, the conflicting results of the two experiments could be due to a methodological error in the playback experiment, because only one playback call of each type was tested. It was recently argued by Kroodsma (1989) that the generality of such response results is limited to that particular pair of calls tested, if there is any variability in responsiveness. However, as the values of the call feature to be tested were very close to the mean value in the populations which they were supposed to represent, it seems unlikely that the lack of preference for either call can be explained by the unattractiveness of the used calls.

*Evolution of planthopper calls*

In many acoustically signalling animals there is only one sender and one receiver. In planthoppers the situation is more complicated in that males and females both produce acoustic signals. In this paper only the female call and the male preference are considered.

This study reveals that the observed variability for *IP1* in the population is to a large extent composed of additive genetic variation. Especially in combination with a genetically correlated male preference, this clearly allows for a potentially high rate of evolutionary change in the mate recognition system, in contrast to Paterson's (1985) expectation of stasis. This might explain the common occurrence of geographic variation in mate recognition characters (West-Eberhard, 1984), and might especially be relevant to the results of Claridge et al. (1985a,b, 1988) on the planthopper species-complex related to *Nilaparvata lugens*. Allopatric populations of this species were found to differ greatly in both male and female calls. The magnitude of the differences in the male calls turned out to be correlated with the degree of sexual isolation between populations. In these isolated populations the mate recognition system must have been subject to rapid evolutionary change (Claridge et al., 1988).

It was suggested by West-Eberhard (1983, 1984) that rapid signal evolution under sexual selection might play a key role in insect speciation. The fact that *Ribautodelphax* males in laboratory experiments do discriminate among female calls of different species (De Winter and Rollenhagen, 1990) indicates that female calls can have an effect in species isolation, and could contribute to speciation if sympatry arose between populations that were previously acoustically differentiated in allopatry. The ecological and geographical isolation of most *Ribautodelphax* species (Den Bieman, 1987a), and the ample genetic variation in the female calls, renders the model of speciation by reinforcement (Dobzhansky, 1940) unlikely here. Thus in this genus the species-specific properties of the female calls may have (had) a secondary effect in species isolation, but were probably not directly involved in the speciation process itself. Incipient speciation would be facilitated if a change in mate recognition components is followed or preceded by a shift in host plant, as seems to be the case in many planthopper taxa.

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# THE GENETIC BASIS AND EVOLUTION OF ACOUSTIC MATE RECOGNITION SIGNALS IN A *RIBAUTODELPHAX* PLANTHOPPER (HOMOPTERA, DELPHACIDAE)

## 2. THE MALE SIGNAL <sup>1</sup>

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### Abstract

Both males and females of *Ribautodelphax imitans* produce species specific calls. This paper addresses the genetics of the acoustic signal of males, and discusses some aspects of the evolution of the whole acoustic mate recognition system in this species. The male call consists of two differently structured parts, the 'chirp-section' and the 'buzz-section', which appeared to vary independently. By means of father-son regression, heritability estimates of six male call parameters were obtained. Characters of the chirp-section were found to have moderately high, significant heritabilities (0.44-0.54), whereas those of the buzz-section were statistically not significant (0.09-0.28). Phenotypic, genetic and environmental correlations among the male call parameters were calculated. One of these characters, number of chirps, appeared to be influenced by sex-linked genes. Some characters of the chirp-section showed significant correlated response to artificial selection for interpulse interval length in the female call reported previously. This genetic correlation constrains independent evolution of male and female calls. Two scenarios for the evolution of acoustic communication in planthoppers are discussed. In one scenario the calls have differentiated as a consequence of founder events; in another scenario the calls have differentiated after a shift in hostplant took place, either in allopatry or sympatry. The existence of a genetic correlation between male and female call characters could accelerate the evolution of the whole acoustic communication system.

### Introduction

For a meaningful discussion on the evolution of mate recognition systems information on the genetics of the different components is essential. As change in specific mate recognition systems relate to speciation (Paterson, 1985), knowledge on the evolution of mate recognition systems may be the key to understanding biological diversity. Many genetic models of speciation have been developed, each with their own assumptions

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about the genetic control of mate recognition systems, but very few experimental data are available to verify such models.

Planthoppers of the genus *Ribautodelphax* are well suited as a system to study such problems. These animals communicate acoustically through substrate-borne vibrations, which comprise an important part of their mate recognition system. The two sexes produce very different calls, but they are both species-specific. Female calls consist of series of pulses, whereas male calls have a more complex structure (Den Bieman, 1986). Males and females also behave differently during distant communication: males are the initiating and active sex, and approach acoustically responding females, while females remain stationary once acoustic contact with a male is established. Playback tests have revealed that males approach only conspecific female calls, whereas most females respond equally well to both conspecific and heterospecific calls of congeneric males, at least during distant communication (De Winter and Rollenhagen, 1990). Thus in the first stage of the sexual behaviour males appear to be more selective than females towards heterospecific acoustic signals of the opposite sex. The relative importance of these calls at close range is still largely unknown.

In a previous paper (De Winter, 1992) the genetic control of the female call of *R. imitans* (Ribaut) was analysed by artificial bi-directional selection for one of the call characters, interpulse interval length. This character was found to be polygenic and highly heritable (mean realized  $h^2 > 0.80$  over 5 generations). Correlated changes occurred in two other features of the female call, and a significant degree of assortative mating among the diverged selection lines was observed after 10 generations of selection.

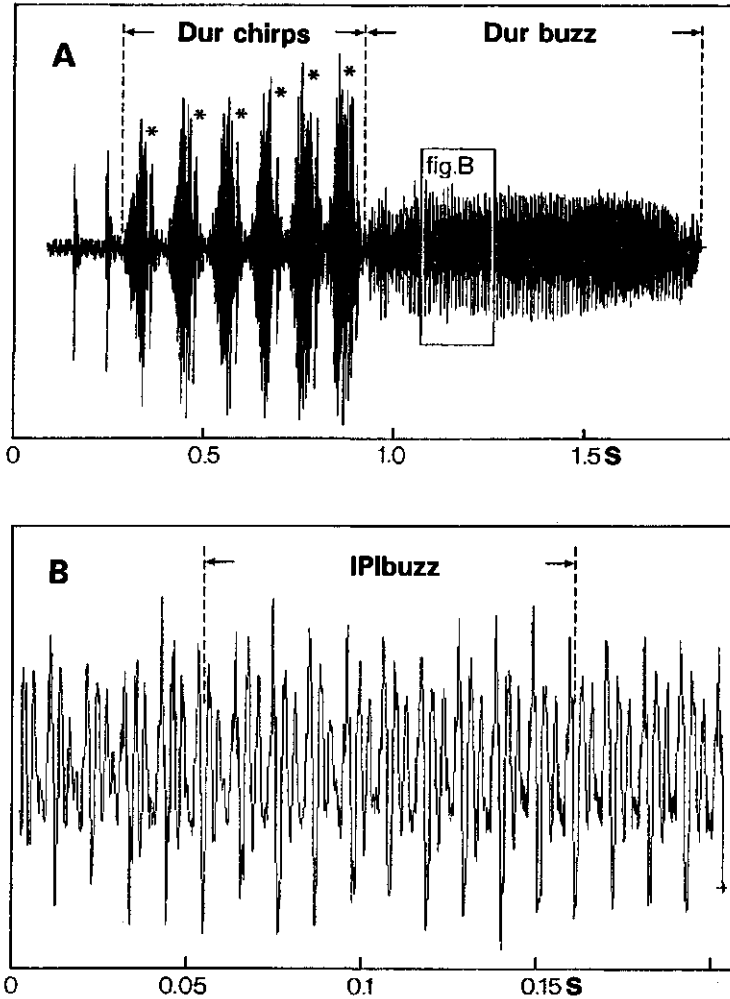
The present paper is aimed at providing data on the genetic control of the male acoustic signal of this species. The different parameters which make up the male call are characterized, estimates of narrow-sense heritabilities of the call characters are provided, phenotypic, genetic and environmental correlations among these are calculated, and the effect of artificial selection for interpulse intervals in female calls on male call characters is examined. In addition, an attempt is made to integrate both the data on males, and those previously obtained for females in a discussion on the evolution of this mate recognition system.

### Material and methods

Information concerning the origin of the *R. imitans* population used in this study and the rearing method is provided by De Winter (1992).

Males were separated from the cultures as fifth instars, and were allowed to mature for at least six days after final ecdysis before attempting to record the calls. Ideally males were recorded when 6-8 days old, but occasionally up to 10-day-old males had to be used.

Signals were recorded in a thermostatic cabinet at  $20 \pm 1^\circ \text{C}$  by means of a accelerometer (Bruel & Kjaer 8307) attached to a stem of the species' hostplant (*Festuca arundinacea fenas*), on a Revox B77 MKII tape recorder, as described by De Vrijer (1984).



**Fig. 5-1.** Oscillogram of a *Ribautodelphax imitans* male call, showing the parameters used. Stars indicate individual chirps ( $No\ chirps = 6$ ). Abbreviations are explained in the text. **A** shows a complete signal, **B** shows part of the buzz-section at an expanded time scale, as indicated in **A**.

Calls of *Ribautodelphax* males are more difficult to obtain than those of females. Males cannot always be induced to call in response to the playback of a conspecific female call. Moreover, the high activity of males often results in their departure from the recording stem after one or two calls. Recapturing the animal and putting it back on the stem sometimes results in disturbance of the animal. These problems severely restrict the number of recordings one can make within a reasonable time.

Within the male call of *R. imitans* two structurally different parts can be distinguished, the 'chirp-section' and the 'buzz-section' (Den Bieman, 1986). In the present study the signal was characterized by the following parameters (Fig. 1): duration of chirp-section



(*Dur chirps*), number of chirps (*No chirps*), mean chirp duration ( $\text{Mean dur chirp} = \text{Dur chirps}/\text{No chirps}$ ), duration of buzz-section (*Dur buzz*), the ratio of the chirp-section duration and buzz duration ( $\text{Dur chirps}/\text{Dur buzz}$ ), and the duration of 10 intervals between pulses in the buzz-section (*IPIbuzz*). Call characters were measured from oscillograms of the calls displayed by a Siemens Oscillomink at a time scale of either 320 mm/s (*IPIbuzz*) or 20 mm/s (all other characters). Parameters derived as ratios were arcsine transformed whenever statistical analysis required a normal distribution of the character.

The mean values and ranges of these call characters, and the phenotypic correlations among them, were obtained by recording 52 males taken at random from the stock culture. From each male five calls were obtained. The repeatability or interclass correlation coefficient was calculated for each song character as the ratio of the among individual variance to total variance, in order to provide an upper limit for the heritability of the character (Falconer, 1981). The signal:noise ratio has to be optimal to allow *IPIbuzz* to be measured with some degree of accuracy. Therefore, *IPIbuzz* was only determined in one or two calls per animal, and no repeatability for this character was calculated.

Narrow sense heritabilities and standard errors of male call characters were estimated as twice the slope and standard error of the regression line of offspring mean on father value (Falconer, 1981). Signals of 40 males were recorded, and each recorded male was confined with a randomly chosen female. From 25 families five sons per family could be recorded. For each animal and call character the values of five calls (with the exception of *IPIbuzz*, see above) were averaged.

Genetic correlations ( $r_A$ ), environmental correlations ( $r_E$ ), and their standard errors were calculated from the formulas given by Falconer (1981).

In an earlier study, female calls were artificially selected for short and long interpulse intervals, with four replicate lines in each direction (De Winter, 1992). During this experiment, the response of male call characters was monitored by recording random samples of males from various selection lines at different stages of the selection procedure. From 19 lines in the 2nd, 5th, 10th and 13th generation of selection, as well as from the unselected population, calls of at least 10 males per line were examined. Because of this fragmentary record of male signals, correlated responses could not be determined for each replicate selection line separately. Instead, the mean values of the male call characters in these 19 lines were correlated with the corresponding mean female interpulse interval values (hereafter termed *IPIfem*), thus providing a measure relating to the degree of genetic correlation between these characters. *IPIfem* as used here corresponds to *IPI-5* as defined elsewhere (De Winter, 1992).

In addition, calls of 20 males per line were analyzed from two lines selected for 10 generations in opposite direction for *IPIfem* (HLD10 and LLA10, De Winter, 1992), and also from both reciprocal F1 crosses between these lines.

## Results

The ranges and means of the male call characters in the unselected stock culture can be read from Table 1. The repeatabilities of all male call characters (*IPIbuzz* excluded) were highly significant (Table 2), which is in accordance with Den Bieman's (1986) results, except for *Mean dur chirp* and *Dur chirps/Dur buzz*, which were not considered in that study. Heritability estimates from father-son regressions are provided in Table 2. Generally the correlation between repeatability and heritability estimates was poor. Only for *No chirps*, *Dur chirps* and *Mean dur chirp* were heritability estimates moderately high, and significantly different from zero, or almost so. For *Dur buzz* and *Dur chirps/Dur buzz* estimates were not significant, but there might well be a heritable component, which was not detected because of the small number of families studied. The heritability estimate of *IPIbuzz* was very low and well exceeded by its standard error.

Table 5-1. Means, coefficients of variation and range (s) of *Ribautodelphax imitans* male call parameters from animals taken at random from the stock culture (n=52).

Variable	Mean	CV	Range
<i>No chirps</i>	7.7	0.16	6.1 - 12.5
<i>Dur chirps</i>	0.76	0.18	0.60 - 1.26
<i>Mean dur chirp</i>	0.098	0.071	0.085 - 0.123
<i>Dur buzz</i>	0.65	0.23	0.25 - 0.96
<i>IPIbuzz</i>	0.103	0.044	0.094 - 0.112
<i>Dur chirps/Dur buzz</i>	1.43	0.37	0.72 - 2.83

Table 3 provides phenotypic, genetic and environmental correlations among male call characters. The phenotypic correlations among the characters suggest that the chirp-section and buzz-section vary independently. Neither of *No chirps*, *Dur chirps* or *Mean dur chirp* was correlated with *Dur buzz*. However, these chirp-section characters were all correlated with *IPIbuzz*, as was *Dur buzz*.

*No chirps* was highly correlated with *Dur chirps*. In fact, the genetic correlation is so high that they might possibly have the same genetic basis. A close genetic similarity is also indicated from the very similar correlations between both *No chirps* and *Dur chirps* and *Dur chirps/Dur buzz*. The significant phenotypic correlations between *Dur chirps/Dur buzz* and the chirp-section characters appeared to be mainly due to genetic causes. Phenotypically *Dur chirps* was significantly correlated with *Mean dur chirp*, in contrast to *No chirps*, but this correlation is probably environmental rather than genetic.

*No chirps* showed a significant negative 'correlated response' to artificial selection for *IPIfem*: the number of chirps tended to increase with decreasing *IPIfem*. Also *Dur chirps* exhibited a slight, but non-significant negative correlation. *Mean dur chirp* was highly

**Table 5-2.** Repeatabilities and heritability estimates from father-son regressions for *Ribautodelphax imitans* male call variables. Significance levels: \*\*\*,  $P < 0.001$ ; \*\*,  $p < 0.01$ ; o,  $P < 0.10$ .

Character	Repeatability	Heritability	SE of $h^2$
<i>No chirps</i>	0.91***	0.48**	0.17
<i>Dur chirps</i>	0.86***	0.54**	0.20
<i>Mean dur chirps</i>	0.74**	0.44°	0.22
<i>Dur buzz</i>	0.71***	0.28	0.26
<i>IPIBuzz</i>	---	0.09	0.36
<i>Dur chirps/Dur buzz</i>	0.81***	0.28	0.20

(and positively) correlated with *IPIfem* (Table 3). This means that an increasing pulse rate in the female call tends to go together with an increase in chirp rate in that of the male. All other male characters did not respond to change in *IPIfem*.

Table 4 shows the values of male call parameters of two lines selected during 10 generations in opposite direction for *IPIfem*, and of the progeny of both reciprocal F1 crosses between them. Males from oppositely selected lines differed significantly in *No chirps* and *Mean dur chirp*, as was to be expected from the 'correlated response' of these characters. They also differed somewhat in *Dur buzz* and *Dur chirps/Dur buzz*, but not at a statistically significant level. None of the latter characters did respond to selection for *IPIfem*, so the difference in *Dur buzz* and *Dur chirps/Dur buzz* between the oppositely selected lines should be due to other causes than genetic correlation, like chance or sampling error.

Only for *No chirps* a significant difference was found between the reciprocal F1 crosses of the oppositely selected *IPIfem* lines. Males from both crosses produced a mean number of chirps close to that in the lines of their mothers. Such a pattern is consistent with either a sex-linked or maternal mode of inheritance. As remarked by Butlin and Hewitt (1988), it is difficult to imagine a mechanism for maternal inheritance for characters not expressed in females. If for this reason it is assumed that there are no maternal effects (there can be no Y-chromosome effect, because *Ribautodelphax* males are XO (Den Bieman, 1988)), the magnitude of the effect of X-linked genes can be estimated. The difference in the effect of the X-chromosome between the reciprocal F1 males is  $1.23 \pm 0.26$ , while the difference between the parental lines is  $1.26 \pm 0.39$ . Thus sex-linked genes appear to contribute very substantially to the difference in *No chirps* between the selection lines.

**Table 5-3.** Phenotypic ( $r_p$ ) ( $N=52$ ), as well as genetic ( $r_A$ ) and environmental ( $r_E$ ) correlations among *Ribautodelphax imitans* male call characters calculated from father-sons regression data ( $N = 25$ ). 'Genetic' correlations between male call characters and *IPlfem*, are calculated from correlated responses to artificial selection for female IPI ( $N = 19$ , see text for explanation). Abbreviations are explained in the text. Genetic and environmental correlations are only calculated for characters with heritability estimates greater than their standard errors. Significance levels: \*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ .

	<i>Dur chirps</i>	<i>Mean dur chirp</i>	<i>Dur buzz</i>	<i>Dur chirps/Dur buzz</i>	<i>IPlbuzz</i>	<i>IPlfem</i>
<i>No chirps</i>	$r_p$	0.95***	-0.03	0.60***	0.48**	
	$r_A(\text{SE})$	1.02 (-0.04)	0.33 (0.36)	0.96 (0.03)	---	-0.54*
	$r_E$	0.90	-0.25	0.40	---	
<i>Dur chirps</i>		0.39**	0.13	0.60***	0.58***	
		0.07 (0.13)	0.23 (0.39)	0.93 (0.05)	---	-0.24
		0.70	0.07	0.41	---	
<i>Mean dur chirp</i>			-0.01	0.14	0.37**	
			-0.11 (0.48)	0.30 (0.38)	---	0.73**
			0.05	0.06	---	
<i>Dur buzz</i>			-0.87***	-0.15 (0.56)	0.39**	
			-1.15		---	0.18
					---	
<i>Dur chirps/Dur buzz</i>					-0.02	
					---	-0.27
					---	

## Discussion

*Structure and genetics of male calls*

An analysis of correlations between characters showed that the two main parts of the male call, the chirp-section and buzz-section, vary largely independently. In view of the very low heritability estimate for *IPIbuzz*, the significant phenotypic correlations between chirp-section characters and *IPIbuzz* are likely to be caused predominantly by environmental correlations, although these are based on a reasonable number of specimens ( $N = 52$ ). This is contrary to Cheverud's (1988) suggestion that phenotypic correlations can be reasonable estimates of genetic correlations with sample sizes equivalent to those used here. For characters of the chirp-section (*No Chirps*, *Dur Chirps* and *Mean dur chirp*) significant or nearly significant, moderately high heritability estimates were obtained, in contrast to the buzz-section characters. This could be the cause of the independent behaviour of the male call sections, and could also explain why only characters of the chirp-section (*No chirps* and especially *Mean dur chirp*) responded to artificial selection for *IPIfem*. However, Houle (1992) recently showed that heritabilities are generally poor predictors of the ability of a character to respond to selection. He suggested instead to use coefficients of additive genetic variation,  $CV_A = 100 V_A/\bar{X}$ . Calculation of  $CV_A$ 's for some male call characters showed that this value for *Dur buzz* is of the same order of that of *Dur chirps* and *No chirps*, whereas that of *IPIbuzz* is considerably lower. The problem, however, is that the values of  $V_A$  are derived from the heritability estimates, which are not statistically significant for *Dur buzz* and *IPIbuzz*; in *Dur buzz* this could be due to the small numbers of families studied. Artificial selection experiments might be more appropriate to study the potential for evolution of these characters.

**Table 5-4.** Values of *Ribautodelphax imitans* male call characters ( $\pm$  SEM) from lines selected for long (HLD10) and short (LLA10) female IPI, and of their reciprocal F1 progeny. Significant differences between the parental lines are indicated in the first column, between reciprocal F1 crosses in the second. \*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ .

	HLD10 (P1)	F1 (P1♀ x P2♂)	F1 (P2♀ x P1♂)	LLA10 (P2)
<i>No chirps</i>	6.94 (0.20)**	6.73 (0.21)**	7.96 (0.26)	8.20 (0.39)
<i>Dur chirps</i>	0.79 (0.03)	0.66 (0.02)*	0.76 (0.03)	0.83 (0.04)
<i>Mean dur chirp</i>	0.114 (0.002)***	0.099 (0.002)	0.095 (0.001)	0.101 (0.002)
<i>Dur buzz</i>	0.67 (0.07)	0.85 (0.03)*	0.75 (0.03)	0.83 (0.05)
<i>IPIbuzz</i>	0.102 (0.006)	0.102 (0.005)	0.101 (0.004)	0.101 (0.006)
<i>Dur chirps/Dur buzz</i>	1.39 (0.20)	0.80 (0.04)***	1.02 (0.03)	1.03 (0.07)

At first sight male and female acoustic signals of *Ribautodelphax* species appear to have little in common. The female call basically consists of a series of pulses, whereas the male call is composed of two structurally more complicated components (Den Bieman, 1986). The existence of a genetic correlation between some male and female call characters would mean that the male and female calls do not evolve independently of each other. This conclusion may hold only under the rearing conditions and selection regimes used for the population studied. Under more heterogeneous field circumstances, and in other populations the correlation may be quite different (Stearns et al., 1991). On the other hand, these results show at least that genetic correlations between male and female mate recognition signals can develop. Obviously, such a genetic correlation could accelerate the evolution of the whole acoustic signalling system.

The X-chromosome appeared to have a large effect on *No chirps*, which character also responded to selection for *IPIfem*. If this genetic correlation resulted from pleiotropy, it could possibly be that one or more sex-linked genes affect characters of both the male and the female call. This common genetic control would appear to influence the 'rhythm' of these calls (pulse rate in the female call, chirp rate in the male call). Although different in details, this situation is reminiscent of that in *Drosophila melanogaster*, where a sex-linked gene, *per*, controls several periodicity characters, from the rhythmically fluctuations of pulses in the male call to the oscillations of circadian rhythms (Kyriacou and Hall, 1986).

The large X-chromosome effect found for *No chirps* suggests that the father-son regression underestimates the true heritability of this character. The X-chromosome cannot contribute to the father-son regression, because males receive the X chromosome from their mothers. Thus the true heritability for this character is probably very substantial.

Contribution of the X-chromosome to characters involved in mate recognition has been found in for example *Drosophila* species (Ewing, 1969; Carson and Lande, 1984; Kyriacou and Hall, 1986), *Chorthippus* grasshoppers (Butlin and Hewitt, 1988), and several Lepidoptera (Löfstedt, 1990). Ewing (1969) argued that sex-linkage could lead to rapid fixation of a new song pattern in a population, because mutations of sex-linked loci affecting the call will be immediately expressed in the hemizygous males. If females heterozygous for the mutation would accept the new song, half of their sons will produce the new call, and all their daughters will respond to it. However, recent models by Charlesworth et al. (1987) appear to indicate that the rate of evolution of sex-linked characters is dependent on the extent that such mutations are favourable and recessive.

#### *Evolution of the acoustic communication system in planthoppers*

Acoustic differentiation of *Ribautodelphax* species can be easily envisaged by founder events. Templeton (1980) has analysed the conditions in a population which would promote the occurrence of founder events, many of which appear to hold in *Ribautodelphax*. Most importantly, the founder population should have sufficient genetic variability to respond to drift and the perturbed genetic environment caused by the

founder event, leading to a rapid shift to a new adaptive peak. Restricting this to the acoustic characters, we have found significant genetic variation for some male and especially female call characters. Other favourable attributes in *Ribautodelphax* are (cf. Templeton, 1980, Table 2): an average female can produce dozens of offspring; the population structure is likely to be subdivided as the result of the host plant distribution; the mate recognition system is likely to be under sexual selection; the recognition of the female signal by males is partially 'learned' (De Winter and Rollenhagen, 1993), and the number of chromosomes ( $2n = 30$ ) is considerable (Den Bieman, 1988). In addition, all species possess a wing length polymorphism; macropterous animals are capable of flying, and appear especially under crowding conditions. Hence, a new population could be established by a single inseminated female flying away from the old population.

However, it seems to be equally possible that acoustic differentiation evolved after a change in host plant took place, either in allopatry or sympatry. Each species of *Ribautodelphax* is generally able to survive and reproduce only on one particular plant species (Den Bieman, 1987a), and the signals could have evolved as the result of different selection regimes at work in the new habitat, or by chance. One argument against the latter theory is that in some planthopper genera, like *Nilaparvata* (Claridge et al., 1985a, b), *Prokelisia* (Heady and Denno, 1991), and *Chloriona* (Gillham et al., 1992), acoustic differentiation apparently has taken place without a host plant shift. In addition, the rather patchy geographic distributions of *Ribautodelphax* species and populations and their sometimes very small ranges, despite the generally common occurrence of the host plants (Den Bieman, 1987a, b), appear to argue in favour of the founder event theory.

In view of the apparent genetic correlation between some characters of the male and female calls, the evolution of this acoustic communication system could be triggered by a change in either of these calls, as a change in the call of one sex is likely to have an effect on that of the other. Such changes may be brought about by the action of direct (for instance sexual selection) or indirect selective forces (like a change in hostplant) on the call characters, or accidentally by genetic drift. This genetic correlation is likely to accelerate the evolution of the whole acoustic communication system, because any initial acoustic differentiation of a population will simultaneously affect the calls of both sexes.

### Acknowledgements

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# ACOUSTIC COMMUNICATION AND MATING BEHAVIOUR WITHIN AND BETWEEN *RIBAUTODELPHAX* PLANTHOPPER SPECIES (HOMOPTERA, DELPHACIDAE) <sup>1</sup>

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## Abstract

This paper describes qualitatively and quantitatively the sexual behaviour of five species of the planthopper genus *Ribautodelphax*. During distant calling both males and females were acoustically active. During close range courtship the male call structure and calling rate remained unchanged, whereas females tended to call less often, and the duration of their calls decreased. Intraspecific courtship involving one male and one female were rather long-winded, and copulation usually took place only after many refusals by females of male copulation attempts. Courtship durations were significantly reduced when a female was confined with two instead of one male. Females did not appear to mate at random with the available males, suggesting that sexual selection might take place. Aggressive behaviour between males was not detected. Specific cues involved in intraspecific mate choice during courtship were not identified but are most likely to be found in characters of the male call. Most non-acoustic behavioural events were shared by all species studied. Pathways of non-acoustic events during courtship were broadly similar among species, but probabilities of transitions between events were often different. Courtships between heterospecific partners were studied in four combinations involving three species. Only 2 out of 28 interspecific trials studied resulted in copulation. Many interspecific trials did not lead to courtships; most courtships that did develop were terminated by the female ignoring or rejecting male behaviours and copulation attempts, not unlike in the lengthy intraspecific courtships. Both in intra- and interspecific courtships males appeared to be less choosy than females. Species recognition takes place during the distant calling phase where males normally approach only conspecific females. The interspecific courtships followed from accidental encounters enabled by the experimental conditions of the mating trials, and are unlikely to occur in nature.

## Introduction

Acoustic communication constitutes a conspicuous part of the reproductive behaviour

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<sup>1</sup>Intended for publication as: A.J. de Winter and P.W.F. de Vrijer. Acoustic communication and mating behaviour within and between *Ribautodelphax* planthopper species (Homoptera, Delphacidae). Behaviour (submitted).

of planthoppers, signals being transmitted as vibrations through the host plants, usually grasses (see Claridge and De Vrijer, 1993). Calling signals are exchanged between receptive males and females, serving to bring them together for mating. Mating receptive females respond acoustically immediately after perceiving a suitable male call. Females usually remain stationary during the exchange of signals, whilst males search actively for responding females. After the male has physically located a female the courtship phase begins, also accompanied by elaborate acoustic activity.

Because species are characterized by calling signals of a specific structure, this type of communication is potentially involved in species recognition (species isolation). In species of the genus *Ribautodelphax*, both sexes produce species-specific attraction calls (Den Bieman, 1986). A study of the importance of these signals in species recognition revealed that females generally respond to heterospecific (but congeneric) male calls at a similar level as to conspecific ones, whereas males significantly prefer conspecific female calls over heterospecific ones (De Winter and Rollenhagen, 1990). Thus, during distant calling *Ribautodelphax* males apparently are more species-selective than females.

Most studies on planthopper acoustic communication have concentrated on calling behaviour. Little is known about close range communication during courtship, i.e. the last phase before mating. Claridge (1985) concluded that in many small Auchenorrhyncha there may be no sharp division in the structure of calling and courtship signals. Such differences have been reported in *Muellerianella* (Booy, 1982), but were not found in *Prokelisia* (Heady and Denno, 1991).

Non-acoustic components of planthopper courtship have also received little attention. Courtship and copulatory behaviour may be used for timing and facilitation of insemination and pair maintenance, but may also have an effect in mate recognition, especially in groups where accidental pair formation is frequent (Alexander, 1967). In certain insects, e.g. *Drosophila* species, it is known that courtship success depends on acoustic and chemical signals, but also on the sequence of various behaviours. Inappropriate responses to different behaviours may contribute to delay or failure of courtship (Ewing, 1983; Jallon, 1984; Welbergen et al., 1987).

In this paper the courtship behaviour of five species of *Ribautodelphax* is described for the first time. In addition, the behaviour between heterospecific males and females is observed. The contribution of non-acoustic behaviours to successful courtship and copulation is examined. The importance of close-range courtship processes for mate preference and species recognition in planthoppers is discussed.

## Materials and methods

### *Species and rearing*

The courtship and copulatory behaviour of the following *Ribautodelphax* species was studied: *R. imitans*, *R. vinealis*, *R. pungens*, *R. angulosus*, and *R. albostratus*. The first four are closely related and are members of the '*R. collinus* complex' (Den Bieman, 1987a). *Ribautodelphax albostratus* is distantly related to this complex, on evidence

from morphology, isozymes, crossability, and acoustic signals (Den Bieman, 1986, 1987a, 1988; Den Bieman and Eggers-Schumacher, 1988). The geographic distributions of all species is western European, but each feeds on a different hostplant (Den Bieman, 1987b).

Observations on heterospecific pairs involved *R. imitans*, and either *R. vinealis*, or *R. albostrigatus*. The former two species are capable of cross-insemination under no-choice circumstances, whereas *R. imitans* and *R. albostrigatus* are not (Den Bieman, 1988).

Rearing conditions and origin of the populations used have been described elsewhere (De Winter and Rollenhagen, 1990). All animals were 'virgin', being isolated from the stock cultures as last instars, and allowed to mature for 6-9 days after final ecdysis before use in the experiments.

### *Observations and event recording*

Planthoppers were observed on a 20 cm long stem of the grass *Festuca arudinacea fenas*. Acoustic signals were recorded using the accelerometer method described by De Vrijer (1984). Non-acoustic behaviour was monitored by means of a video-camera using a 105 mm macro-lens. The acoustic signals were recorded on the audio track of the video tape.

Because of the limited size of the observation stem, it usually took a male only a short while to locate a female; therefore events were not quantified during the attraction phase, as this is likely to be an unnatural situation. The observations were continued until copulation was terminated, or up to 20 minutes after the male(s) and female had met.

The behavioural events, as observed from the video recordings, were scored and analysed using the event recording software package 'The Observer' version 2.0 (Noldus, 1990) on a PC. The behavioural events recognized are listed in Table 1. Only the duration of the courtship (i.e. from the moment the sexes meet till the beginning of the successful copulation attempt), and the duration of the copulation (i.e. from the beginning of the successful copulation attempt till the moment of separation) were measured. Of the other events only the frequency of occurrence was tallied. The locomotor behaviour of males was not scored, because males were active for most of the time. The female behaviour *Kick* usually resulted in the male falling down from the stem, and was considered to be the end of courtship. Uninterrupted sequences of the same non-acoustic event were scored as one event. However, when such an event was repeated after one or more acoustic signals, it was scored twice.

Halfway through the experiments it appeared that mating trials involving one male and one female took longer to complete than in the cases where a female was confined with two males (see below). The remaining intraspecific trials were recorded in a 'female choice' design, involving one female and two males. Interspecific trials were conducted with one male and one female only. A few trials involved both a conspecific and heterospecific male.

Table 6-1. Male and female behavioural acts performed during courtship.

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**Male events:**

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<i>Mcall</i>	calling
<i>Mtouch</i>	touching female with forelegs
<i>Atcop</i>	attempting to copulate
<i>Int</i>	interaction with other male
<i>Loc</i>	localisation of female

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**Female events:**

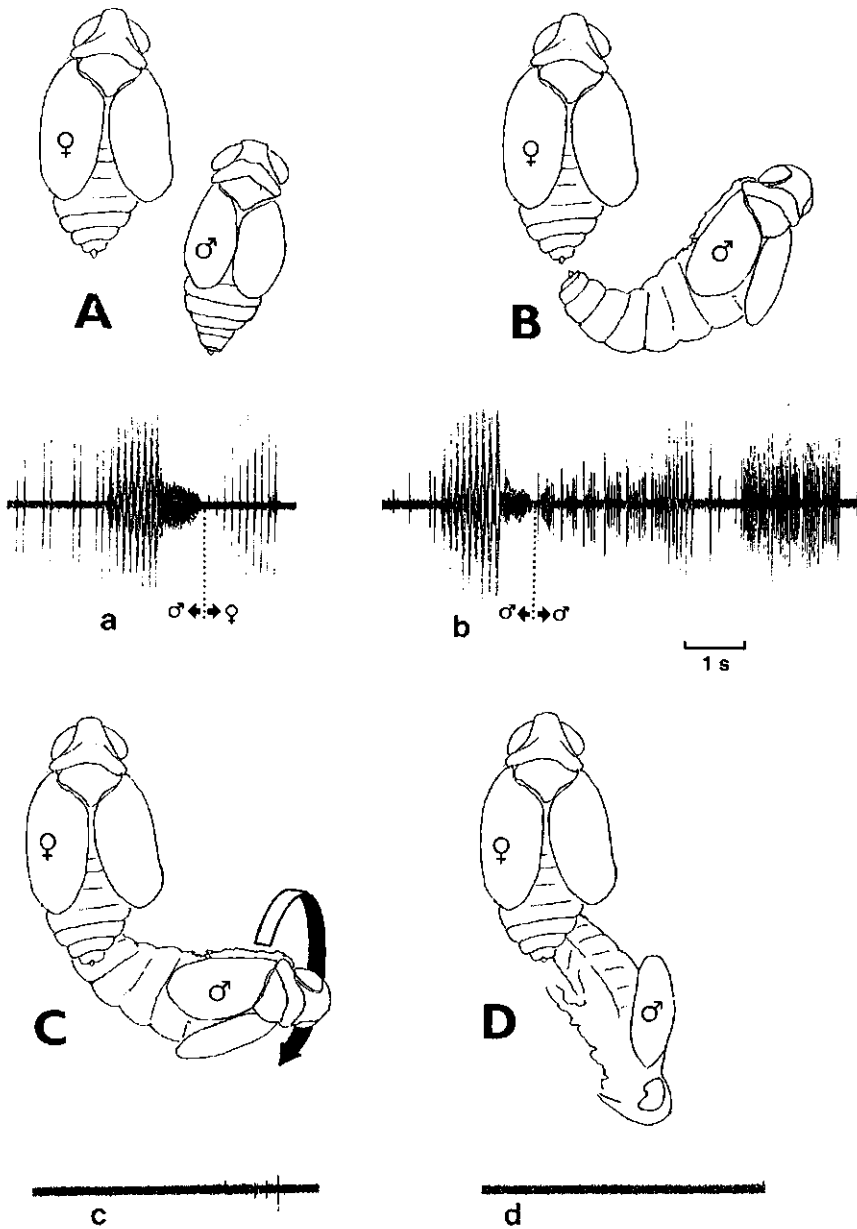
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<i>Fcall</i>	response calling, usually expressed as:
<i>Rnafecal</i>	ratio of numbers of male calls and female response calls
<i>Spon</i>	spontaneous calling, i.e. calling later than 10 s after a male call
<i>Walk</i>	walking
<i>Ftouch</i>	touching male with fore or hindlegs
<i>Kick</i>	kicking away male with hindleg
<i>Shake</i>	shaking of abdomen in reaction to <i>Mtouch</i> or <i>Atcop</i>
<i>Posture</i>	lifting the abdomen in reaction to <i>Atcop</i>

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**Sequential analysis**

From the sequential data, transition frequencies of all dyadic transitions between non-acoustic events were determined for each mating observation, assuming that the probability of the occurrence of an event is only dependent upon the event immediately preceeding it. Data of all trials per species or combination of species were pooled into transition frequency matrices, which were treated as contingency tables. Expected frequencies of transitions were calculated from the actual frequencies, and the significance of transitions between different events was determined by computing adjusted residuals for each cell, and testing these against the 1% standard normal distribution deviate (Everitt, 1977). The frequency of each transition was calculated as a conditional probability, by dividing the number of transitions from the first to the second act by the total number of transitions originating from the first act (Wood et al., 1980). Both significant and non-significant transitions were included in kinematic diagrams of behavioural sequences. Acoustic events were not considered in the sequential analysis, because these would complicate the kinetic diagrams too much. Thus, repetitions of events, even immediately after one or more acoustic displays, were treated as one event only. In other words, all frequencies on the diagonal of the transition matrix were taken to be zero.



**Fig. 6-1.** Schematic representation of successive behaviours of *Ribautodelphax imitans* during successful copulation attempts (A-D), and oscillograms of calls produced (a-d). A, orientation of male towards female; a, male calls, female responds with short series of pulses. B, extending and bending of abdomen by male; b, male call, followed by pre-copulatory sound. C, coupling of genitalia; c, no acoustic activity. D, copulation; d, no acoustic activity.

In order to quantify comparisons among the diagrams of behavioural sequences,

conspecific and heterospecific combinations were clustered by UPGMA according to the conditional probabilities of all non-acoustic behavioural transitions showing variation among species.

### Results

#### *General description of intraspecific sexual behaviour*

Two phases were recognized within the sexual behaviour of *Ribautodelphax*: distant communication between potential mates (including orientation and approach of males towards females), and courtship in the strict sense, normally resulting in copulation.

Under the experimental conditions, the first phase was usually short. In all conspecific, acoustically communicating couples, the male(s) succeeded in finding the female. Courtship started when the male(s) and female were within touching range.

At close range, the male calling frequency and the structure of the male call remained unchanged from that during distant communication. However, in all species, apart from *R. albostrigatus*, up to half of the individuals produced an extra acoustic element, consisting of a series of simple pulses immediately following the normal call. Presence or absence of this feature did not appear to be related to mating success. The frequency of occurrence and length of the female response calls usually decreased considerably as courtship proceeded, the calls losing much of their species specificity. Occasionally females ceased calling completely, apparently without interrupting the normal sequence of courtship events.

After the first localisation of the female, courtship was always initiated by a male tapping the female's body with his forelegs. Females reacted either by walking away, or by tapping or striking the male's body by means of their forelegs, after turning around when approached from behind. When orientated parallel to the female, males were also touched by the hindlegs. Occasionally, females kicked the male away by a sudden stroke from the hindleg. When females did not react (at least visibly), males tried to copulate. However, early in courtship this rarely happened, and only in one case was an early copulation attempt successful (*R. imitans*, in the presence of a second male). Usually it took several cycles of physical contacts between the sexes, walking away by the female, and renewed localisations by the male, before males attempted to copulate. In contrast to several other planthoppers (Claridge and De Vrijer, 1993), wing fluttering was never observed. During copulation attempts, males of all species, except *R. albostrigatus*, produced a typical pre-copulation sound (Fig. 1b).

Females rejected copulation by either walking away, or by lifting the abdomen in a position too high to allow coupling of the genital structures, while sometimes hindering the male with a hindleg. In addition to the rejection behaviours performed by the other species, *R. albostrigatus* females frequently reacted to touching or copulation attempts by vigorously shaking their abdomens.

Copulation attempts were successful only if the male was halfway behind the female, either on the right or left side, and both male and female were oriented with their heads pointing in the same direction (Fig. 1A). The male then extended the abdomen and bent

it towards the base of the ovipositor underneath the female, while she lifted her abdomen (Fig. 1B). Males occasionally tried to copulate when the partners were oriented head to head. Normally, only after several attempts did the female allow coupling of the genitalia, during which time the male made a 90° axial turn, and ended up with his body facing the opposite direction to that of the female (Fig. 1B, C). The attachment of genital structures during copulation was rather strong, as the partners remained coupled even when the male lost contact with the substrate. Copulation lasted less than one minute in all species observed. During and after copulation no sounds were produced. After parting, the male pressed his genital segment against the substrate.

**Table 6-2.** Median frequencies and ranges of behavioural acts per 20 minutes of courtship in different *Ribautodelphax* species. Differences among species are tested by a Kruskal-Wallis one-way analysis of variance. **A.** Observations involving one male and one female (*R. imitans* (N=12), *R. vinealis* (N=5), and *R. angulosus* (N=5)). **B.** Observations involving one female and two males (*R. imitans* (N=25), *R. pungens* (N=5), and *R. albostratus* (N=9)). Significance levels: \*\*\*,  $P < 0.001$ ; \*,  $P < 0.05$ ; °,  $P < 0.10$ .

<b>A</b>				
	<i>R. imitans</i>	<i>R. vinealis</i>	<i>R. angulosus</i>	H
<i>Mcall</i>	121.6 (87.9-156.8)	77.2 (27.8-125.4)	111.9 (91.6-143.0)	5.92°
<i>Mtouch</i>	51.6 (24.3-77.8)	29.4 (11.5-46.2)	52.1 (40.9-61.3)	8.32*
<i>Atcop</i>	11.5 (2.2-18.3)	20.2 (8.6-39.6)	9.9 (1.9-33.0)	3.12
<i>Rmafecal</i>	1.1 (1-1.2)	2.4 (1.7-9.5)	1.4 (1.2-1.8)	16.98***
<i>Spon</i>	5.4 (0-10.3)	8.0 (0-21.1)	1.9 (1.2-13.2)	0.18
<i>Ftouch</i>	32.0 (0-58.3)	21.7 (11.5-52.8)	17.8 (9.7-41.4)	0.59
<i>Walk</i>	26.3 (9.9-41.5)	19.3 (6.6-25.8)	41.8 (25.3-66.1)	8.16*
<b>B</b>				
	<i>R. imitans</i>	<i>R. pungens</i>	<i>R. albostratus</i>	H
<i>Mcall</i>	171.2 (93.8-222.2)	300.4 (217.6-427.1)	84.8 (59.2-121.7)	22.96***
<i>Mtouch</i>	44.6 (14-76.7)	27.6 (26.4-53.4)	40.6 (21.7-68.0)	1.80
<i>Atcop</i>	13.1 (8.2-55.8)	34.7 (6.9-37.5)	14.5 (9.3-22.0)	0.83
<i>Rmafecal</i>	1.2 (1-2.2)	15.7 (2.4-31.5)	1.6 (1.2-15.0)	14.80***
<i>Spon</i>	0 (0-5.1)	0 (0-3.4)	2.2 (0-21.8)	8.17*
<i>Ftouch</i>	36.9 (0-72.1)	32.7 (0-50.5)	15.3 (4.4-26.4)	6.54*
<i>Walk</i>	23.4 (0-70.0)	18.9 (0-22.4)	21.7 (8.0-58.7)	4.45
<i>Inu</i>	3.0 (0-39.1)	0 (0-3.2)	3.0 (0-9.1)	0.47



Courtships were found to be rather lengthy. In only 3 out of 12 cases were single *R. imitans* males allowed to mate within 20 minutes. In most of the remaining cases females after some time tended to produce longer response calls, and many females occasionally produced spontaneous calls. This behaviour might be interpreted as an advertisement for other males. For this reason experiments were made with two instead of one male per trial. This resulted in a significant reduction in courtship duration, in that now 18 out of 25 (72 %) *R. imitans* courtships were successfully completed within 20 minutes ( $X^2$  with Yates correction, 1 d.f. = 2.71,  $P < 0.05$ ).

*Ribautodelphax* males did not produce rivalry songs, or otherwise behave aggressively while competing for a female. Both males called during the initial stage of courtship, and the female usually responded to calls of both males, if there was an ample period of time between the male calls. However, males often called more or less simultaneously, to which the female responded only once. Often both males touched the female initially. In the majority of trials only one male continued to court the female, the other male remaining stationary at a distance, or leaving the recording stem. The calling frequency of the unsuccessful male dropped, and his calls were ignored by the female. In two cases (in *R. imitans*), both males courted the female for a prolonged period, but only in one trial did this appear to contribute to a delayed courtship success. Here males frequently made physical contact with their forelegs, and even made copulatory movements towards each other.

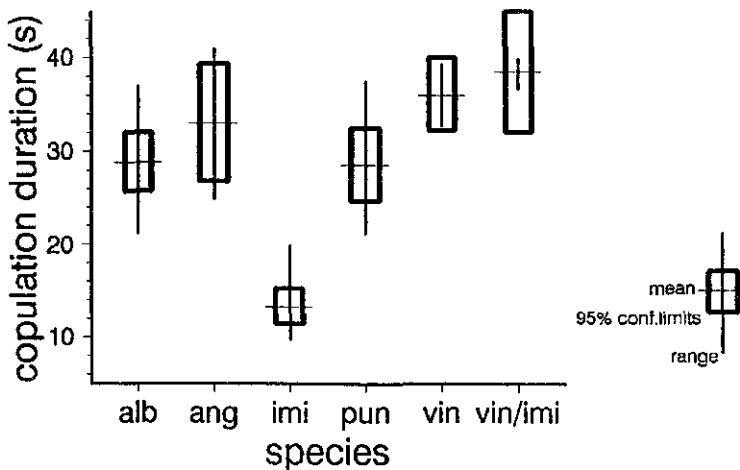


Fig. 6-2. Conspecific copulation durations of different *Ribautodelphax* species, and of one heterospecific copulation ( $N=2$ ) between *R. vinealis*♀ and *R. imitans*♂, with their means, ranges and 95 % confidence limits. Abbreviations: imi, *R. imitans* ( $N=22$ ); alb, *R. albostratus* ( $N=9$ ); pun, *R. pungens* ( $N=5$ ); vin, *R. vinealis* ( $N=5$ ); ang, *R. angulosus* ( $N=2$ ).

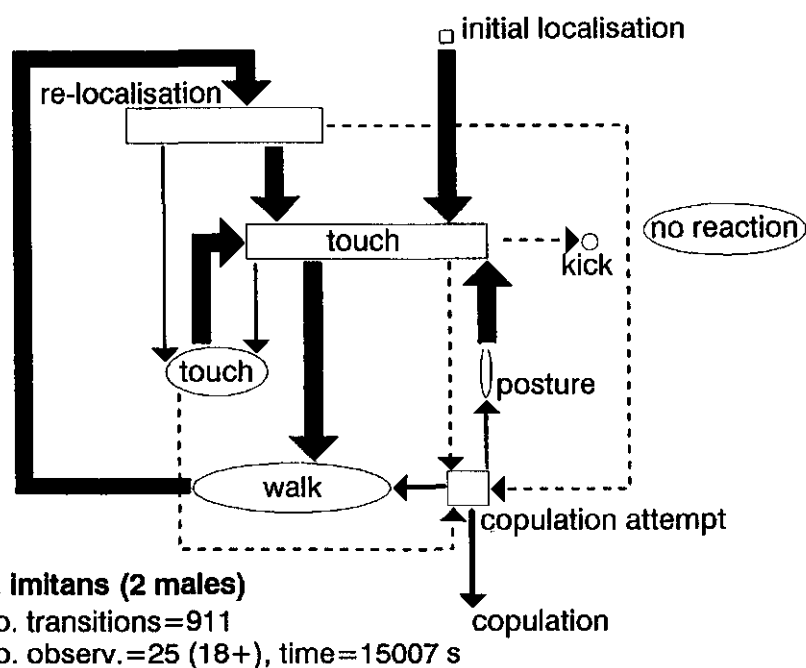
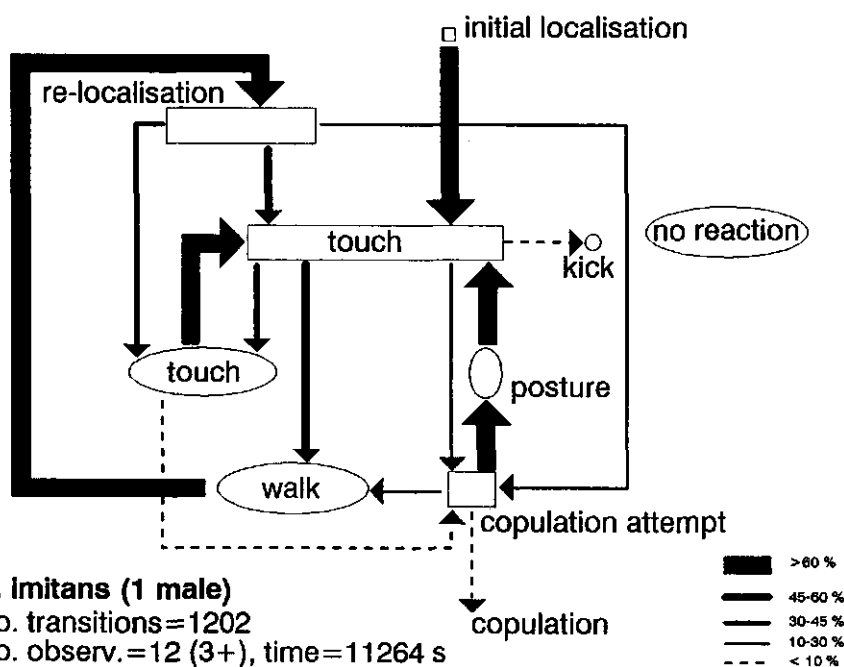
*Quantitative and sequential aspects*

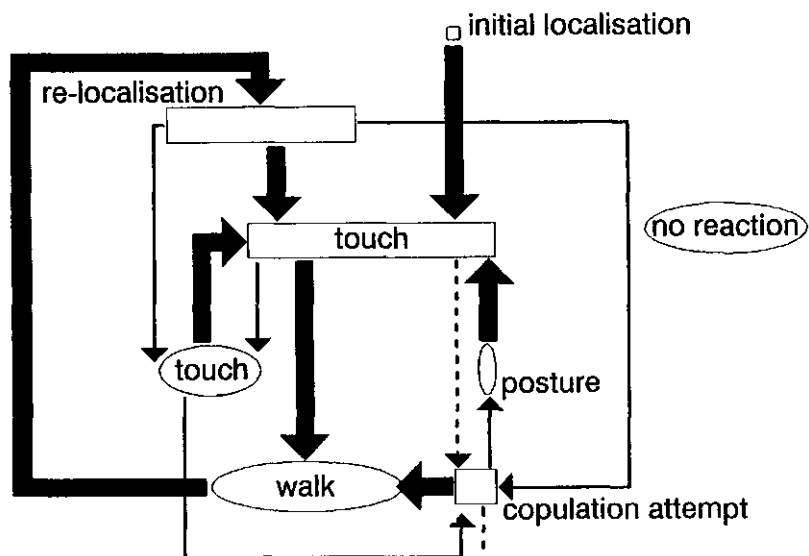
Because observations were made either with one or two males, frequencies of behavioural elements were compared among the two groups (A and B respectively) separately (Tables 2A and 2B). Only in *R. imitans* both types of trials were examined. Trials involving two males had a significantly higher incidence of male calls (one-tailed Mann-Whitney test,  $Z = 3.01$ ,  $P = 0.013$ ). The increase was clearly less than two-fold, which might be due to the fact that one of the males often became less active once a female was occupied with the other. The incidence of the female response calls did not differ significantly, however ( $Z = 1.48$ ,  $P = 0.069$ ), probably because males often called simultaneously, in which case females did not respond to each individual male call. Consequently, trials involving two males tended to have higher ratios of male:female call frequencies ( $Z = 2.79$ ,  $P = 0.003$ ). Trials with single males had a significantly higher incidence of spontaneous female calls ( $Z = -4.20$ ,  $P = 0.00003$ ). No differences were found with respect to the number of copulation attempts, male and female touching, and female locomotor activity (in all cases  $P > 0.19$ ). Such comparative data were not available for the other species.

Apart from the species-specific acoustic male and female signals (Den Bieman (1986, 1987), differences in sexual behaviour among *Ribautodelphax* species were small. Copulation duration was found to differ significantly among species (one-way ANOVA,  $F_{[4,36]} = 44.08$ ,  $P < 0.001$ ), which is mainly due to the relatively short copulation of *R. imitans*; the other species do not differ in this respect (Fig. 2,  $F_{[3,16]} = 2.13$ ,  $P = 0.14$ ).

The frequency of male calls during courtship differed among species. *Ribautodelphax vinealis* tended to have the lowest calling rate among the species in group A. In group B, *R. albostratus* had the lowest calling frequency, whereas *R. pungens* had by far the highest one. Both *R. vinealis* and *R. albostratus* have relatively long male and female calls, whereas those of *R. pungens* are by far the shortest of the species studied (Den Bieman, 1986, 1987c). This suggests that male calling rate is correlated with the length of the calls. During courtship, most females did not respond to each male call, in contrast to the attraction phase (De Winter and Rollenhagen, 1990). The ratio of numbers of male and female calls varied greatly among species. In *R. imitans* the ratio was closest to one, whereas in *R. vinealis* and especially in *R. pungens* it was much higher. Frequencies of other behavioural elements differed little among species.

**Figs. 6-3 to 6-8.** Kinetic diagrams of non-acoustic events in courtships of different *Ribautodelphax* species. Rectangles represent behaviours by males, ovals behaviours by females, the width of which (except for *Kick*) represents the relative frequencies of the events. The width of the arrows indicate the magnitude of conditional transition probabilities, the actual values of which are given in Table 3. In principle, male events are followed by female events and vice versa, except when females did not visibly react. The total number of behavioural transitions and the total observation time are provided, as well as the number of trials (number of successful ones+). ➡ ➡ ➡ ➡ ➡ ➡ ➡ ➡ ➡

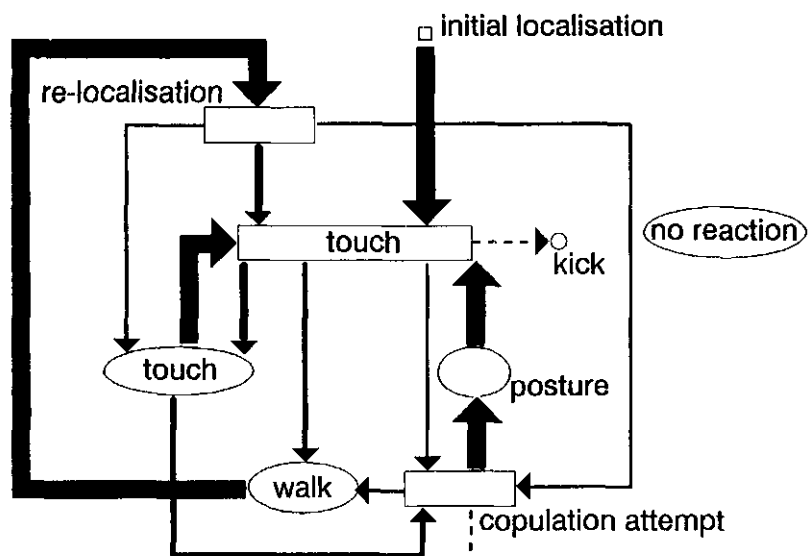




***R. angulosus* (1 male)**

No. transitions=443

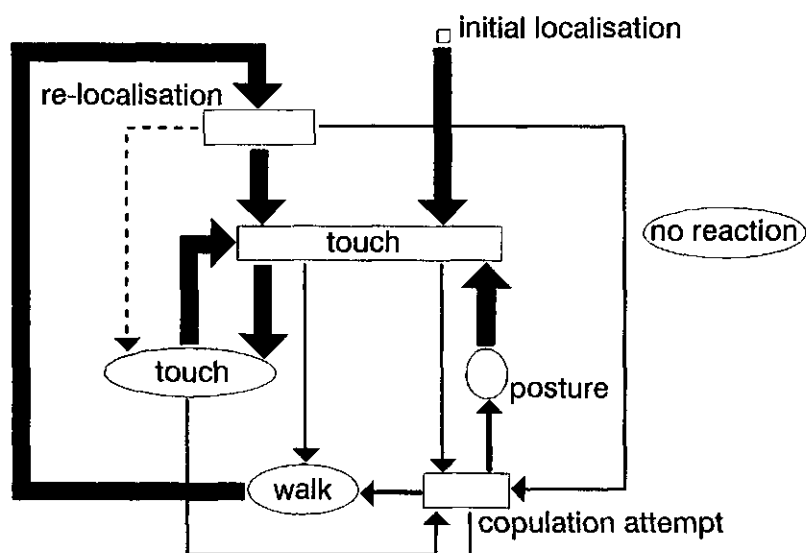
No. observ.=5 (2+), time=4470 s



***R. vinealls* (1 male)**

No. transitions=440

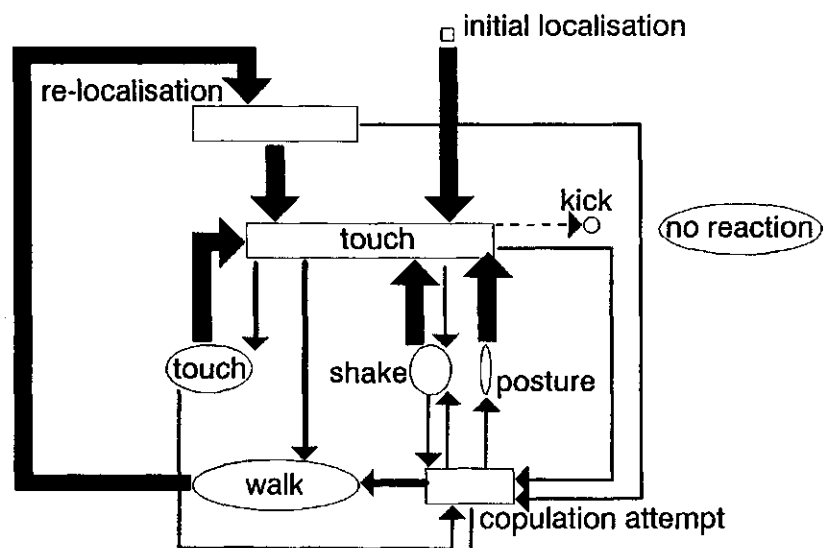
No. observ.=5 (3+), time=5763 s



***R. pungens* (2 males)**

No. transitions=194

No. observ.=5(5+), time=1842 s



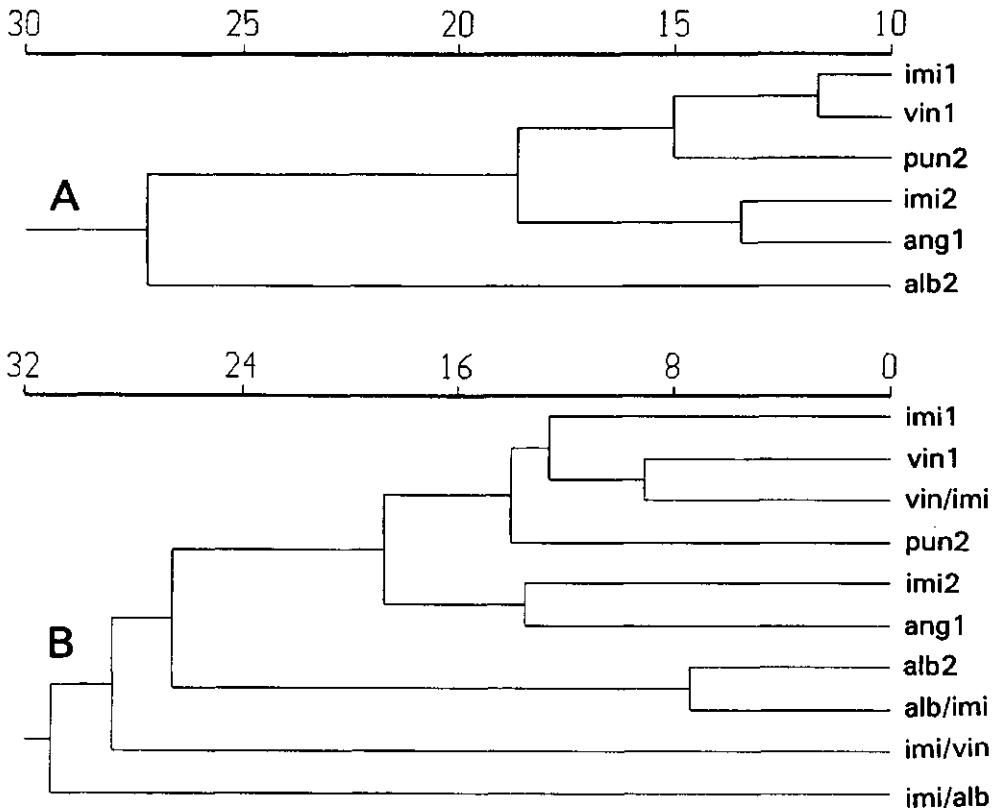
***R. albostratus* (2 males)**

No. transitions=780

No. observ.=9 (8+), time=7803 s

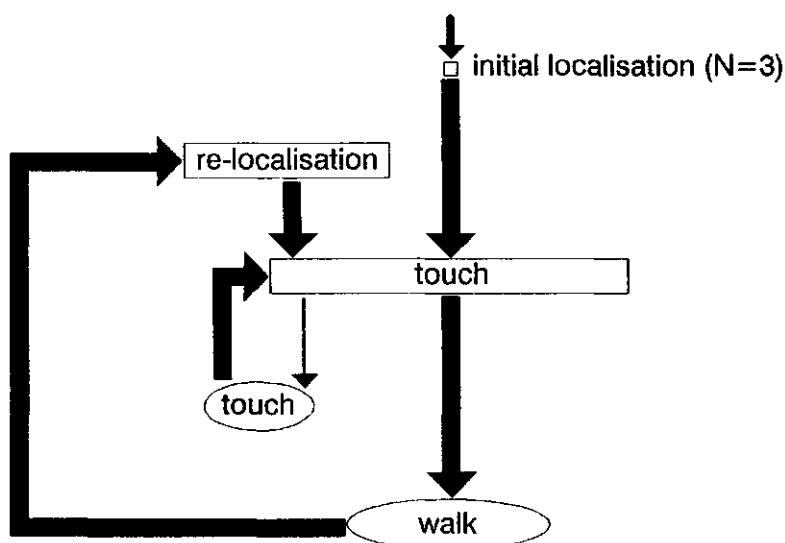
**Table 6-3.** Conditional transition probabilities in courtship of different *Ribautodelphax* species and combinations of species. See text for abbreviations of behavioural elements. Species are abbreviated by their first three letters. In heterospecific combinations the female species is given first. Significant transitions are marked \* ( $P < 0.01$ ).

Species (No. males):												
	imi (1)	imi (2)	vin (1)	ang (1)	pun (2)	alb (2)	imi/vin	vin/imi	imi/alb	alb/imi		
(1st)Loc - Mtouch	100	100	100	100	100	100	67	80	75	57		
(re)Loc - Mtouch	57*	67*	55*	74*	77*	79*	15*	60*	20	75*		
Loc - Frouch	30*	27*	29	15	4	-	-	6	10	-		
Loc - Atcop	13*	6	16	11*	19	21*	-	34*	70*	25*		
Mtouch - Frouch	37*	19	50*	29*	62*	34*	15	41*	43*	21*		
Mtouch - Walk	52*	72*	35*	68*	26*	43*	85*	41*	43	56*		
Mtouch - Shake	-	-	-	-	-	10*	-	-	-	9		
Mtouch - Kick	1	3*	1	-	-	1	-	2	-	4*		
Frouch - Mtouch	93*	91*	65*	87*	79*	85*	100*	67*	75*	87*		
Frouch - Arcop	7	9	35*	13	21	15	-	33*	25	13		
Arcop - Walk	24	31	23	63*	37*	54*	-	35*	100*	54*		
Arcop - Posture	69*	25*	68*	29*	41*	12*	-	60*	-	28*		
Arcop - Shake	-	-	-	-	-	24*	-	-	-	16*		
Arcop - Cop	7*	44*	9*	8*	22*	10*	-	5*	-	-		
Arcop - Shake	-	-	-	-	-	-	-	-	-	2		
Shake - Mtouch	-	-	-	-	-	80*	-	-	-	78*		



**Fig. 6-9.** Dendrograms resulting from UPGMA clustering of courtships of *Ribautodelphax* species according to conditional transition probabilities between behavioural elements. **A**, intraspecific courtships. **B**, intraspecific and interspecific courtships. For abbreviations see legend to Fig. 2.

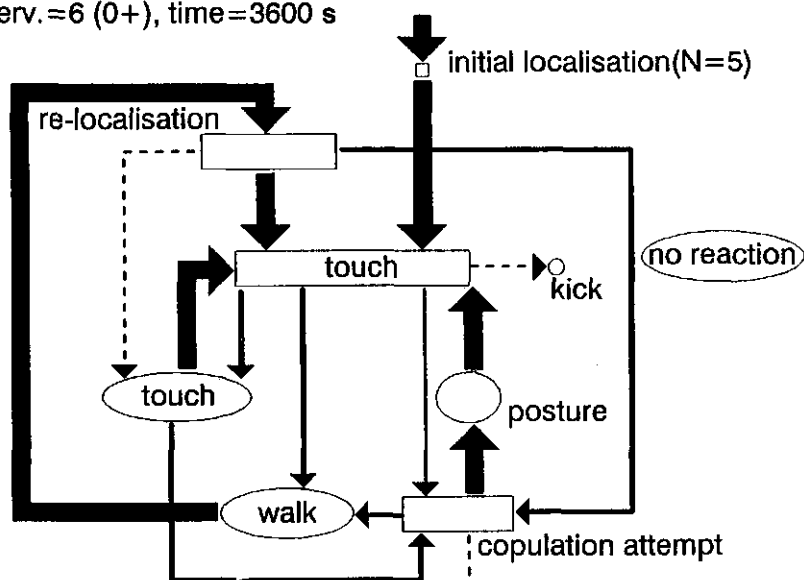
Kinetic diagrams of the non-acoustic courtship behaviours of different species are given in Figs. 3-8. Most behavioural elements were shared by all species studied, and the pathways were broadly the same, but the probabilities of transitions between events often differed. Conditional probabilities of transitions are listed in Table 3. Results of UPGMA clustering of the species according to conditional probabilities of transitions between events are given in Fig 9A. *Ribautodelphax albostratus* was placed in a separate subcluster, as was to be expected, because of the unique behavioural element *Shake*, and the lack of transitions between (re-)loc and *Ftouch* in this species. Of the remaining species, *R. imitans*2♂ was grouped together with *R. angulosus*, and not with *R. imitans*1♂, which was placed in another subcluster with *R. vinealis* and *R. pungens*.



***R. imitans* female, *R. vinealis* male**

No. transitions=31

No. observ.=6 (0+), time=3600 s



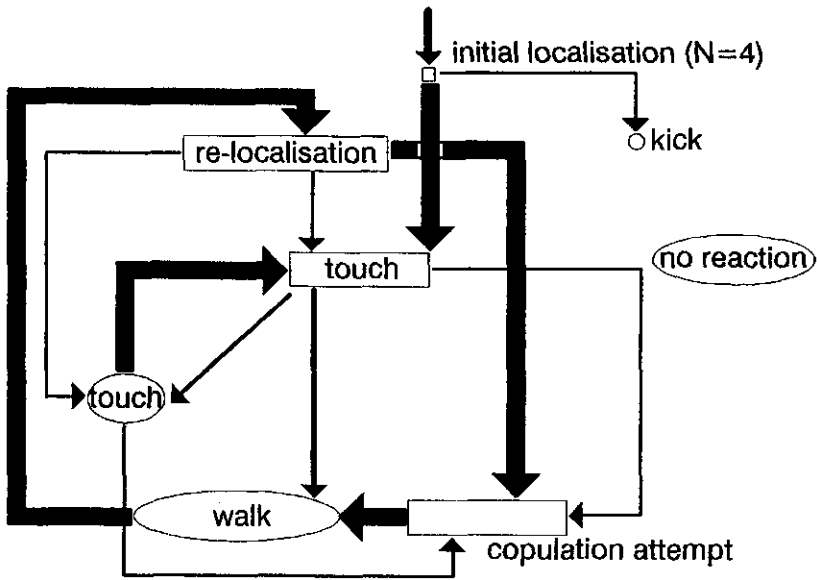
***R. vinealis* female, *R. imitans* male**

No. transitions=270

No. observ.=6 (2+), time=5808 s

Figs. 6-10 to 6-13. Kinetic diagrams of interspecific courtships of *Ribautodelphax* species. For explanation see legend to Figs. 3-8.

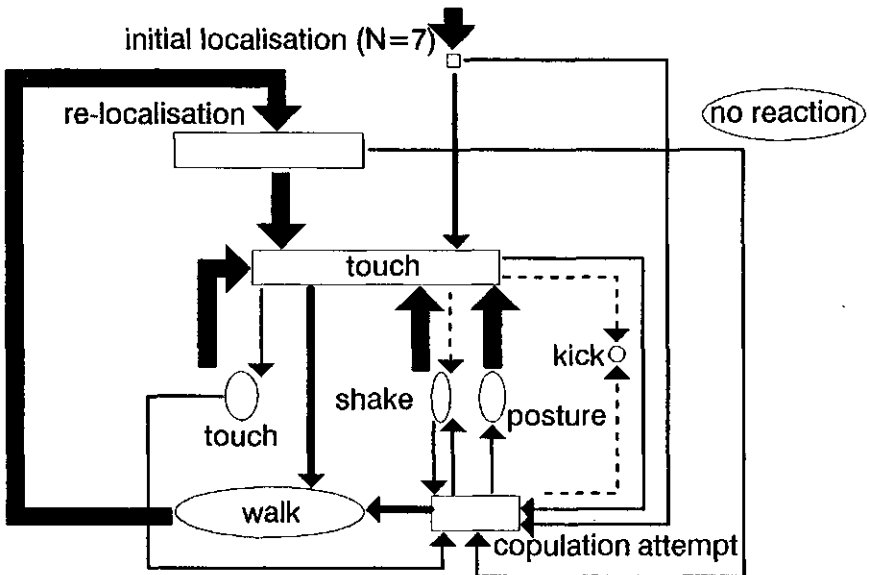




***R. imitans* female, *R. albostratus* male**

No. transitions=39

No. observ.=7 (0+), time=4800 s



***R. albostratus* female, *R. imitans* male**

No. transitions=395

No. observ.=9 (0+), time=8400 s

The frequency of the total number of non-acoustic behavioural transitions per time spent in courtship varied from  $0.11 \text{ s}^{-1}$  (*R. pungens* and *R. imitans*1♂) to  $0.06 \text{ s}^{-1}$  (*R. imitans*2♂), while the others had values in between (0.08 in *R. vinealis*; 0.10 in *R. angulosus* and *R. albostratus*). The considerable lower overall non-acoustic activity in *R. imitans*2♂ compared to *R. imitans*1♂ could result partly from the higher male calling rate in *R. imitans*2♂, because during calling no other behaviours are performed. However, in *R. pungens*, the very high calling rate was accompanied by a high non-acoustic activity. Therefore the high activity of single *R. imitans* males seems more likely to result from the greater number of refusals by females in such trials.

### *Interspecific courtships*

In courtships between heterospecific partners, activity varied greatly among individual observations as well as among the combinations of species observed; even reciprocals of the same combination differed conspicuously (Table 4), with obvious consequences for the kinetic diagrams of courtship of the four combinations studied (Figs. 10-13, conditional transition probabilities in Table 3). Except for two cases (see below), interspecific courtships did not result in copulation. There appeared to be no general cause for the unsuccessful encounters between heterospecific pairs. Males always produced attraction calls. In most cases, females did answer to at least some of the male calls. In 17-50 % of the respective combinations of species, the sexual behaviour was terminated in the distant calling phase, because the male did not effectively search for the female, and eventually ignored her. In 20-43 % of the pairs that reached the courtship phase, nothing further happened, because the male ignored the female, even when she continued calling, or because the female kicked the male away (*imitans*♀/*albostratus*♂, one case only). The majority of the latter encounters were probably accidental, these failures resulting from insufficient stimulation of males during the distant calling phase. The remaining interspecific pairs engaged more or less actively in courtship.

When interspecific courtships developed, they were terminated sometimes by the male, but more often by the female. In some cases the female left the recording stem after one or more cycles of touching each other, without copulation attempts taking place, which never occurred during conspecific courtships. In others, both sexes initially engaged in a more or less normal courtship, but after several unsuccessful copulation attempts the female completely ceased to react to the male, but remained stationary, and was ignored by the male after a while. In other such cases, the male terminated courtship by moving away from the female. Some seemingly more normal courtships continued without success for the total observation period (20 minutes). These causes of unsuccessful courtship were observed in all combinations, except in *imitans*♀/*vinealis*♂, where males never attempted to copulate, and the frequency of the few behavioural events occurring was rather low. In the other three combinations, behavioural acts were sometimes performed at about the same or even higher rates as in the conspecifics, but more often the rate of events was much lower. However, because rates of events were also rather variable among successful intraspecific trials, it seems difficult to draw

conclusions about the exact cause of failure.

**Table 6-4.** Median frequencies and ranges of behavioural acts per 20 minutes of courtship in different heterospecific combinations of *Ribautodelphax* species.

	vin♀/imi♂	imi♀/vin♂	alb♀/imi♂	imi♀/alb♂
<i>Mcall</i>	45 (2-144)	12.5 (3-18)	31 (3-178)	10 (3-87)
<i>Mtouch</i>	18 (0-63)	1 (0-8)	3 (0-74)	0 (0-3)
<i>Atcop</i>	7.5 (0-38)	0 (0)	0 (0-25)	0 (0-12)
<i>Rmafecal</i>	8.4 (1-24)	1.1 (1-1.8)	3.5 (3.2-30.7)	13.2 (1.4-25)
<i>Spon</i>	3.5 (0-9)	0.5 (0-11)	0 (0-3)	0 (0-6)
<i>Ftouch</i>	8.5 (0-30)	0 (0-1)	0 (0-16)	0 (0-2)
<i>Walk</i>	4 (0-60)	1.5 (0-7)	1 (0-65)	1 (0-11)

Two trials, both involving *R. imitans* males and *R. vinealis* females, resulted in copulation within 20 minutes after courtship started. Copulation durations (36.8 and 39.9 s) were within the range of that of conspecific *R. vinealis*. As copulation durations were much shorter in *R. imitans* (Fig. 2), this suggests that copulation duration is mainly determined by the female. Dissection of both mated females revealed the presence of sperm in only one of them. In this combination, rates of events in the successful pairs were much higher than in the unsuccessful pairs except for one, in which courtship was intensive until the female kicked the male from the stem. In the successful pairs the duration of female response calls during courtship dropped, unlike in the unsuccessful ones, suggestive of a higher sexual arousal of the females in the former pairs. The sequences of non-acoustic behavioural events were broadly similar, but in both successful couples females refused courtship attempts equally often by walking away or by taking a rejecting posture, whereas in the unsuccessful pairs females nearly always performed the latter behaviour.

Interspecific courtships involving *R. imitans* females were much less intense than those with either *R. vinealis* or *R. albostratus* females. Or, to put it differently, courtships involving *R. imitans* males were more vigorous than those involving either *R. vinealis* or *R. albostratus* males (Table 4). Clustering by UPGMA of all intraspecific and interspecific combinations according to transition probabilities of non-acoustic behaviours showed the interspecific combinations involving *R. imitans* females to differ greatly both from each other, and from all other ones. The remaining interspecific courtships were most similar to the conspecific ones of the female species (Fig. 9B), suggesting that the course of courtship, including courtship success, is mainly determined by the female.

#### *Courtship in choice situations*

In two observations, involving a *R. imitans* female and one male each of *R. imitans*

and *R. vinealis*, both males called, but only the conspecific male approached and courted the female, whereas the heterospecific male moved away from the recording stem without interfering. This shows that in choice situations interspecific courtships are unlikely to take place, as was to be expected from a previous experiment where two *Ribautodelphax* species were reared together for ten generations without any indication of hybridization (De Winter and Rollenhagen, 1990).

## Discussion

### *Intraspecific behaviour*

Distant communication (mate location) and courtship represent two distinct phases in the sexual behaviour of *Ribautodelphax* planthoppers, both with respect to acoustic and non-acoustic events. The distant communication phase is characterized by male calling and active searching for the female, while females remain stationary, and acoustically respond to almost each male call (De Winter and Rollenhagen, 1990). Female response calls during distant calling are very important in eliciting male searching, as males never approach silent females. At close range, both the frequency and the length of female response calls tended to drop. Instead, females became more active in performing non-acoustic behaviours in response to the advances of males, who maintained their acoustic activity.

During courtship, the behavioural displays did not proceed in a fixed unidirectional sequence. Usually many, often different, cycles of various male and female behaviours occurred before courtship finally resulted in copulation. Unfortunately no data have been published on courtship durations in other planthopper taxa, but compared to the genera *Javesella* and *Chloriona* (unpublished data), that of *Ribautodelphax* was rather long. The frequency and sequence of interactions between male and female events showed a great deal of variability among pairs. The main reason for the lengthy courtships in all species studied appeared to be the initial reluctance by the female to accept the advances of the male.

In all species studied females rejected copulation attempts either by walking away, or by lifting their abdomens to prevent successful coupling of genitalia. Only *R. albostrigatus* females also shook their abdomens, a way of rejection shared by planthoppers in some other genera (Claridge and De Vrijer, 1993). There appeared to be no clear hierarchy in these behaviours among species, because they occurred in different species in different relative frequencies. Within species, these differences might be related to copulation success. In courtships of a *R. imitans* female with one male, *Posture* occurred more often than in those with two males. In the combination *vinealis*♀/*imitans*♂ females of unsuccessful pairs also performed the *Posture* behaviour much more often than the successful ones. Females also performed *Walk* and *Shake* (in *R. albostrigatus*) behaviours in reaction to being touched by the male.

The *Touch* behaviour was performed with different intensities. It generally seemed to be a more gentle gesture initially than later on during a courtship. However, because

the distinction was not always clear, they were treated as the same event, but might actually include two different behaviours. In all species, *Mtouch* was the most common non-acoustic courtship event. After location of the female, males always first touched the female, especially the abdomen. This behaviour was frequently repeated later on in courtship. Similar behaviours have been observed in various insects, notably Diptera where they are involved in reception of the chemical substances present in the cuticular wax, that are important for courtship success (e.g. Carlson et al, 1978; Schlein et al., 1981; Venard et al, 1989, and references therein). From this analogy, both *Mtouch* and *Ftouch* behaviours might be suspected to be involved in the perception of contact pheromones.

As far as could be observed, copulations were performed in a similar fashion in all *Ribautodelphax* species studied, which is rather different from that found in *Prokelisia* planthoppers (Heady and Denno, 1991). Copulation durations differed among *Ribautodelphax* species only in that it lasts significantly shorter in *R. imitans* than in the others. Copulation durations vary greatly among planthoppers, from about one second in *Javesella pellucida*, to more than one hour in *Muellerianella* species (Claridge and De Vrijer, 1993). Viewed in this light, the differences among *Ribautodelphax* species are relatively small. Drosopoulos (1985) found a positive correlation between copulation duration and the time between subsequent copulations, but as we only used virgin animals this cannot explain the differences found between *Ribautodelphax* species. There is presently no explanation for these large differences among planthopper taxa.

Visual cues could contribute to stimulation of the male in initiating or continuing courtship. Once males were in close proximity of the female, they sometimes started courtship even when the female did not respond acoustically, or belonged to a different species. Females of different *Ribautodelphax* species do not differ in external appearance, thus, if at all, vision seems unlikely to be a specific recognition cue.

At close range, both the female call length and the intensity of calling tended to decrease. Even when the female stopped calling altogether, the male(s) continued calling intensively. Thus, female calling during courtship seems to be less important for mating success, whereas male calling is likely to be vital in maintaining or enhancing the receptive state of the female.

### *Intraspecific mate preference*

Courtships in *Ribautodelphax* species proceeded quicker when two males instead of one were confined with a female. Although both males usually interacted acoustically with the female, in most cases only one continued courting her, while the other stayed behind or even left the recording stem. This finding contrasts strongly with observations on other planthoppers, like *Muellerianella* (Booy, 1982), *Nilaparvata* (Ichikawa, 1982), and *Javesella* (De Vrijer, unpublished data), where males become involved in extensive agonistic behaviour, most clearly demonstrated by the production of typical rivalry signals, also termed 'aggressive songs' (Ichikawa, 1982), which may even impede a normal courtship. In *Ribautodelphax* aggressive male-male interactions appeared to be

completely absent. No rivalry song was ever observed in the mating trials and even dozens of males confined in one cage could only be observed chorussing together in apparent harmony (unpublished observation).

When looking for explanations for the shortening of courtship duration, some possibilities seem improbable. There was no correlation between courtship duration and the total number of male calls or the male calling rate. This excludes a simple relation between the amount of male calling and the time necessary for a female to reach a receptive state. Since not all trials involving two males were successful within the observation period, the simple fact of hearing two different males also cannot alone be sufficient for a female to become receptive to mating.

A further element that should be considered is the finding that in the successful trials the male which finally mated with the female was not necessarily the first one that approached her. This suggests that some kind of mate preference (sexual selection) may be involved. It would therefore be interesting to find out whether males differ in attractiveness for the female. Evidence for differences in attractiveness among males was found in *Muellerianella*, where the same males were repeatedly preferred by different females (Drosopoulos, 1985). This could explain why occasionally single *Ribautodelphax* males (supposedly attractive ones) were accepted by a female without much delay, whereas some courtships with two males (supposedly equally attractive ones) were rather protracted. Conspicuous struggle is not the only option for competing males. When the chances of winning contests vary, reciprocal assessment of the status of competing males could diminish the need to engage in agonistic behaviour (West-Eberhard, 1979; Moore and Breed, 1986). Males could e.g. differ in social status by being dominant or subordinate, as was found in the cockroach *Nauphoeta cinerea* (Moore and Breed, 1986), where males assess their relative status from the pheromones they produce, and thereby avoid physical struggle.

The cues by which females potentially might be able to distinguish between different males, and by which males might assess their relative attractiveness, are likely to be found in the male call. Calls show significant variation among males, but relatively little within individual males for all temporal parameters that have been analyzed (Den Bieman, 1986; De winter, Chapter 5). However, a preliminary analysis of the correlation between male call characters and mating success failed to reveal simple thresholds, possibly due to the limited number of trials observed or to differences in preferences of individual females. At close range, contact pheromones might also be involved, which could be assessed during the touching behaviour. However, physical contact between all mating partners did not always take place. Hence, male calls could potentially be under sexual selection during courtship, although at present it remains unclear whether non-random mating is caused by female choice or a subtle form of male contest. However, since females ultimately decide whether they allow copulation or not, we should perhaps classify this as a female choice system.

#### *Interspecific behaviour and species recognition*

Only a part (33 - 67 %) of all interspecific trials were aborted during the distant

calling phase, which appears to be in conflict with earlier experimental observations where males did not show searching behaviour in response to heterospecific female playback calls (De Winter and Rollenhagen, 1990). However, in the experimental conditions of the mating trials described in this paper chances for accidental encounters between males and females were high. Accidental encounters are probably rare in nature, where different species of *Ribautodelphax* live on different host plants (Den Bieman, 1987b). Even if they occur, acoustic contacts between species are not likely to elicit searching behaviour of males. Males became much less selective once they reached the courtship stage, possibly stimulated by non-acoustic (visual, chemical) cues.

Most of the close range interactions between species did not lead to normal courtship and copulation within the experimental observation period, largely due to female rejection of male courtship and copulation attempts. It is probably not accidental that the few successful interspecific trials were observed between those species that showed the most resemblance in the sequence of courtship behaviour. There is, however, little reason to infer a close range isolation mechanism (*sensu* Dobzhansky, 1940) that specifically evolved as an adaptation against hybridization, firstly because accidental encounters are probably rare, and secondly because mate recognition largely takes place during distant calling. More likely, the explanation for the interspecific courtship failures should be sought in the effect of those factors that are also responsible for the mate selection within species.

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## GENERAL DISCUSSION: ACOUSTIC COMMUNICATION AND EVOLUTIONARY PROCESSES IN PLANTHOPPERS

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### Introduction

This thesis was aimed at answering three interrelated questions: 1. Do planthopper calls have a function in species recognition (sexual isolation) and mate preference? 2. What forces have caused differentiation of acoustic signals? 3. What is the relationship between divergence in acoustic signals and speciation?

In most acoustically signalling animals one sex, usually the male, broadcasts the call, which is perceived by the other sex, usually the female. In all delphacid genera studied both males and females produce acoustic signals. The male calls are species-specific (Claridge and De Vrijer, 1993); the female calls have received less attention but at least in some genera (including *Ribautodelphax*) these are species-specific as well. Because in planthoppers both sexes are signaller *and* receiver, there is not only the problem of how the signals and receivers are tuned (see also Butlin & Ritchie (1989) for a discussion on this topic), but also, whether call differentiation is brought about by the same processes in both sexes.

The following discussion is an attempt to answer these questions by taking the results on *Ribautodelphax* from previous chapters and the available data of other planthopper genera into account.

### Effects of calls: species recognition and mate preference

It is important to distinguish between recognition and preference, as was very recently stressed by Ryan and Rand (1993). Mate recognition can be defined as a behavioural response indicating that one individual considers another as an appropriate mate, even if mistakenly. Mate preference can be defined as a behavioural response indicating that an individual intends to mate with one individual rather than another (Ryan and Rand, 1993). Preference is distinguished from recognition in that it implies a comparison.

Both male and female acoustic signals are important for successful reproduction of *Ribautodelphax* species, but their roles change between different phases of the sexual behaviour.

Exchange of calls during the distant calling phase is especially important in bringing potential mates together. Male calling signals induce female response calls, by which females signal their receptive state and elicit male searching behaviour. In choice experiments *Ribautodelphax* males significantly more often approached conspecific female calls than heterospecific ones. This was even more pronounced after previous experience of a conspecific female call, whereas choice behaviour appeared not to be

affected by experience of a heterospecific call. When exposed to both calls separately, males only approached the conspecific call (De Winter and Rollenhagen, 1990, 1993). Thus, males apparently do not recognize heterospecific females as appropriate mates.

In contrast, females showed generally a positive response to heterospecific male *Ribautodelphax* calls. Male calls of closely related species belonging to the *R. collinus*-complex share a similar basic structure, whereas the call of a more distantly related species, *R. albostratus*, clearly deviates in some respects (Den Bieman, 1986). Response levels of both *R. collinus* and *R. albostratus* females to each others male calls were much lower than of females to male calls within the complex, where heterospecific response levels were about as high as conspecific ones (De Winter and Rollenhagen, 1990). Because in these experiments females were exposed to each of the call types separately, it appears that within the *collinus*-complex most females recognize male calls of all *Ribautodelphax* males as belonging to potentially appropriate mates, in contrast to the more strongly deviating male calls of some related genera which did not elicit any response (De Winter and Rollenhagen, 1990). In view of the intermediate response to *R. albostratus* males, mate recognition by females appears to be related to the degree of distinctiveness of the male calls.

When conspecific mating receptive partners are in close proximity (chapter 6), both the number and especially the length of female response calls tend to drop. Occasionally a female ceased calling completely, without impairing male courtship activity and courtship success. Hence, female acoustic signals appear to be less important during courtship. In contrast, the intensity of male calling remained high during courtship, and is therefore likely to serve in sustaining and enhancing the receptive state of the female.

*Ribautodelphax* females were found to mate faster in the presence of more than one male, and observations of courtship behaviour suggest that they do not mate randomly with the available males (chapter 6). This might suggest that during close range courtship females exhibit a preference, which would provide opportunity for sexual selection. However, females appeared not to choose actively among males, nor did males behave aggressively towards each other. The only feasible way by which sexual selection might take place is that males assess their relative attractiveness or social status from individual differences in their calls, which would affect their chances in acquiring a mate. Because females ultimately decide whether mating takes place or not, this would have to be classified as a female choice system.

Such preferences of females for certain male call characteristics could contribute to the rejection of heterospecifics. No-choice interspecific courtships were generally unsuccessful as the result of the reluctance by females to respond properly to male courtship behaviour and to accept copulation attempts (chapter 6). Males appeared to be relatively unselective during this phase. These interspecific courtships resulted from accidental encounters caused by the experimental conditions, rather than from attraction of males to heterospecific female calling. Hence, under natural conditions courtship between heterospecific partners seems unlikely to occur.

Female preferences for male call characteristics could explain the significant degree of assortative mating observed in mate preference tests after artificial selection for long and short *IPI* in the female call (De Winter, 1992). In an additional test co-selected males did

not show a significant preference for female playback calls with *IPIs* close to those occurring in their selection lines. As some male call characters also responded to selection, it seems possible that the assortative mating among individuals of the selection lines was due to female preference for the changed male call parameters, rather than to the preference of males for changed *IPIs* in the female calls.

This study did not seek direct evidence of preferences of *Ribautodelphax* males for call parameters in conspecific females, although the peculiar findings by Den Bieman (1987b) in species with mixed populations of diploids and triploids, discussed later, suggest that such preferences do exist.

These observations might be taken as support for Ryan and Rand's (1993) suggestion that assortative mating results from different interactions between signals and receivers in different situations, and that species recognition and sexual selection are not mutually exclusive processes.

## Evolution of acoustic communication

### *Differentiation of signals*

Studies on the genetic control of acoustic communication revealed that several important parameters of the male and female call have a significant heritable component, and thus are potentially evolutionary plastic (De Winter, 1992; chapter 5). For reasons outlined elsewhere (chapter 5) planthopper calls might well have obtained their species-specificity by responding to genetic drift and/or natural selection in small allopatric populations, for example as the result of founder events. However, sexual selection might also affect call characters of males, because at close range *Ribautodelphax* females did not seem to mate randomly with the available males (see above). Because preferences for sexually selected characters are likely to be different among different populations and species (Kirkpatrick, 1987), sexual selection could be responsible for species-specificity of male call characters. Since it also appeared that some characters of male and female calls are genetically not completely independent (chapter 5), the problem of what factors are responsible for call differentiation seems an inextricable one. In fact, these forces may be different among species or genera of planthoppers.

The idea that the species-specificity of signals is caused by selection against hybrids after secondary contact between previously isolated populations (Dobzhansky, 1940), has been generally accepted until recently. This theory of reinforcement appears to be unlikely in planthoppers. In several genera acoustic differentiation has occurred despite the fact that the species are geographically or ecologically isolated. The differentiation of acoustic signals as well as the assortative mating observed under experimental conditions among different species are thus accidental by-products of within-species processes.

### *Preferences and recognition*

The observation that females are less selective than males towards heterospecific calls during distant calling seems to be at odds with the theory that the sex with the higher

parental investment should be more discriminating (Trivers, 1972). Apart from costs involved in searching, there is no obvious greater investment by the male sex relative to that of females. Therefore one would expect *Ribautodelphax* females to respond to heterospecific male calls at a much lower level, as has been reported in some other planthopper genera like *Nilaparvata* (Claridge et al., 1989) and *Prokelisia* (Heady and Denno, 1991).

One explanation for the situation in *Ribautodelphax* can be derived from a reasoning by Von Schilcher and Dow (1977), who tried to explain a similar observation in *Drosophila*. They argued that the initiating and more active sex should be more choosy, because it is advantageous to recognize a conspecific partner at an early stage in the sexual behaviour. In many animals females are attracted to advertizing males, but there are a number of insect groups where males actively approach signalling females, enabling them to avoid heterospecific females if female signals are species-specific. In the latter case there is likely to be selection for males to prefer conspecific signals, even if such males are not necessarily the more highly investing sex; the indiscriminate approach of males to signalling females of different species would lead to a waste of time and energy for both sexes. However, this theory does not explain why in other planthoppers females are at least as discriminating as males.

This theory also seems inappropriate because it was designed for situations where closely related species live syntopically. For *Ribautodelphax* species the chances of meeting other congenics appear to be small, in view of their strong host plant specificity (Den Bieman, 1987a). It seems more likely that females generally recognize heterospecific male *Ribautodelphax* calls because there is little selection for precise species recognition. Females can thus afford to attract as many potential mates as possible to choose among. In the situation where closely related species do live syntopically one would expect females not to respond to heterospecific males, which seems to be the simplest way to avoid heterospecific encounters. This might explain the female selectiveness against heterospecific male calls reported in two *Prokelisia* planthopper species, which live sympatrically on the same host plant (Heady and Denno, 1991). In the latter situation precise recognition of conspecifics is likely to be brought about by interactions between related species (reproductive character displacement in the sense of Butlin, 1989), rather than by reinforcement.

In many planthopper taxa precise recognition of signals would appear to be at least as important for males as for females. Even in genera where females were reported to be less responsive towards heterospecific male calls than in *Ribautodelphax*, males seemed to be selective as well (Ichikawa et al., 1975; Heady and Denno, 1991), although male responses to female calls have been less thoroughly studied. One obvious reason might be that males cannot afford to approach any signal resembling that of a conspecific female, because searching behaviour incurs costs in energy and risk of predation (Bell, 1990). This holds even in groups where related species are unlikely to meet each other in the field, like in *Ribautodelphax*. There are usually fewer mating-receptive females than males present in a population because, in contrast to males, females mate only once during their lives. Fine tuning to a specific call type would increase the chances of finding a maximally attractive female, as the fraction of such females (i.e. presumably

those with call characteristics close to the population mean) will decrease as the season progresses (De Jong and Sabelis, 1991). Tuning to a specific call type also avoids being attracted to female calls of syntopic members of other delphacid genera, which all have broadly similar calls. Thus, likely selective forces responsible for the precise recognition of female calls by males might be efficiency and inter-male competition for increasingly scarce mating receptive females.

Although artificial bi-directional selection for a female call character in *R. imitans* resulted in some degree of assortative mating, this appeared not to be the result of preferences of co-selected males for playback calls of females from their own selection lines (De Winter, 1992). Therefore it seems to be unlikely that species recognition is brought about by pleiotropic effects of the same genes coding for both signal and receiver characteristics ('genetic coupling'), or even close linkage of such genes.

### *Acoustic communication and the coexistence of diploid and triploid planthoppers*

Sperm-dependent parthenogenesis or pseudogamy has been reported in a variety of plants and animals, including planthoppers (Stenseth et al., 1985). In two species of *Ribautodelphax* (*R. pungens* and *R. imitantoides*) triploid pseudogamous females live in association with diploid populations as reproductive parasites (Den Bieman, 1988a). Variation in the female calls was found to be much greater within and between populations with associated triploids than in populations of diploid species, and may even exceed the differences existing between species (Den Bieman, 1987b). This variation exhibits a discontinuous pattern; up to three different call 'types' were found in a single population containing both karyotypes, and Den Bieman (1987b) suggested that this variation might relate to the preference of males for diploid females.

The ratio diploids:triploids was found to differ considerably between populations, but to be relatively constant over several generations (Den Bieman, 1988c). Triploids potentially have a twofold reproductive advantage over diploid females, which would theoretically lead to the elimination of the diploids, eventually resulting in the demise of the triploids themselves because that would deprive them of their sperm resource. Several models have provided possible mechanisms by which a stable coexistence may be established and maintained, including male preference for sexual females, and fitness differences between both types of females under different ecological conditions (Stenseth et al., 1985; Kirkendall and Stenseth, 1990). *Ribautodelphax* males were reported to discriminate between the two female types, but on what criteria is uncertain (Den Bieman, 1988c). In field collected material usually all diploid females were found to be inseminated, whereas in triploids the fraction inseminated was less than 50 % (Den Bieman, 1988c). The present studies on the genetic control of the acoustic communication in *Ribautodelphax* provide a possible explanation for both the coexistence of diploids and triploids, and the large variance of female calls in such populations.

In contrast to most animals where pseudogamous triploids are thought to have arisen by hybridization between different species (Stenseth et al., 1985), it is assumed that the triploid *Ribautodelphax* females have an autopolyploid origin, which is more consistent with the available data for this genus (summarized in Den Bieman and Eggers-Schumacher,

1987). The arising of a triploid clone is likely to be a relatively rare event, because usually only one or two, rarely three, electrophoretically and acoustically recognizable clones occur within populations (Den Bieman and Eggers-Schumacher, 1987; Den Bieman, 1987b). It is further assumed that males can distinguish between different female calls (De Winter and Rollenhagen, 1990), and that the male preference for a female call, like the recognition (De Winter and Rollenhagen, 1993) is genetically determined.

In females of a related sexually reproducing species, *R. imitans*, phenotypic variation in the call characters was found to be largely composed of additive genetic variation (De Winter, 1992), which ensures that selection on the call can have a significant effect in the next generation. The preference of a male for a female call will be inherited only if he mates with a diploid female. Assuming an initially gaussian distribution for a female call character, a triploid clone has the highest chance of originating from a female with call characters close to the population mean. Because of the higher productivity of the triploids, and because most males will initially prefer female calls near the population mean, the frequency of triploids will rapidly increase at the expense of the diploid females with this call character. Males choosing the common call type will thus be increasingly strongly selected against, because they risk wasting reproductive effort, and even genetic death. This is likely to result in a bimodal male preference function, and eventually in a bimodal distribution of the female call character, while the old common call will gradually disappear. A diploid female with a call character at one of the new peaks may produce a new triploid clone with a different call; these triploids will become more common, until they will again cause a change of the male preference function, and of the female call character distribution. The preference of males for aberrant calls will result in a higher insemination level of diploid females because these calls are likely to be produced by diploid females, which may counterbalance the twofold reproductive advantage of the triploids. Such a dynamic 'armsrace' might prevent the extinction of either karyotype, as well as explain the unusual pattern of call variation in populations of the species in which they occur.

### Scenarios of speciation in planthoppers

In view of their importance in species recognition, acoustic signals are to be considered as components of the specific mate recognition system of planthoppers (in the sense of Paterson, 1985). Speciation could have resulted directly from a change in the acoustic communication system in small allopatric populations through founder events, which appears to be in agreement with Paterson's (1985) view that speciation occurs when a species' mate recognition system has changed. However, the acoustic mate recognition system might need to change further in order to reach complete speciation (i.e. non-recognition of members of other populations as potential mates), which could rapidly take place, for instance by sexual selection (Lande, 1981; West-Eberhard, 1983, 1984; De Jong and Sabelis, 1991).

In *Ribautodelphax* an alternative theory seems to be equally likely at first sight. Speciation might have resulted from a shift to a new host plant after which further

genetic divergence occurred, including acoustic differentiation. Each *Ribautodelphax* species is generally able to survive and reproduce only on one particular plant species (Den Bieman, 1987a), and the signals could have evolved as the result of different selection regimes at work in the new habitat, or by chance, because the founding population is likely to have been small. However, in some planthopper genera, like *Nilaparvata* (Claridge et al., 1985a, b), *Prokelisia* (Heady and Denno, 1991), and *Chloriona* (Gillham et al., 1992), acoustic differentiation has taken place without a host plant shift.

Additional circumstantial evidence against the primary role of the host plants in planthopper speciation comes from the apparent differences in interspecific hybridization possibilities within different genera. In both *Prokelisia* (Heady and Denno, 1991) and *Chloriona* (Gillham, pers. comm.) it has not been possible to produce hybrids between different species confined under no-choice conditions in small containers. In genera with species living on different host plants interspecific hybridization occurs more or less easily under such circumstances (Booy, 1982; Den Bieman, 1988b). In the genera with syntopic species the reluctance to mate with heterospecifics is apparently stronger compared to the genera with host plant-specific species, possibly by more precise recognition of acoustic signals, or by the presence of additional recognition cues. For reasons of parsimony, it seems likely that species of genera now living syntopically on the same host plant have first diverged in allopatry to such an extent that they can coexist at secondary contact. For such syntopic species the possibility of a reinforcement scenario, or, more likely, one that involves reproductive character displacement, cannot be excluded at the present state of knowledge.

Thus, it appears that the primary factor in planthopper speciation was a change of the acoustic mate recognition system in allopatry. Incipient speciation can be facilitated by a host plant shift. The example of *Nilaparvata* might serve as an illustration of this: geographically widely separated populations all feeding on rice have developed acoustic differences and can be easily hybridized in the laboratory (Claridge et al., 1985a), whilst two closely related sympatric species live on different host plants (Claridge et al., 1985b). However, the situation in *Prokelisia* and *Chloriona* shows that a host plant shift is not a prerequisite for speciation to occur.

The process of speciation is viewed as an undirected change of the specific mate recognition system in small isolated populations, which can take place by various causes, up to the point where other populations are no longer recognized. This is in accordance with Paterson's recognition species concept, except that the mate recognition system, at least some of its components, appears to be less evolutionary stable than envisaged by that author.

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## SUMMARY

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Delphacidae (Homoptera), commonly referred to as planthoppers, are herbivores, which usually feed on grasses and sedges. During sexual behaviour males and females communicate by exchanging low-frequency vibrational signals, which are transmitted through the substrate, normally the host plant. This thesis deals with the acoustic behaviour of one planthopper genus, *Ribautodelphax*, where both male and females have been found to produce species-specific calls, which differ between species in temporal parameters. As in other planthoppers, the acoustic signals of males and females are rather different. Male calls are more complicated, and consist of at least two structurally different elements, a variable number of 'chirps', followed by a 'buzz' of variable length, hence termed the 'chirp-section' and the 'buzz-section', respectively. The female call consists of a series of simple pulses, which differs between species in interpulse interval length (*IPi*), signal duration, and modulation of pulse repetition rate within the signal.

This study was aimed at answering the following central, interrelated questions:

1. Do planthopper calls have a function in species recognition (sexual isolation) and mate preference?
2. What forces have caused differentiation of acoustic signals?
3. What is the relationship between divergence in acoustic signals and speciation?.

It was confirmed that, during the first phase of the sexual behaviour (the so-called distant calling phase), these calls are especially important in bringing potential mating partners together. Males called first, and mating-receptive females responded acoustically. The male then started searching actively for the female and continued to exchange calls with her, during which the female remained sedentary until the male came in close range. Females only responded to conspecific male calls when they are virgin and old enough. In populations of two closely related species, the development of female responsiveness with age corresponded fairly well with that of insemination levels, which shows acoustic response levels to be good indicators of mating receptiveness.

At close range (courtship in the strict sense), males remained acoustically active, but the female signal length and calling frequency tended to decrease, and some females ceased responding altogether. This suggests that during courtship the male call serves in maintaining and enhancing the female's receptiveness, and that the female call is less important. At this stage of sexual behaviour females appeared to be rather cautious, and usually only allowed copulation after many refusals. Courtships were clearly shorter when a female was confined with two males instead of one. Females seemed not to mate randomly with the available males, leaving open the possibility of some form of sexual selection. In the absence of other obvious cues, it seems possible that females might prefer males on the basis of their acoustic signals, but possibly due to the limited number

of observations no trend in preference was found. However, females did not actively choose between males, nor did males behave in any way aggressively towards each other. After both males called initially, usually only one male continued courtship, leaving the possibility that males first assess their relative attractiveness or social status from their calls.

Different closely related *Ribautodelphax* species performed the same behaviours during courtship, but differed more or less in the frequencies of transitions between behavioural events. A more distantly related species deviated more strongly in transition frequencies, as well as by exhibiting a behavioural element not shared by the other species.

Many combinations of *Ribautodelphax* species are known to be able to produce viable and fertile interspecific hybrids under no choice conditions. However, when both conspecific and heterospecific partners are available, interspecific matings rarely take place, if at all. After rearing two species together for 10 generations no indication was found of introgression having occurred. Thus, recognition of conspecifics takes place before mating. Most females exposed to playbacks of heterospecific male calls responded about as well as to conspecific calls. In contrast, males were found to approach only playbacks of conspecific female calls, or, in a two-way choice experiment, chose significantly more often for the conspecific call. In an additional experiment males were continuously exposed to either a conspecific or a heterospecific female playback call during their development from egg to adult. After this treatment both types of males preferred the conspecific female call over the heterospecific one, but males with experience of the conspecific call did this significantly more often. Males primed with the heterospecific female call performed similarly to acoustically naive males. This shows that recognition of conspecific female signals by males is largely genetic, but can be improved to some extent by previous experience of the conspecific signal, whereas the recognition mechanism is not affected by heterospecific signals. Thus, the acoustic communication between the sexes forms at least part of the specific mate recognition system of these species. Apparently, species recognition in *Ribautodelphax* results primarily from the male preference for conspecific female calls. This is a surprising result, because females only mate once during their lives, in contrast to males. In the absence of any obvious male parental investment, apart from costs involved in searching, the females would be expected to be more selective.

At close range, most interspecific courtships observed did not result in copulation, which is likely to be a by-product of within-species choosyness by females towards acoustic or non-acoustic performances of males. Such interspecific encounters are unlikely to occur under natural conditions, because at that stage recognition has already taken place.

Artificial bi-directional selection for large and small *IPIs* in the female call of *R. imitans* was very successful, resulting in non-overlapping distributions of the character after only five generations. The mean of realized heritability estimates of all selection lines over this period was above 80%, and still above 50% over 10 generations. *IPI* proved to be a polygenic character, controlled by at least six independently segregating genetic factors. Other female call characters, like signal duration, and modulation of

pulse repetition rate within the signal exhibited correlated responses. After the experiment a significant degree of symmetrical assortative mating was found in mate preference tests between males and females from oppositely selected lines, but co-selected males did not show a significant preference for female playback calls with *IPIs* close to those occurring in their selection lines. Some characters of the chirp-section of the male call also appeared to exhibit a correlated change, suggesting that male and female call characters do not evolve independently. It seems possible that the assortative mating among individuals of the selection lines is due to female preference for the changed male call parameters, rather than to the preference of males for changed *IPIs* in the female calls.

The genetic control of male call characters in *R. imitans* was studied by father-sons regression. Heritability estimates of the chirp-section characters were statistically significant (0.44-0.54), in contrast to those of the buzz-section (0.09-0.28). Phenotypic, genetic, and environmental correlations calculated among male call characters suggest that chirp-section and buzz-section characters vary independently. One chirp-section character, number of chirps, appeared to be influenced by sex-linked loci. This means that the heritability estimate obtained by father-sons regression probably underestimates the true heritability of this character.

The possibility that the calls have evolved as adaptations to prevent hybridization (reinforcement) appears to be unlikely, for reasons like the apparent genetic plasticity of call characters, the observation that females inseminated by heterospecific males produce both viable and fertile offspring, and the fact that these species live ecologically isolated. It seems more probable that the calls obtained their species-specificity as the result of selection and chance, e.g. after founder events. Potentially, sexual selection might also have contributed to the differentiation of at least the male call. In view of the genetic correlation between some male and female call characters found in *R. imitans*, the possibility that change in the call of one sex might affect that of the other cannot be excluded.

The observation that, during distant calling, males are much more selective than females with respect to calls of other species appears to be best explained from the need to be as efficient as possible in finding a proper conspecific mate. Because *Ribautodelphax* species are confined to different host plants, the chances of meeting other related species are slim, and selection for precise species recognition is likely to be weak. In order to attract as many as possible males to choose among, it might be sufficient for females to recognize the calls of congeners, which have basically the same structure. For males precise recognition is likely to be more important. There are usually less mating-receptive females than males present in a population, because females mate only once during their lives, in contrast to males. Tuning to a specific call type would increase the chances of finding as many as possible attractive females, as the fraction of such females (i.e. presumably those with call characteristics close to the population mean), is likely to decrease as the season progresses. In species living syntopically on the same host plant, females might be expected to be at least as selective as males towards heterospecific calls of the other sex, because that would seem to be the most economic and safe way to avoid heterospecific encounters. In two species of the

planthopper genus *Prokelisia*, which share the same hostplant, this indeed seems to be the case.

In populations of two *Ribautodelphax* species, where diploid males and females live associated with triploid gynogenetic females, a peculiar use of the acoustic communication system in a within species context appears to occur. The triploids occasionally arise spontaneously in diploid populations, and need to mate with diploid males, but produce only identical triploid females. Because of their two-fold reproductive advantage, triploids potentially can outcompete the diploid females, which would also lead to their own demise. However, the ratio diploid:triploid females in the field was reported to be stable over time. A model is suggested explaining this stable coexistence by a dynamic 'armsrace' involving the female calls, enabled by the genetic plasticity of the female call, and driven by the selection pressure on males to prefer female calls deviating from the population mean, thereby avoiding mating with the otherwise indistinguishable triploids. This would also explain the peculiar occurrence of several different female call types within and between populations of the species in which such triploids occur.

In view of the potential effect of acoustic signals in species recognition, the evolution of the acoustic communication system might be the primary force behind speciation in planthoppers. However, a confounding factor in *Ribautodelphax* is that, although the species studied live potentially sympatrically, each is confined to one particular host plant species, on which they feed and oviposit and therefore are unlikely to meet related species in the field. Hence, call differentiation could have taken place as the result of isolation after a change to a new host. However, comparative evidence from related genera shows that acoustic differentiation can also occur without a host plant shift. It therefore seems inevitable to conclude that the change of the acoustic communication system in allopatry is indeed the main factor in planthopper speciation. Although speciation will be facilitated by a host plant shift, because it obstructs secondary contact, it appears to be no prerequisite. Species of genera living syntopically on the same host appear to have developed more rigid recognition systems than members of genera which are ecologically or geographically isolated, because interspecific inseminations were reported to be extremely rare or non-existent, even under no-choice conditions. In these species the specific mate recognition system has apparently changed sufficiently in allopatry to enable coexistence with congeners after secondary contact. The process of speciation is viewed as undirected change of the specific mate recognition system in small isolated populations up to the point where other populations are no longer recognized, which is in accordance with Paterson's recognition species concept, except that the mate recognition system, at least some of its components, appears to be less evolutionary stable than envisaged by that author.

## SAMENVATTING

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Delphaciden of spoorcicaden vormen een groep van herbivore insecten, die sterk gebonden zijn aan hun voedselplanten, voornamelijk grassen en zeggen. Tijdens de balts communiceren de mannetjes en vrouwtjes middels laagfrequente trillingssignalen, die via een vast substraat, normaliter de voedselplant, worden getransporteerd. Dit proefschrift gaat over het akoestisch gedrag in het spoorcicadengeslacht *Ribautodelphax*, waarvan bekend is dat zowel de mannetjes als de vrouwtjes soortspecifieke signalen produceren, die tussen soorten verschillen in temporele parameters. De signalen van mannetje en vrouwtje zijn zeer verschillend van structuur. Het meer gecompliceerde mannelijke signaal bestaat uit twee verschillende onderdelen, een variabel aantal 'chirps', de 'chirp-sectie', direct gevolgd door een 'buzz' van variabele duur, de 'buzz-sectie'. Het vrouwelijk signaal bestaat uit een reeks eenvoudige pulsen, en soorten verschillen van elkaar in de afstand tussen twee opeenvolgende pulsen (het zgn. interpulse-interval, *IPi*), de duur van het signaal, en in veranderingen van *IPi* binnen het signaal.

Het hier beschreven onderzoek was gericht op de beantwoording van de volgende nauwvervlochten vragen:

1. Wat voor rol spelen deze signalen in processen binnen de soort, leveren ze een bijdrage aan de herkenning van andere soorten (reproductieve isolatie) en wat is de relatieve bijdrage van de mannelijke en vrouwelijke signalen aan deze processen?
2. Hoe is de overerving van de signalen en de signaalpreferenties georganiseerd, en door welke evolutionaire processen is hun soortspecificiteit ontstaan?
3. Hebben deze signalen op een of andere manier bijgedragen aan het soortvormingsproces?

Uit eerder werk aan verwante geslachten is gebleken dat de akoestische communicatie een belangrijke rol speelt in het bij elkaar brengen van paringsbereide mannetjes en vrouwtjes. Dit bleek ook bij *Ribautodelphax*-soorten het geval. Mannetjes roepen eerst, waarop paringsbereide vrouwtjes antwoorden. Vervolgens gaat het mannetje op zoek naar het vrouwtje, terwijl de uitwisseling van signalen doorgaat. Het vrouwtje verplaatst zich niet, tot het mannetje haar gevonden heeft. Vrouwtjes antwoorden alleen als ze maagdelijk zijn en een zekere leeftijd bezitten. Uit experimenten bleek het responsniveau een goede maat voor de paringsbereidheid van het vrouwtje te zijn.

Wanneer de beide seksen in elkaars directe nabijheid zijn, begint de eigenlijke baltsfase. Het mannetje blijft akoestisch actief, maar bij het vrouwtje nemen zowel de duur van het signaal als de roepfrequentie duidelijk af en soms stopt ze helemaal met antwoorden. Dit suggereert dat de mannelijke roep tijdens de baltsfase een rol speelt in de instandhouding en versterking van de paringsbereidheid van het vrouwtje, terwijl het vrouwelijk signaal in deze fase van het paringsgedrag minder belangrijk is. Gedurende de baltsfase is het vrouwtje duidelijk 'voorzichtiger' dan het mannetje en staat meestal pas na veel weigeringen de copulatie toe. De duur van de balts bleek duidelijk korter in de aanwezigheid van twee in plaats van één mannetje. Vrouwtjes lijken niet willekeurig met

één van de aanwezige mannetjes te paren, hetgeen erop zou kunnen wijzen dat één of andere vorm van seksuele selectie plaatsvindt. Er zijn geen duidelijke aanwijzingen gevonden voor het bestaan van andere kenmerken waarvoor vrouwtjes een preferentie zouden kunnen hebben. Daardoor lijkt het mogelijk dat vrouwtjes bepaalde mannetjes verkiezen op basis van hun roepgedrag. Er kon echter geen duidelijke trend in geprefereerde roepkenmerken worden aangetoond. Nauwverwante *Ribautodelphax*-soorten vertoonden globaal hetzelfde baltsgedrag, maar verschilden in meer of mindere mate in de frequenties van alternatieve overgangen tussen de diverse gedragselementen. De balts van een minder verwante soort week sterker af in de volgorde van gedragingen en bevatte bovendien een gedragselement dat bij de andere soorten ontbreekt.

Onder gedwongen omstandigheden blijken tussen veel combinaties van *Ribautodelphax*-soorten interspecifieke kruisingen mogelijk te zijn, resulterend in levensvatbare en fertiele hybriden. Als echter een keuze mogelijk is tussen conspecifieke en heterospecifieke partners, gebeurt dit niet of nauwelijks. Twee goed kruisende soorten werden gedurende 10 generaties samen gekweekt, waarna geen aanwijzing voor het optreden van introgressie kon worden gevonden. Herkenning van de eigen soort treedt dus voor de paring op. Vrouwtjes bleken ongeveer even goed op playback-signalen van heterospecifieke als van conspecifieke mannetjes te antwoorden. Mannetjes zochten echter alleen naar playback-signalen van conspecifieke vrouwtjes, of, in een keuze-experiment, bleken significant vaker het conspecifieke signaal te benaderen. In een ander experiment werden mannetjes gedurende hun ontwikkeling van ei tot adult continu blootgesteld aan signalen van hetzij conspecifieke hetzij heterospecifieke vrouwtjes. Na afloop bleken de meeste mannetjes van beide behandelingen in een keuze-experiment het conspecifieke signaal te prefereren, maar mannetjes met ervaring met het conspecifieke signaal deden dit significant vaker. Mannetjes met een heterospecifieke signaalervaring vertoonden hetzelfde keuzepatroon als akoestisch naïve mannetjes. Dit toont aan dat de herkenning door mannetjes van het conspecifieke vrouwelijke signaal grotendeels genetisch bepaald is, maar preciezer kan worden door ervaring, terwijl het herkenningsmechanisme niet beïnvloed lijkt te worden door een heterospecifieke signaal. De akoestisch communicatie tussen de seksen is dus een onderdeel van het soortspecifieke partner-herkenningssysteem van *Ribautodelphax*-soorten. Het niet optreden van paringen met andere soorten is blijkbaar vooral het gevolg van de preferentie van het mannetje voor het conspecifieke vrouwelijk roepsignaal. Dit is een verrassend resultaat, omdat vrouwtjes, in tegenstelling tot mannetjes, slechts éénmaal tijdens hun leven paren. Omdat mannetjes relatief weinig in hun nakomelingen investeren, behalve middels kosten verbonden aan hun zoekgedrag, zou men verwachten dat vrouwtjes selectiever zijn dan mannetjes.

Kunstmatige bidirectionele selectie voor grote en kleine *IPIs* in de vrouwelijke roep van de soort *R. imitans* resulteerde al na vijf generaties in niet-overlappende verdelingen voor het kenmerk. Het gemiddelde van de 'realized heritability'-schattingen van alle selectielijnen over deze periode was hoger dan 80% en hoger dan 50% na tien generaties, hetgeen aantoont dat de variatie voor dit kenmerk binnen de populatie voor een groot deel additief genetisch van aard is. *IPi* bleek een polygeen kenmerk te zijn dat



gecodeerd wordt door tenminste zes onafhankelijk segregerende genetische factoren. Andere kenmerken van de vrouwelijke roep, zoals de signaalduur en de verandering van pulsrequentie binnen het signaal, veranderden mee. Paringsvoorkeursproeven na afloop van het selectie-experiment tussen mannetjes en vrouwtjes afkomstig uit de diverse selectielijnen lieten een significante mate van symmetrische assortatieve paring zien. De meegeselecteerde mannetjes vertoonden echter geen significante voorkeur voor playback-signalen van vrouwtjes afkomstig uit hun eigen selectielijnen. Ook bepaalde kenmerken van de chirp-sectie van het mannelijk signaal bleken te zijn meegeselecteerd, hetgeen suggereert dat het vrouwelijk en mannelijk signaal niet geheel onafhankelijk van elkaar evolueren. Dit laat de mogelijkheid open dat de assortatieve paring als gevolg van selectie voor *IPI* het gevolg is van vrouwelijke preferentie voor de meeveranderde mannelijke roepkenmerken, in plaats van door mannelijke preferentie voor de veranderde vrouwelijke roep.

De genetische basis van de mannelijke zang van *R. imitans* is onderzocht door regressie van zangkenmerken van vaders op zonen. De heritability-schattingen voor kenmerken van de chirp-sectie bleken statistisch significant (0.44-0.54), in tegenstelling tot die van de buzz-sectie (0.09-0.28). Uit berekeningen van fenotypische-, genotypische- en omgevingscorrelaties tussen de kenmerken van het mannelijke signaal bleek dat de chirp-sectie en buzz-sectie onafhankelijk van elkaar variëren en evolueren. Een chirp-sectie-kenmerk, het aantal chirps, bleek te worden beïnvloed door genetische factoren op het X-chromosoom. Dit zou erop kunnen wijzen dat de heritability-schatting voor dit kenmerk, zoals verkregen middels vader-zoon regressie, te laag is.

Om redenen als de klaarblijkelijke genetische plasticiteit van de zang, het gegeven dat heterospecifieke paringen levensvatbare en fertiele nakomelingen opleveren, en het feit dat *Ribautodelphax*-soorten ecologisch van elkaar geïsoleerd zijn, lijkt de klassieke theorie dat deze geluiden geëvolueerd zijn als adaptaties ter voorkoming van interspecifieke kruisingen (speciatie door 'reinforcement') onwaarschijnlijk. De beschikbare gegevens wijzen er eerder op dat de geluiden hun soortspecifiteit te danken hebben aan processen binnen de soort, zoals selectie en toeval, b.v. als gevolg van 'founder'-gebeurtenissen. Met name bij de differentiatie van het mannelijke signaal zou ook seksuele selectie mogelijk een rol kunnen spelen. Gezien de gevonden genetische correlatie tussen mannelijke en vrouwelijke zangelementen bij *R. imitans*, bestaat de mogelijkheid dat een genetische verandering van het signaal in de ene sekse tevens een signaalverandering in de andere sekse tot gevolg heeft.

De observatie dat mannetjes veel selectiever zijn met betrekking tot heterospecifieke signalen van de andere sekse dan vrouwtjes, lijkt het best te kunnen worden verklaard vanuit de noodzaak om op een zo efficiënt mogelijke manier een geschikte conspecifieke partner te vinden. Omdat *Ribautodelphax*-soorten alleen op hun soortspecifieke voedselplant voorkomen, lijkt de kans om in het veld nauwverwante soorten tegen te komen klein. Om zoveel mogelijk mannetjes aan te trekken om een keuze uit te maken, is het voor vrouwtjes voldoende om congenerieke mannelijke signalen te herkennen, waarvan de basale structuur binnen het genus hetzelfde is. Voor mannetjes is precieze herkenning van het vrouwelijke signaal waarschijnlijk belangrijker. Omdat vrouwtjes

slechts éénmaal tijdens hun leven paren, in tegenstelling tot mannetjes, zijn er in een populatie meestal meer paringsbereide mannetjes dan vrouwtjes aanwezig. Door scherp te reageren op vrouwelijke signalen, verhoogt het mannetje zijn kansen op het vinden van zoveel mogelijk attractieve vrouwtjes, daar de fractie van dergelijke vrouwtjes (degenen met signaalkarakteristieken het dichtst bij het populatiegemiddelde) afneemt naarmate het seizoen voortschrijdt. Bij spoorcicadensoorten die syntopisch op dezelfde voedselplant leven, zou men verwachten dat vrouwtjes tenminste zo selectief zijn als mannetjes, omdat dat de meest efficiënte en veilige manier lijkt te zijn om heterospecifieke paringen te vermijden. Bij twee soorten van het geslacht *Prokelisia* die op dezelfde voedselplant leven, zou dit inderdaad het geval zijn.

In populaties van een tweetal *Ribautodelphax*-soorten, waar diploide mannetjes en vrouwtjes geassocieerd leven met triploide gynogenetische vrouwtjes, lijkt een bijzonder geval van het gebruik van akoestische signalen binnen de soort voor te komen. Triploide vrouwtjes ontstaan af en toe spontaan in diploide populaties. Om zich voort te planten moeten ze paren met diploide mannetjes, maar ze produceren uitsluitend identieke triploide vrouwtjes. Door dit tweevoudig reproductief voordeel zijn de aseksuele vrouwtjes theoretisch in staat de diploide vrouwtjes weg te concurreren, hetgeen zou leiden tot hun eigen ondergang. Uit eerder onderzoek is echter gebleken dat de verhouding diploiden:triploiden in de tijd constant blijft. Ter verklaring van deze stabiele samenleving wordt een theorie gepresenteerd, die ervan uitgaat dat deze vorm van samenleving in stand blijft door een soort 'wapenwedloop' in roepkenmerken tussen beide typen vrouwtjes. Dit wordt mogelijk gemaakt door de genetische plasticiteit van het vrouwelijk signaal en de selectiedruk op mannetjes om paringen met triploiden te vermijden door vrouwelijke signalen te prefereren met kenmerken die afwijken van het populatiegemiddelde. Dit zou een verklaring kunnen geven voor het merkwaardige optreden van diverse, zeer verschillende vrouwelijke zangtypen binnen en tussen populaties van soorten waarbij triploiden voorkomen.

Gezien hun potentiële effect bij de soortsherkenning zouden akoestische signalen een belangrijke factor kunnen zijn bij de soortsvorming van spoorcicaden. Bij *Ribautodelphax*-soorten speelt in deze discussie de sterke waardplantbinding een verwarrende rol. De differentiatie van de akoestische signalen zou het gevolg kunnen zijn van de isolatie van populaties door hun overstap naar een nieuwe voedselplant. Onderzoek aan verwante genera laat echter zien dat akoestische differentiatie ook kan optreden zonder verandering van waardplant. Verwante soorten die naast elkaar op dezelfde voedselplant leven, lijken veel sterkere herkenningsmechanismen te hebben ontwikkeld, gezien het feit dat bij dergelijke groepen interspecifieke inseminaties niet of uiterst zelden voorkomen, zelfs onder gedwongen omstandigheden. Het lijkt daarom het meest waarschijnlijk dat speciatie veroorzaakt wordt door akoestische differentiatie in allopatrie. Hoewel soortsvorming wordt vergemakkelijkt door verandering van voedselplant, hetgeen verhindert dat de soorten secundair weer in contact komen, is dit geen noodzaak. Bij verwante soorten die op dezelfde waardplant voorkomen is het soortsspecifieke partner-herkenningssysteem klaarblijkelijk zover veranderd dat het mogelijk is geworden om na secundair contact in stabiele coëxistentie te leven. Het

soortvormingsproces wordt gezien als het zodanig veranderen van het soortsspecifieke partner-herkenningssysteem, dat dieren van andere populaties niet langer worden herkend als potentiële partners. Dit komt overeen met het 'recognition'-soortconcept van Paterson, behalve dat het soortsspecifieke partner-herkenningssysteem, althans een aantal componenten daarvan, minder evolutionair stabiel lijkt te zijn dan door deze auteur wordt verondersteld.

## PUBLICATIONS

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The contents of this dissertation have been, or will be, published as the following articles:

- De Winter, A.J., 1987. Artificial bi-directional selection on a female acoustic character in a *Ribautodelphax* planthopper species (Homoptera, Delphacidae). Proc. 6th Intern. Meeting on Insect Sound and Communication, Gamle Avernoes, Denmark: 44.
- De Winter, A.J., 1988. Preliminary data on the significance of acoustic behaviour in the genus *Ribautodelphax* (Homoptera, Delphacidae). Proc. 6th Auchen. Meeting, Turin, Italy: 337-339.
- De Winter, A.J. & Rollenhagen, T., 1990. The importance of male and female acoustic behaviour for sexual isolation in *Ribautodelphax* planthoppers (Homoptera, Delphacidae). Biol. J. Linn. Soc. 40: 191-206.
- De Winter, A.J., 1992a. Genetic studies on acoustic differentiation in *Ribautodelphax* planthoppers (Homoptera, Delphacidae). Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam 3: 116-120.
- De Winter, A.J., 1992b. The genetic basis and evolution of acoustic mate recognition signals in a *Ribautodelphax* planthopper (Homoptera, Delphacidae) 1. The female signal. J. Evol. Biol. 5: 249-265.
- De Winter, A.J. & Rollenhagen, T., 1993. Different preferences for species-specific female calls in acoustically experienced and naive male *Ribautodelphax* planthoppers (Homoptera, Delphacidae). J. Insect Behav. 6: 411-419.
- De Winter, A.J. The genetic basis and evolution of acoustic mate recognition signals in a *Ribautodelphax* planthopper (Homoptera, Delphacidae) 2. The male signal. (submitted to J. Evol. Biol.).
- De Winter, A.J. & De Vrijer, P.W.F. Acoustic communication and mating behaviour within and between *Ribautodelphax* planthopper species (Homoptera, Delphacidae) (submitted to Behaviour).

## NAWOORD/POSTSCRIPT

Dit proefschrift is met 'ups and downs' tot stand gekomen. Het tragisch overlijden in 1987 van mijn oorspronkelijke promotor, Prof. René Cobben, betekende een zware tegenslag. René liet me de vrijheid om de oorspronkelijke opzet van het project om te buigen in een meer genetische richting. Zijn kennis, vriendschap en gewillig oor voor allerhande problemen te moeten missen was niet makkelijk. Een extra financiële ondersteuning van BION maakte het mogelijk een aantal korte bezoeken te brengen aan de University of Wales (Cardiff) om begeleiding te zoeken. I have learned a great deal from Prof. Mike Claridge, Roger Butlin, Jeroen den Hollander, en John Morgan. I am grateful for their kindness, hospitality and friendship.

Aanvankelijk heeft Prof. Louis Schoonhoven de taak van begeleider op zich genomen, totdat eind 1990 Rory Post de nieuwe hoogleraar Diertaxonomie werd. Ik ben beiden dankbaar dat ze zich van deze taak hebben willen kwijten, ondanks dat ze niet van aanvang aan betrokken waren bij mijn onderzoek.

Belangrijke wetenschappelijke ondersteuning heb ik gekregen van de volgende personen, door het kritisch lezen van een of meerdere manuscripten en/of het voeren van stimulerende discussies: Paul Brakefield, Roger Butlin, Mike Claridge, Folchert van Dijken, Malcolm Gillham, Rolf Hoekstra, Jeroen den Hollander, Graham Holloway, Mart de Jong, Rory Post, Mike Ritchie, Richard Stouthamer, Louise Vet en Peter de Vrijer.

Ook de sectie Diertaxonomie heeft veel bijgedragen aan het welslagen van dit project, niet in de laatste plaats vanwege de goede sfeer tijdens het werk en daarbuiten. Peter de Vrijer was steun en toeverlaat voor de dagelijkse ditjes en datjes, naast de al gememoreerde wetenschappelijke begeleiding. Theodoor Heijerman heeft me een groot deel van de tijd als kamergenoot moeten dulden, hetgeen niet altijd even prettig moet zijn geweest, omdat activiteiten als het openen van de deur of het verschuiven van een stoel tijdens geluidsopnamen zeer storende trillingen teweegbrengen. Ook knutselde hij diverse malen 'even' een handig programmaatje in elkaar ter bespoediging van de analyse van resultaten. Julius Pattiapon heeft aanvankelijk geholpen bij het maken van geluids- en video-registraties. Na het vertrek van Patti heeft Thomas Rollenhagen een jaar bij de sectie gewerkt. Dankzij zijn technisch vernuft en werklust konden een aantal belangrijke experimenten succesvol worden afgerond c.q. uitgevoerd en is de sectie een aantal handige apparaten rijker. De enige student die ik in het kader van dit project heb mogen begeleiden is Paul Beuk. Zijn video-opnamen van het baltsgedrag hebben essentieel bijgedragen aan de totstandkoming van het hoofdstuk over dit onderwerp. Kees den Bieman heeft mij bij de aanvang van het onderzoek veel kennis over *Ribautodelphax* bijgebracht. Wouter Tigges en Hanneke van Heest zorgden ervoor dat ik vrijwel nooit zonder beesten of planten zat. Dankzij de genereuze voorziening door Wouter van bessenstruiken, druivenstekken, aardbeien-, tomaten-, asperge-, en andere plantjes heb ik deze periode gezond gegeten.

De afrondingsfase werd veraangenaamd door de prettige contacten met mijn burens

Yde Jongema, Trisha Martin, Andrew Polaszek, Jan Rozeboom en Mike Wilson.

De medewerkers van de vakgroep Entomologie en de gecombineerde diensten ben ik dankbaar voor de prettige sfeer, tijdens én buiten de koffiepauzes en het verlenen van diverse diensten en gunsten. Een speciaal woord van dank verdient Ans Klunder-Wind voor de manier waarop ze efficiënt management weet te combineren met een warme persoonlijke belangstelling.

Lieve Josine, jij hebt zonder twijfel het meest moeten afzien bij de totstandkoming van dit proefschrift, met name na de geboorte van George, toen je je kunstenaarschap tijdelijk moest opgeven. Zonder jouw maningen, raad, begrip en kritiek zou dit boekje er nu niet zijn en zouden de diverse manuscripten nog een groot aantal inconsistenties en fouten bevatten.

Ik draag dit werk op aan mijn ouders, die me de gelegenheid boden om bioloog te worden en me daarin op alle mogelijke manieren hebben ondersteund. Het is triest dat mijn vader de afronding niet meer heeft mogen meemaken.

## CURRICULUM VITAE

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Antonius Johannes (Ton) de Winter werd op 9 januari 1956 te Amsterdam geboren. In dezelfde plaats doorliep hij het Gymnasium-B aan het St. Ignatius College. In 1975 begon hij de studie biologie aan de toenmalige Landbouwhogeschool Wageningen. Tijdens de doctoraalfase deed hij een verzwaard hoofdvak populatiegenetica aan de Rijksuniversiteit Utrecht onder begeleiding van Dr. F.R. van Dijken en Prof. W. Scharloo (Vakgroep Populatie- en Evolutiebiologie), en vervolgens een hoofdvak Diertaxonomie bij de Vakgroep Entomologie aan de Landbouwhogeschool Wageningen, onder begeleiding van Ir. C.F.M. den Bieman en Prof. R.H. Cobben. In januari 1984 behaalde hij het doctoraalexamen biologie (met lof). Van juli 1985 tot juli 1988 deed hij (een groot deel van) het in dit proefschrift beschreven onderzoek, dat gefinancierd werd door BION (NWO). Van oktober 1989 tot januari 1990 werkte hij als 'assistent curator' aan de revisie van de collectie niet-mariene Afrikaanse mollusken op het Muséum National d'Histoire Naturelle te Parijs. Van 1990 tot 1992 was hij tijdelijk aangesteld als universitair docent bij de Vakgroep Entomologie (Sectie Diertaxonomie) van de Landbouwuniversiteit Wageningen. Momenteel is hij werkzaam als vertaler (Duits-Nederlands).

Naast het hier beschreven onderzoek heeft hij zich sinds 1980 actief bezig gehouden met de systematiek en ecologie van niet-mariene Mollusca, hetgeen geresulteerd heeft in zo'n 35 publicaties.