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Genetic Control and Evolution of Acoustic Signals in Planthoppers (Homoptera: Delphacidae)

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Abstract. Acoustic signals are part of the specific mate recognition system of planthoppers. The genetic control of acoustic signal characters was studied in the planthopper *Ribautodelphax imitans*. Artificial selection for interpulse interval in the female call revealed a large additive genetic component for this polygenic character. Other female call characters showed a correlated response. Some male call characters also appeared to be genetically correlated with the female character selected for, despite the rather different structure of male and female calls. Parent-offspring regression provided significant heritability estimates for those male call characters that also responded to artificial selection in the female call, one of which appeared to be influenced by sex-linked genes. It is argued that the differentiation of this mate recognition system in planthopper populations and species could be the result of founder effects, enabled by the genetic plasticity of the call characters and the existence of a wing length dimorphism in these animals.

Key words: genetic control, mate recognition system, speciation, *Ribautodelphax*, acoustic signals.

Introduction

In planthoppers both sexes communicate by means of low-frequency substrate vibrations (Ichikawa 1976; Claridge 1985). Three kinds of acoustic signals can occur in this group: calling (attraction), rivalry (aggression), and courtship signals. Calling signals mainly serve to bring mating-receptive partners together. Males usually call first. Only receptive, virgin females respond. While alternate calling (duetting) takes place, the male actively searches for the female, which remains stationary once the sexes are in acoustic contact. Rivalry and courtship signals have been less thoroughly studied. In various planthopper genera, the male calls have been shown to be species-specific, and to be important in species-recognition (Claridge et al. 1985; De Vrijer 1984; Heady and Denno 1991).

At least in the Palaearctic genus *Ribautodelphax*, the calling signals of both sexes are species-specific (Den Bieman 1986). From experiments it appeared that *Ribautodelphax* females generally respond nearly as well to conspecific as to heterospecific (but congeneric) male playback calling signals; in contrast, males were found to approach only conspecific female playback calling signals (De Winter and Rollenhagen 1990). Many species of *Ribautodelphax* can be hybridized under no-choice conditions and produce fully viable and fertile hybrid offspring; however, in a choice situation involving both conspecific and heterospecific potential partners, hybridization has never been observed (Den Bieman 1988; De Winter and Rollenhagen 1990). Apparently the female calling signal in *Ribautodelphax* species is also an important component of the specific mate recognition system (SMRS) in the sense of Paterson (1985).

There is a growing interest in the evolution of SMRS components, because the acquirement of a new recognition system is thought to relate to speciation (Paterson 1985). In order to understand how mate recognition systems evolve, data on the genetic basis of the components are crucial, but unfortunately are available for few organisms, and theoretical models (e.g. Lande 1981a; De Jong and Sabelis 1991) have rarely taken actual data into account.

This paper describes a study on the genetic control of characters of male and female calling signals in the species *Ribautodelphax imitans* (Ribaut), and provides a tentative scenario of signal evolution and speciation in planthoppers, in which group the wing length dimorphism is thought to play an important role.

Materials and Methods

Calling signals of female planthoppers (female calls) consist of a series of pulses. In the genus *Ribautodelphax* they differ among species in strophe duration, interpulse interval, and gradual changes in pulse repetition frequency within the signal. The genetic control of the female call was studied in a population of *R. imitans* from southern France, which had been cultured for 14 generations before the start of the experiment (De Winter 1992). The female call in this species consists of at least 50 pulses, with decreasing interpulse intervals (IPIs) towards the end of the signal (Fig. 1).

The genetic basis of the most conspicuous character, IPI, was studied by artificial bi-directional selection for long (HL) and short (LL) duration of 10 IPI's between the 41 st and 51 st pulse. The experiment consisted of four replicate lines in each direction. In each generation the calls of 40 females per line were recorded at 20°C and ten females having extreme IPI lengths were selected as parents for the next generation. The experiment was continued for at least 11 generations (up to 14 generations in some lines). During the experiment some lines were examined for correlated changes in other female call characters. Four estimates of the minimum number of segregating genetic factors contributing to IPI were obtained by examining the F_1 and F_2 female progeny of reciprocal crosses between two 10th generation HL and LL lines. In addition, mate preference tests were performed by confining either two HL or two LL males with two females of each line for three hours, and examining the female spermathecae for the presence of sperm. Such males were also given a two-way choice between playback calls of these females.

Calling signals of male planthopper (male calls) are generally more complex than those of females. In *Ribautodelphax* the male call consists of two structurally different parts, termed the 'chirp-section' and the 'buzz-section' (Fig. 2). The species differ in the duration of the chirp-section, the number of chirps, the mean chirp duration, and in the duration of the buzz (Den Bieman 1986).

Narrow-sense heritabilities of a number of male call characters were estimated by plotting the offspring means on father values in 25 families (De Winter 1994 and in press). The effect of the selection on female IPI on male call characters was studied by recording 19 samples of at least 10 males from various selection lines at different stages of the selection experiment. The values of the male call characters in these samples were correlated with the corresponding mean female IPI values obtained for these lines.

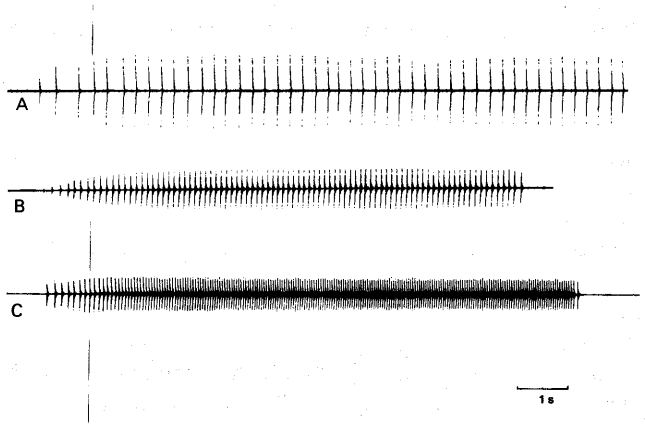

Fig. 1. Examples of oscillograms of female calling signals of *Ribautodelphax imitans* from the unselected population (B), and from lines after six generations of selection for long (A, only first part shown) and short (C) interpulse interval (IPI, see text).

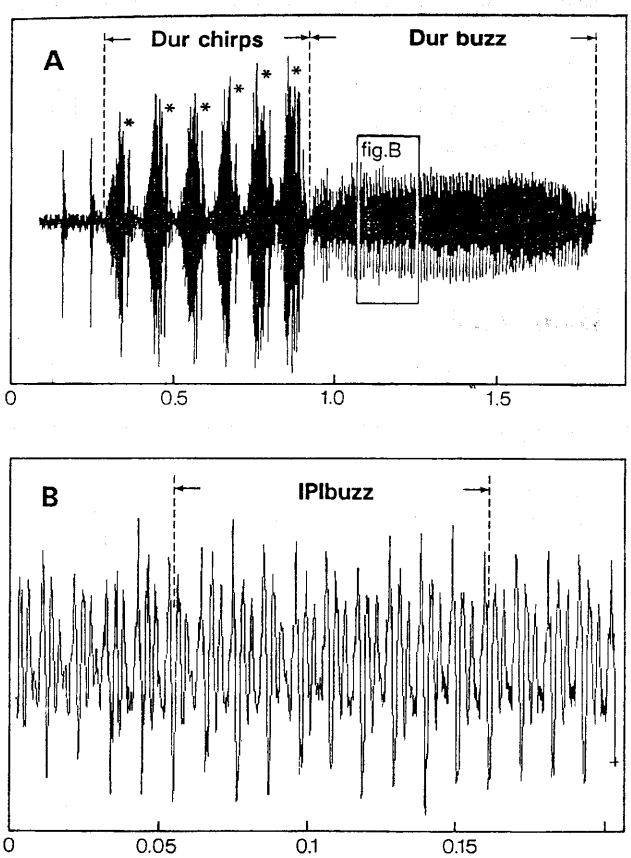

Fig. 2. Oscillogram of a male calling signal of *Ribautodelphax imitans*, showing the parameters used (Dur chirps, duration of chirp section; Dur buzz, duration of buzz-section; IPI buzz, duration of 10 IPIs in the buzz-section; stars indicate individual chirps). A shows a complete signal, B shows part of the buzz-section at an expanded time scale, as indicated in A.

Results

Selection for both long and short female IPI was very successful. The response to selection in the replicate lines was remarkably similar. Already after five generations of selection the ranges of the IPI values in the HL and LL lines became non-overlapping (Fig. 3). After ten generations the IPI values were at or beyond the range of values occurring in the entire genus (Den Bieman 1986; cf. Fig. 1).

Realized heritability estimates for IPI were very high, ranging from 0.51 to 0.88 in the HL lines, and from 0.91 to unity in LL lines over 5 generations of selection. Calculated over 10 generations they were somewhat lower, but still considerable (0.47–0.85). Although the response to selection in LL lines was less rapid than in HL lines, the heritability estimates in LL lines were consistently higher than in HL lines.

Using the formulae provided by Lande (1981b), four estimates were obtained for the minimum number of segregating genetic factors determining the IPI character (range 5.2–7.8), showing that this character is polygenic, as was expected from the almost linear course of the response to selection over generations.

Other female call characters showed correlated responses, especially in change in IPI within the signal, measured as the duration of 10 IPIs between the 11th and

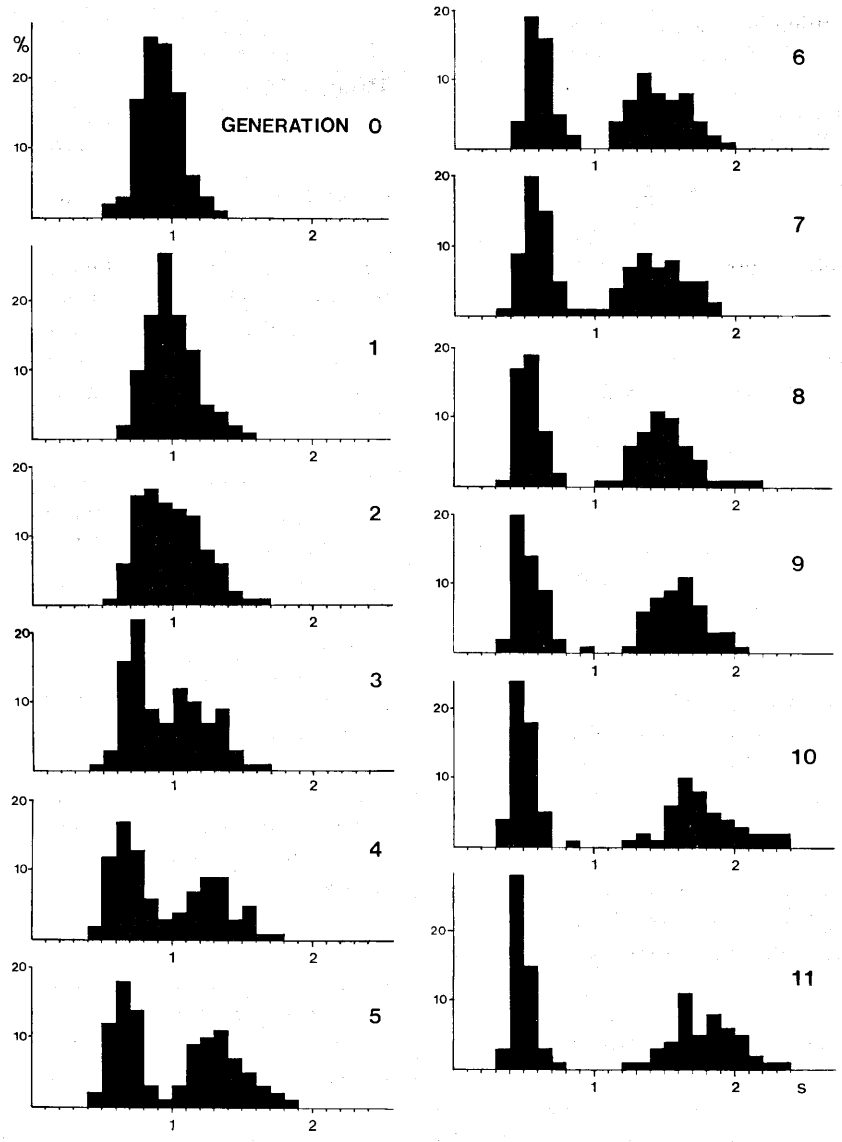

Fig. 3. Frequency distributions of IPI in the unselected population ($N=160$), and in 11 successive generations of bi-directional selection. Data of all selection lines were combined per generation ($N=320$).

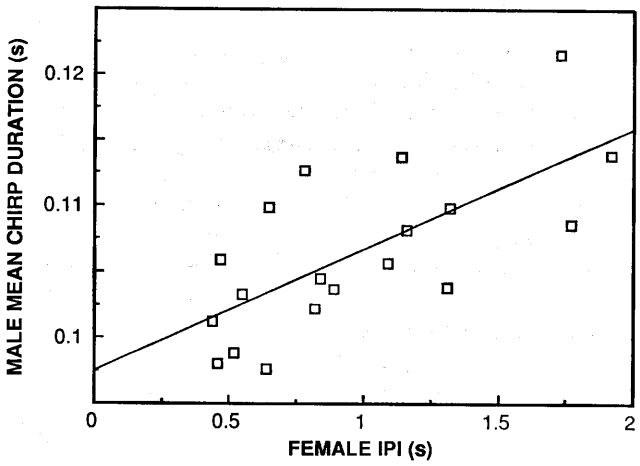

Fig. 4. Mean chirp duration of 19 samples of males recorded from various selection lines for female IPI at different stages of the selection experiment, plotted against the corresponding mean female IPI values in these lines. The regression of male chirp duration (y) on female IPI (x) is $y=0.0092\ x+0.097$, $r^2=0.48$, $P=0.001$.

21st pulse, which closely followed the course of the selection criterion over all generations of selection. The duration of the calls in the HL and LL lines also diverged significantly, which was mainly due to a significant increase in call duration in the HL lines, whilst those in the LL lines showed a slight decrease. Thus, the main three characters which constitute the female call appear to be genetically associated.

Sexual isolation tests between males and females of HL and LL lines indicated a significant departure from random mating, in that HL females were more often inseminated by HL males, and LL females more often by LL males [Joint Isolation Index (Malogolowkin-Cohen et al. 1965) $I=0.40\pm0.095$, $P<0.001$]. However, when such males were provided with a two-way choice between the playback-signals of these females, they failed to show a significant preference ($I=0.15\pm0.10$, $P>0.10$).

Two chirp-section characters of the male call also responded to selection for female IPI. In the selection lines, the number of chirps was negatively correlated with female IPI, whereas mean chirp duration was positively correlated with IPI (Fig. 4). This means that an increasing pulse rate in the female call tends to go together with an increase in chirp rate in the male call.

Only for characters of the chirp-section (number of chirps, duration of the chirp-section, and mean chirp duration) significant or nearly significant heritability estimates were obtained (0.48, 0.54 and 0.44, respectively). Chirp-section and buzz-section characters appeared to vary independently of each other; only interpulse interval in the buzz-section (IPI buzz) was phenotypically correlated with the three chirp-section characters, but in view of the low heritability estimate for IPI buzz (0.09), the correlations are probably environmental rather than genetic.

Two 10 th generation HL and LL selection lines, which diverged significantly in number of chirps and mean chirp duration, were hybridized. The reciprocal F_1 crosses differed significantly in the former trait; males from both crosses produced a mean number of chirps close to that in the line of their mothers. Such a pattern is consistent with either a sex-linked or a maternal mode of inheritance. If indeed the sex chromosome, which males receive from their mothers, has a significant effect on the number of chirps, then the heritability estimate obtained by father-sons regression probably underestimates the true heritability for this character.

Discussion

Genetic control of acoustic characters

These studies indicate that several important parameters of the male and female call in *R. imitans* have an important heritable component. Thus, a significant proportion of the observed phenotypic variation for these characters consists of additive genetic variation, which can potentially respond to various evolutionary forces. This is a surprising result, because theoretists have claimed that mate recognition characters, or characters important for the species' fitness, should be under stabilizing or directional selection, which is expected to reduce the amount of genetic variation severely (Cade 1984; Paterson 1978, 1985).

At present it remains unclear which mechanisms maintain the additive genetic variation for call characters in this population. There might be trade-offs between call characters and life-history traits, like for example fecundity. The noticeable reduction in numbers of offspring in most lines selected for IPI after about 10 generations, and the return in the direction of the original IPI values after suspending selection (De Winter 1992) would seem to support this view. Another likely option is that the genetic variation in the field is maintained by environmental fluctuations. Temperature is known to affect the IPI phenotype in *Javesella* planthoppers (De Vrijer 1984). Hence, the genetic variation might be buffered against selection through phenotypic variation caused by temperature fluctuations in the field.

Another surprising result is that some male and female call characters appear to be genetically correlated, despite the fact that these calls have very different structures, and are produced in a different way (Mitomi et al. 1984). Whether this is due to pleiotropy or to linkage disequilibrium is unclear. If male and female calls do not evolve independently, this might accelerate the evolution

of the entire acoustic mate recognition system.

These correlations within and between male and female call characters might provide an explanation for the conflicting results between the mate and call preference tests performed after the selection experiment for female IPI. Coselected (and unselected) males did not show a significant preference for female playback calls differing profoundly in IPI, but insemination tests showed significant and symmetrical assortative mating between males and females from similar selection lines. Although selection for IPI was very successful, it apparently did not succeed in disturbing the specific association between the characters that make up the female call, because the other characters, call duration and change of IPI within the call, exhibited a correlated response. Instead, the assortment observed could be due to a preference by females for coselected male call characters (sexual selection). From detailed observations of the courtship behaviour in several *Ribautodelphax* species it appeared that females are much more choosy than males during close-range contact, in contrast to during the distant calling phase (De Winter 1994). At close-range, males maintain their acoustic activity, whereas the duration of female calls and female calling rate significantly drop.

Evolution of the acoustic mate recognition system in planthoppers

These experiments suggest that this acoustic communication system can potentially be subject to rapid evolutionary change. In agreement with this, Claridge et al. (1985) reported considerable geographic variation in male and female call characters between some populations of the planthopper *Nilaparvata lugens* in Australia and southern Asia. They also found hybridization success between these populations to be negatively correlated with differences in pulse repetition frequency in the male calls. Also in *Ribautodelphax* species, especially in those living in association with gynogenetic triploids, interpopulation differences in acoustic characters have been found (Den Bieman 1986, 1987c).

Acoustic differentiation of *Ribautodelphax* populations, and possibly in many other planthoppers, can be easily envisaged by founder effects. The most important condition for founder effects to occur is the presence of ample genetic variation in the original population (Templeton 1981). Most planthopper species, including those of the genus *Ribautodelphax*, possess a wing length polymorphism with both (flightless) brachypterous and macropterous adults; macropterous adults occur especially under crowding conditions (Denno and Roderick 1990). Founder populations can arise from single inseminated macropterous females that fly away to a previously unoccupied habitat, and the acoustic characters could respond to genetic drift, the perturbation of the genetic environment caused by the founder event, and the different selection pressures in the new habitat. Such events might break up the specific assocations between call characters. If the acoustic signals evolve to the extent that they are no longer recognized by members of the original population, speciation will have taken place, even if secondary contact will never arise (Paterson 1985). In *Ribautodelphax* such a scenario is supported by the rather patchy distribution of populations and species, and their sometimes very small ranges, despite the generally common occurrence of the host plants (Den Bieman 1987a, b).

An alternative speciation scenario in many planthoppers would involve a change to a new host plant. Each *Ribautodelphax* species can only survive and reproduce on one particular species or genus of host plant (Den Bieman 1987a). Hence, speciation might have resulted from a shift to a new host plant, after which further acoustic differentiation took place. However, in some groups of planthoppers like *Nilaparvata* (Claridge et al. 1985), *Prokelisia* (Heady and Denno 1991) and *Chloriona* (Gillham and De Vrijer 1995) acoustic differentiation and speciation have apparently taken place without a previous host plant shift. The process of speciation in planthoppers is therefore viewed as an undirected change of the acoustic mate recognition system in small isolated populations, up to the point where signals of other populations are no longer recognized. This is in accordance with Paterson's (1975) recognition species concept, except that the SMRS, at least some of its components, appears to be less evolutionary stable than envisaged by that author.

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