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The genetic basis and evolution of acoustic mate recognition signals in a *Ribautodelphax* planthopper (Homoptera, Delphacidae) 1. The female call

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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 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 alterna-

tive 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 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, in press) explicitly examined the consequences for speciation. None of these models (except De Jong and Sabelis, in press) 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 hostplants (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 Pyrenées 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 hostplant, *Festuca arudinacea fenas* in a greenhouse under long-day conditions (18 hours light) at $20 \pm 2°$ 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 $20 \pm 1°$ 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.

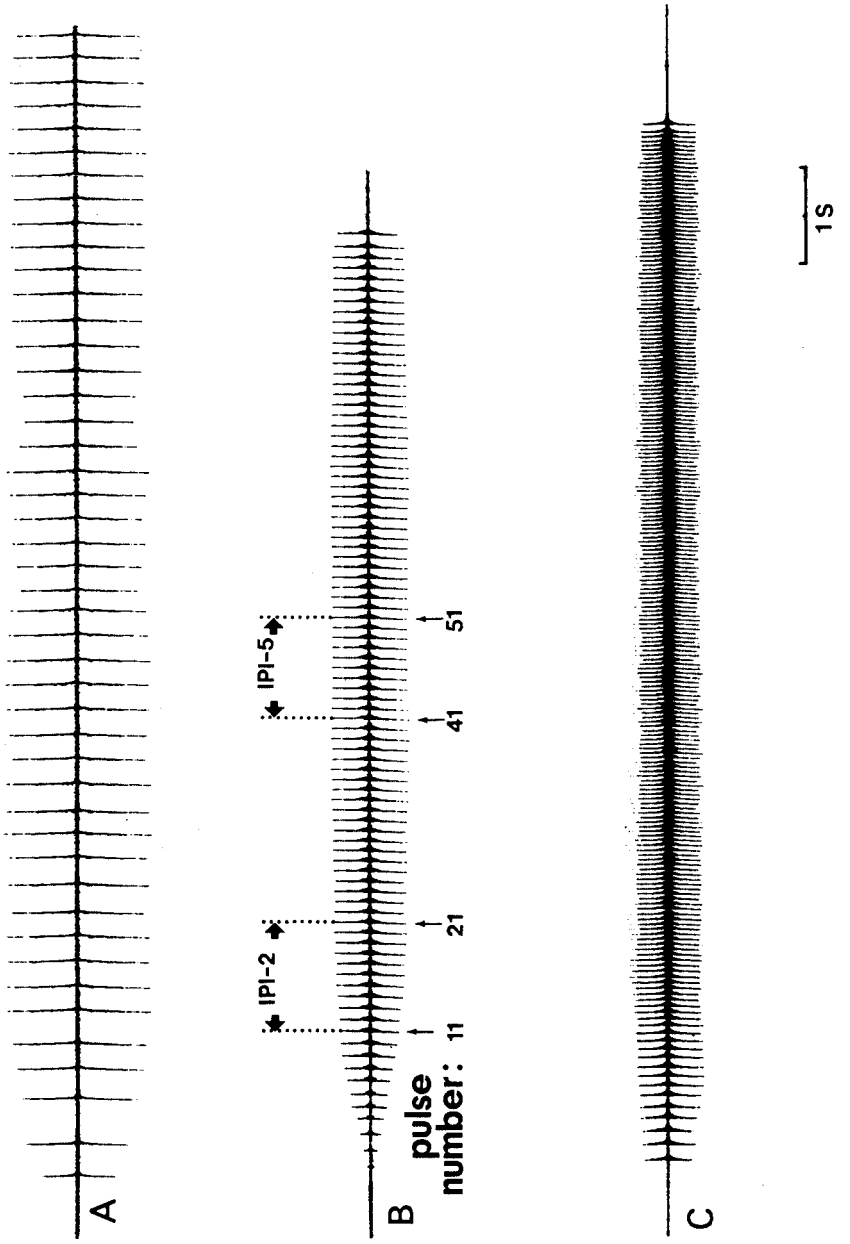

Fig. 1. Examples of oscillograms of female calling strophes from the unselected population (**B**), and extremes from lines after six generations of selection for long (**A**, only first part shown) and short IPI-5 (**C**).

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 stroph 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.

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' test 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 hostplant. 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 scorces in these lines. The setup consisted of three interconnected grass stems of the hostplant. 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 IPI'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 replicate lines were remarkably similar (Fig. 2). After only 5 generations of selection the ranges of the LL and HL lines became non-overlapping. 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 differentials were halved because selection was applied to females only (Falconer, 1981). Heritability estimates over 0–5 genera-

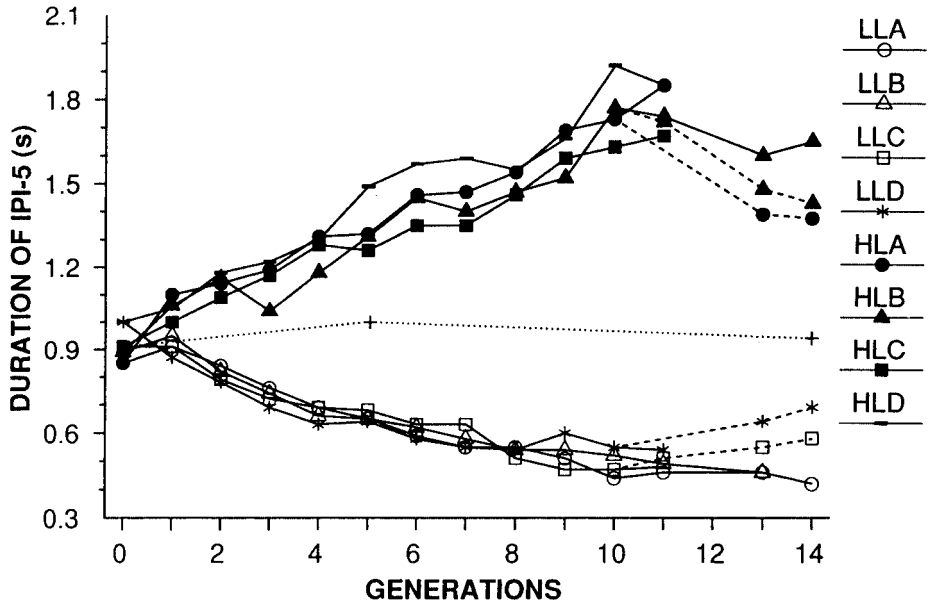

Fig. 2. Mean IPI-5 duration (s) plotted against generations of selection. Solid lines: response to selection. Dashed line: selection relaxed. Dotted line: unselected base population.

Table 1. Means, coefficients of variation (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
	Mean	0.85	0.42	1.73
A	CV	0.14	0.12	0.13
	Range	0.59–1.13	0.32–0.56	1.55–2.40
	Mean	0.89	0.52	1.77
B	CV	0.13	0.13	0.13
	Range	0.69–1.18	0.36–0.66	1.36–2.33
	Mean	0.91	0.47	1.63
C	CV	0.13	0.15	0.15
	Range	0.59–1.21	0.33–0.63	1.18–2.28
	Mean	1.00	0.55	1.92
D	CV	0.15	0.22	0.12
	Range	0.71–1.34	0.38–0.92	1.46 2.30

tions 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.

Table 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), as well as the mean and SE from the replicates in each direction.

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

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 (Fig. 2). 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 × HLD10, and LLC10 × HLB10) and their reciprocals. From means 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

Table 3. Means, variances and sample sizes (N) of log transformed IPI 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

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 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

Table 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)

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 & 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. 3.

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 ($R = 0.997$, $P \ll 0.001$).

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,

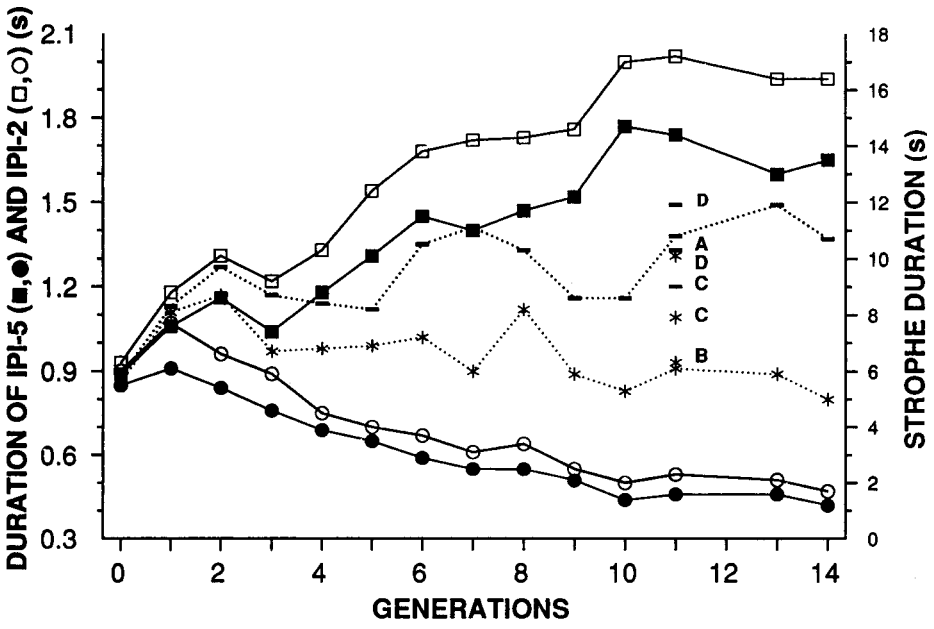

Fig. 3. Mean IPI-5 duration, and correlated changes in mean IPI-2 (solid lines) and strophe duration (dotted lines) in LLA (circles, asterisk's) and HLB (squares, dashes) plotted against generations of selection. Strophe durations in other replicate lines are only given for generation 11 (LL: asterisk's, HL: dashes). Individual strophe durations were log transformed, and the antilog of the generation means are shown.

and from 0.65–0.93 in LLA. These correlations did not change consistently over successive generations of selection.

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. 3).

Within generations, phenotypic correlations between IPI-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 phenotyic correlations between IPI-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 IPI 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 (in press) 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 wit 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, are more likely 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 males 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 & 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 (see above), 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 result 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 IPI 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 hostplant, as seems to be the case in many planthopper taxa.

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