Biological and taxonomic differentiation in the *Ribautodelphax collinus* complex (Homoptera, Delphacidae)

C. F. M. den Bieman

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C. C. Oosterlee,
in het openbaar te verdedigen
op woensdag 28 oktober 1987
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen.

Stellingen

1. De redenering dat onderzoek van parthenogenetische dieren leidt tot inzicht in de evolutie van seksuele voortplanting is discutabel, omdat, voor zover bekend, alle parthenogenetische dieren ontstaan zijn uit seksuele vormen.
   Lynch, M. (1984). Quart. Rev. Biol. 59: 257-290.

2. Selectieve partnerkeuze speelt een belangrijke rol bij de handhaving van populaties, die bestaan uit biseksuele en pseudogame vormen.
   Dit proefschrift.

3. Akoestische signalen van vrouwtjes krijgen ten onrechte weinig aandacht als soortspecifiek kenmerk bij Auchenorrhyncha.
   Dit proefschrift.

4. De grote intraspecifieke variatie in akoestische signalen van vrouwtjes van *Ribautodelphax pungens* en *R. imitantoides* is in tegenspraak met Patersons stelling dat 'soortherkenningssignalen' gebufferd zijn tegen snelle veranderingen.
   Paterson, H. E. H. (1985). Transvaal Mus. Mon. 4: 21-29.

5. Pseudogamie is een vorm van parthenogenese, die voorkomt dat de parthenogenetische vorm de seksuele oudersoort(en) verdringt.

6. Het belang van interspecifieke concurrentie tussen fytofagen wordt vaak overschat.
   Jermy, T. (1985). Z. zool. Syst. Evolut.-forsch. 23: 275-285.

7. De bepaling van de volgorde van kenmerkverandering van een 'multistate' electroforetisch locus mag niet gebaseerd worden op de relatieve verandering in electroforetische mobiliteit.
   Green, D. M. (1986). Syst. Zool. 35: 283-296.

8. Discussies over het ontstaan van polyploïde dieren zijn gefixeerd op een hybride oorsprong en houden te weinig rekening met een mogelijke autopolyploïde oorsprong.
   Bullini, L. (1985). Boll. Zool. 52: 121-137.

9. Seksuele voortplanting is ook van belang voor het voortbestaan van parthenogenetische vormen, omdat hierdoor de kansen op genetische variabiliteit van polyfyletische parthenogeneten vergroot worden.
   Vrijenhoek, R. C. (1984). In: Population biology and evolution, 217-231. Springer Verlag.
   Dit proefschrift.

10. De lange duur van taxonomische promotieonderzoeken en het grote aantal publikaties per onderzoek illusteren de complexiteit van de onderzochte materie.
    Storms, J. J. H. (1986). Vakbl. Biol. 66: 405-407.

11. De geringe hoeveelheid geld zowel nationaal als internationaal beschikbaar voor de taxonomie én de omvang van haar taak én haar economisch belang.
    Wilson, E. O. (1986). Science 230: 1227. Andersen, A. (1987). Nature 329: 6.

12. De neiging om het verzamelen van insekten aan banden te leggen via de nieuwe Natuurbeschermingswet leidt tot een verminderende belangstelling voor deze diergroep en schaadt daardoor hun bescherming.

13. Bij de discussie over de kosten van kinderopvang wordt te weinig rekening gehouden met de kosten van de traditionele kinderopvang.

14. Knarsentanden zal verstommen als de tandarts uit het ziekenfondspakket verdwijnt.

Proefschrift van C. F. M. den Bieman.
Biological and taxonomic differentiation in the *Ribautodelphax collinus* complex (Homoptera, Delphacidae).

Wageningen, 28 oktober 1987.

Aan Marianne
Aan mijn ouders

Ik ben Marianne Ham dankbaar voor de steun en stimulans tijdens dit onderzoek. Zonder jouw hulp zou het veldwerk, de basis van mijn onderzoek, nooit zo sukeesvol hebben kunnen verlopen.

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INLEIDING

Dit proefschrift maakt deel uit van het biosystematisch onderzoek, dat verricht wordt door de sektie Diertaxonomie van de vakgroep Entomologie, Landbouwuniversiteit Wageningen. Belangrijke aandachtspunten in dit onderzoek zijn o.a. de karakterisering van nauwverwante soorten op basis van biologisch zinvolle kriteria en de rekonstruktie van fylogenetische relaties binnen en tussen soortskomplexen. De resultaten van dit onderzoek vormen een bijdrage aan diskussies over het begrip "soort" en over het verloop van soortsvormingsprocessen.

Uit de familie van de spoorcikaden (Delphacidae) zijn voor dit biosystematisch onderzoek enkele genera gekozen, die een reeks met een toenemende mate van interspecifieke differentiatie vormen. Het Ribautodelphax collinus komplex vertegenwoordigt daarbij een basaal nivo van differentiatie.

Bij de aanvang van dit onderzoek omvatte het R. collinus komplex in Europa een viertal beschreven soorten, die slechts een geringe mate van morfologische divergentie vertonen. Variabiliteit in bepaalde diagnostische kenmerken leidde tot twijfel over de taxonomische status van deze soorten. Faunistisch onderzoek in vnl. het Mediterrane gebied resulteerde in de ontdekking van een aantal nieuwe vormen, waarvan de taxonomische status onduidelijk was. Dit onderzoek draagt bij aan een oplossing van deze taxonomische verwarring.

De biosystematiek baseert zich op het "biologisch soortsconcept": soorten zijn eenheden, die in het veld geheel of in belangrijke mate reproduktief geisoleerd zijn van elkaar. Dankzij deze reproduktieve isolatie kunnen soorten verschillen ontwikkelen en blijven de verschillen gehandhaafd. Diverse mechanismen kunnen bijdragen aan het ontstaan van isolatie tussen soorten bv. verschillen in herkenningssignalen, in habitats en in genitaalbouw.

Om de mate van differentiatie tussen de taxa uit het R. collinus komplex te kunnen bepalen zijn deze taxa vergeleken wat betreft: ekologie m.n. waardplantrelaties; geluidssignalen; kruisbaarheid; karyologie; allozymen en morfologie. Voor een evaluatie van de verschillen is het

belangrijk ook de variatie binnen en tussen conspecifieke populaties te bestuderen en daarom is dit onderzoek op ruime geografische schaal opgezet. Deze onderzoeken resulteren in een taxonomische analyse van het R. collinus komplex in het laatste hoofdstuk. Verder leiden deze vergelijkende onderzoeken mogelijk ook tot meer inzicht in de aard van de mechanismen, die bij spoorcikaden een belangrijke rol spelen bij de soortsvorming en bij het handhaven van de reproduktieve isolatie.

De ontdekking van zgn. pseudogame triploide Ribautodelphax ♀♀ leidde tot een verbreding van het onderzoek. Deze ♀♀ vertonen een afwijkende en bizonder zeldzame vorm van parthenogenetische voortplanting. De pseudogame triploide ♀♀ moeten geïnsemineerd worden door ♂♂ om tot produktie te kunnen komen, maar het sperma dient waarschijnlijk slechts om de eiontwikkeling te stimuleren. De pseudogame triploide ♀♀ produceren alleen vrouwelijke nakomelingen en de ♂♂ dragen hieraan genetisch niets bij. De pseudogame ♀♀ zijn dus voor hun voortplanting afhankelijk van de ♂♂ van een verwante bisexuele soort en zij gedragen zich als "reproduktieve parasieten". Theoretisch kunnen deze pseudogame triploide ♀♀ op twee manieren ontstaan zijn nl. uit één soort (autopolyploid) of door kruising tussen verschillende soorten (allopolyploid). Dit aspekt is uitgebreid bestudeerd. Verder zijn de ekologische relaties geanalyseerd, die bestaan tussen de pseudogame triploide ♀♀ en de bisexuele diploide populatie, die fungeert als spermadonor.

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Host plant relations in the planthopper genus *Ribautodelphax* (Homoptera, Delphacidae)

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ABSTRACT. 1. Host plant relations of closely related species and pseudo-gamous triploid forms of the planthopper genus *Ribautodelphax* were studied.

2. From field data and experimental results it is concluded that eleven European *Ribautodelphax* species and undescribed taxa are monophagous or oligophagous on different grass species. Only two *Ribautodelphax* species are found on the same grass species in the field.

3. Differential host plant relations indicate that four recently discovered taxa are probably true species.

4. *Ribautodelphax* species show a more restricted host plant range in choice experiments compared with breeding tests.

5. Choice experiments indicate no differences in host plant preference between males and females of two *Ribautodelphax* species.

6. No differences are found in host plant relations between bisexual species and associated pseudogamous triploid forms.

Key words. Delphacidae, planthoppers, *Ribautodelphax*, host plants, pseudogamy, triploidy, ecological isolation.

Introduction

Planthoppers (Delphacidae) and leafhoppers (Cicadellidae) often dominate the insect fauna of temperate grasslands (Waloff & Solomon, 1973; Morris, 1971). However, the biology of only few species has been studied. Differences in host plant relations might be important for the reproductive isolation of congeneric species (Booij, 1982a; Nault, 1985). The whole life cycle of planthoppers is associated with their host

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plants. Plants are not only food resources but also play an important role as a medium for substrate-borne acoustic communication between the sexes (Claridge, 1985; Booij, 1982b). Mating and oviposition also takes place on the host plant.

Taxonomy

There are nine species in the planthopper genus *Ribautodelphax* Wagner in western Europe (Nast, 1972). Within this genus a group of morphologically similar species is recognized, as the *R.collinus* complex, to which five European species belong (den Bieman,

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C. F. M. den Bieman

unpublished). Recently several new taxa of this complex were discovered in Europe.

Variation in diagnostic characters and small morphological differences between the species of the *R.collinus* complex raised many identification problems and questioned the taxonomic status of the known species and of the new taxa. Solving of these problems requires an extensive analysis of biological differentiation in for example, ecology, acoustics and hybridization capacity. Taxonomical conclusions logically constitute the final part of such a study, and will be discussed when all experimental results are available. To enable discussion of the four new taxa of uncertain status, they are referred by numbers 1–4.

In addition to the diploid bisexual species two pseudogamous triploid all-female forms belong to the *R.collinus* complex (den Bieman, 1984). Each of these forms is associated with a bisexual species on which the triploid females depend for sperm. The triploids are indicated with a combination of 3N and the name of their bisexual associate.

Ecology

Ecological information on *Ribautodelphax* species is scarce in the literature and it is often unreliable because of problems of identification. Most authors (Linnavuori, 1952; Kontkanen, 1950; Marchand, 1953; Remane, 1958; Schwoerbel, 1957; Waloff & Solomon, 1973) indicate only the vegetation type or the dominant grasses at their sampling sites. The xerothermophilous preference of *Ribautodelphax* species is often reported. Host plant records are given for *R.collinus* (Boheman) (Wagner, 1935), *R.imitans* (Ribaut) (Abdul-Nour, 1971), and *R. fanari* nom.nud. and *R.falakron* nom.nud. in Drosopoulos *et al.* (1985).

Material and Methods

During 1980–84 over a hundred populations of *Ribautodelphax* were sampled in western and southern Europe. A list of localities, including ecological information, is available on request. The host plants of the different *Ribautodelphax* species can only be recorded in a complex vegetation by extracting the planthoppers with a pooter directly from a known grass species.

The origin of the *Ribautodelphax* and plant populations used for the experiments are given in Table 1. Detailed information on these populations will be given in a later paper.

Cultures of the *R.collinus* complex were kept on grasses reared from seed collected in the field. *R.albostriatus* and *R.pallens* were reared on cultivars of their host plants. Inbreeding in all rearings was minimized by exchange of males between parallel rearings at each generation. Stock cultures were kept in a glasshouse at 20±1°C, 18 h light, r.h. 70–80% and experiments were carried out under similar conditions. Insects of the third or fourth generation reared in the laboratory were used for experiments. Some of the *R.pallens* belonged to the fourteenth generation and *R.pungens* population A to the seventh generation.

Host plant choice

Host plant choice experiments were carried out in a cage, 90×160×100 cm, with walls of black plastic sheets and covered with fine gauze. A sodium lamp (Philips G 92/2, SONIT, 400 W) supplied light centrally from above.

Six plants of each of the twelve grass species tested were placed at regular distances from each other in the cage. The plants were randomly distributed over the cage, but with two restrictions. Each grass species occurred not more than three times at the cage sides to reduce edge effects, and to avoid clustering not more than two plants of the same grass species were allowed to be next to each other. Leaves of neighbouring plants touched each other. In each experiment plants were cut to equal height and none of them were in flower. Plants were placed in moist sand on which the insects could walk.

Planthoppers were isolated on their natural food plants as fifth instars. The resulting adults, 6–10 days old, were tested. Virgin females do not oviposit at this age. Males and females of each species were tested separately, but macropterous and brachypterous individuals were tested together. On each plant two or three animals were released and re-collected after 48 h. Choice experiments with the delphacid *Nilaparvata lugens* (Stål) (Sogawa & Pathak, 1970) showed that the distribution of the adults over the tested plants was stable after 24 h.

The host plant choice of hybrids was tested in a comparable experiment in a smaller cage,

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TABLE 1. Field data on host plant relations of *Ribautodelphax*. Figure in parentheses: number of localities per country. *Origin of *Ribautodelphax* and plant populations used for experiments. Plant names after Tutin *et al.* (1980).

Ribautodelphax	Host plant	Countries
R.collinus complex		
collinus (Boheman)	*Agrostis capillaris* L. (=*tenuis* Sibth)	Netherlands* (5), Belgium (1), France (3), Yugoslavia (1), Bulgaria (1), Greece (1)
angulosus (Ribaut)	*Anthoxanthum odoratum* L.	Netherlands* (1), Greece (1)
pungens (Ribaut) and 3N	*Brachypodium pinnatum* (L.) Beauv.	Netherlands* (7), Belgium (8) France (9), German F.R. (6), Yugoslavia (7)
	Brachypodium phoenicoides (L.) Roemer & Schultes	France* (4), Greece (1)
	Brachypodium sylvaticum (Hudson) Beauv.	Greece* (3)
imitans (Ribaut)	*Festuca arundinacea fenas*† (Lag.) Arcangeli	France* (4), Greece (1)
fanari nom.nud.	*Elymus pycnanthus* (Godron) Melderis	France* (1)
	Elymus hispidus (Opiz.) Melderis	Greece* (2)
taxon 1	*Agrostis vinealis* Schreber	Netherlands* (2)
taxon 2	*Arrhenatherum elatius* (L.) Beauv.	Greece* (1)
taxon 3	*Festuca rubra rubra* L.	France* (1)
taxon 4 and 3N	*Brachypodium phoenicoides*	France* (21), Spain (7)
Other species		
albostriatus (Fieber)	*Poa pratensis* L.	Netherlands* (1), Belgium (3), France (4), German F.R. (4), Greece (1), Yugoslavia (1), Spain (1)
pallens (Stål)	*Festuca ovina* L.	Norway* (2), Finland (1)
falakron nom.nud.	*Festuca cyllenica* Boiss. & Helder	Greece (2): Drosopoulous *et al.* (1985)

† My earlier report (den Bieman, 1981) of *Arrhenatherum elatius* was a misidentification.

31×45×40 cm, with three plants of the two grass species to be tested.

The choice experiments were analysed with a χ^2-test. Significance levels are indicated as: *$P<0.05$, **$P<0.01$, ***$P<0.001$ and NS $P>0.05$.

Breeding experiments

The breeding tests were comparable to the experiments of Booij (1982a) with *Muellerianella*. Males and females were separated in the fifth larval stage and ten animals from both sexes, 7–9 days old, were placed in a cage with the grass species to be tested. The pre-oviposition period appeared to be 5–6 days. After 20 days the surviving adults were removed and counted. Cages were inspected daily for hatched larvae. The period between hatching of the first larva and emergence of the first adult was taken

as a measure of the time for larval development. Approximately 1 week after the appearance of the first adult the number of offspring was counted to assess reproductive capacity. Each experiment was replicated once.

Results

Host plants in the field

Most of the known species and the newly discovered taxa of the *R.collinus* complex appeared to be monophagous (Table 1). Two species, *R.pungens* and *R.fanari*, were found on more than one grass species. The report of *Leymus racemosus* (Lam.) Tzvelev as the host plant of *R.fanari* (Drosopoulos *et al.*, 1985) could not be confirmed, despite intensive searching at their sampling localities. *R.pungens* and taxon 4 were the only taxa found on the same grass species, *Brachypodium phoenicoides*. No other host

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plant species were found although many kinds of biotopes were examined.

Geographical variation in host plant relations was observed in *R.pungens* (Table 1). In Greece *R.pungens* was found at three localities on *Brachypodium sylvaticum*. *B.sylvaticum* also occurs in west and central Europe, but in these areas *R.pungens* was never found on it. In both areas *B.sylvaticum* is mostly found along wood-paths, forest borders, etc. (Tutin *et al.*, 1980), while in Greece it occurs in more exposed localities.

The grass species from which a *Ribautodelphax* population was collected is here called its 'field host plant'. Two *Ribautodelphax* taxa were collected only from one subspecies of their host plants (Table 1). These subspecies will be considered as the 'field host plant' of these taxa, particularly as these grasses belong to the taxonomically complicated genus *Festuca* (Tutin *et al.*, 1980).

Host plant choice experiments

The preferences of fourteen *Ribautodelphax* species and populations were tested on twelve species of field host plants. *Elymus pycnanthus* was not available. The majority of the choice experiments were made on females. Females choose a plant species for feeding and for oviposition, but because oviposition and feeding behaviour may conflict only virgin females were tested unless stated otherwise.

R.angulosus, imitans, fanari, albostriatus, taxon 1 and 2 strongly preferred their field host plant (Fig. 1a). On each of the other grass species only 0–7% of the insects were recaptured, often on plants adjacent to the field host plants. *R.collinus* showed a preference of only 36.0% for its field host plant (Fig. 1), and no clear preference for a second or third alternative host plant.

Three populations of *R.pungens* collected from different *Brachypodium* species were tested (Fig. 1d–f). Population A strongly preferred its field host plant. Percentage recaptures of population C on its field host plant and on *B. pinnatum* were not significantly different for females ($\chi^2_{(1)}$: 3.34^{NS}) and males ($\chi^2_{(1)}$: 0.91^{NS}). Population B preferred *B.pinnatum* over its field host plant ($\chi^2_{(1)}$: 6.50*).

Populations of taxon 4 were sampled only from *B.phoenicoides* (Table 1) but could be

reared successfully on *F.a.fenas*. A comparison was made between individuals from population A reared for four generations on *B.phoenicoides* and individuals from population B reared for the same number of generations on *F.a.fenas*. Both populations came from the same type of habitat only 48 km apart. The host plant preference of these two populations (Fig. 1h–i) differed significantly ($\chi^2_{(4)}$: 80.36***). Population A preferred its field host plant, while population B was frequently collected on *F.a.fenas*.

The host plant preference of triploid females (Fig. 1d and i) was not significantly different from that of their diploid associates either in *pungens* ($\chi^2_{(7)}$:9.17^{NS}) or in taxon 4 ($\chi^2_{(4)}$:3.16^{NS}).

The host plant preference of males and females was compared in two species (Fig. 1f and k). No significant differences were found between both sexes of *R.fanari* ($\chi^2_{(1)}$:3.58^{NS}) and *R.pungens* population C ($\chi^2_{(3)}$:5.24^{NS}).

Females of taxon 2 were used for a comparison of the host plant preference of mated and virgin females (Fig. 1j). Both groups showed an equally strong preference for their field host plant and mated females frequently oviposited in it.

Macropters and brachypters of only four species showed significant differences in host preference, *R.angulosus* ($\chi^2_{(1)}$:15.6***), *R.collinus* ($\chi^2_{(6)}$:18.5**), *R.imitans* ($\chi^2_{(1)}$:7.95**) and *R.albostriatus* ($\chi^2_{(5)}$:32.8***). Percentages of recapture of brachypters were always higher on the field host plant in all four species.

Host plant choice by hybrid planthoppers

Numerous hybrids between some *Ribautodelphax* species were produced, while the majority of the species either did not hybridize or produced only a low number of hybrids (den Bieman, unpublished). *R.imitans* and taxon 1 were selected for these experiments because of their hybridization potential and their strong preference for their field host plants (Fig. 1b and g).

Hybrids between females of taxon 1 and males of *imitans* were reared in cages with the field host plants of both parent species. Male and female hybrids showed a strong preference for *F.a.fenas* (Table 2) and no significant difference was found between the sexes ($\chi^2_{(1)}$:2.59^{NS}). F2 also strongly preferred *F.a.fenas* but more animals were collected on *A. vinealis*. The choices made by the F1
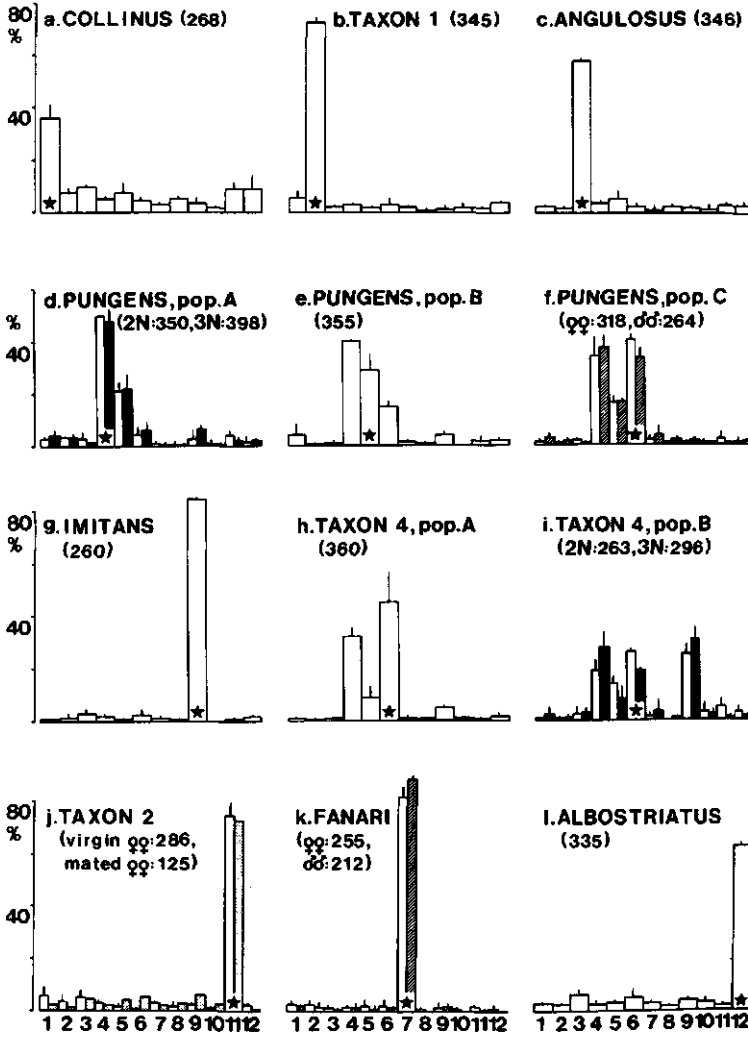
FIG. 1. Host plant choice of *Ribautodelphax* (mean percentage and standard deviation). Each experiment is replicated once, except *R.fanari* (twice) and taxon 2, mated females (no replicate). Figure in parentheses: total number of animals recaptured. 1–12: Plant species: 1, *Agrostis capillaris*; 2, *A.vinealis*; 3, *Anthoxanthum odoratum*; 4, *Brachypodium pinnatum*; 5, *B.sylvaticum*; 6, *B.phoenicoides*; 7, *Elymus hispidus*; 8, *Festuca rubra rubra*; 9, *F.arundinacea fenas*; 10, *F.ovina*; 11, *Arrhenatherum elatius*; 12, *Poa pratensis*. ★ Field host plant; □, virgin 2N females; ■, virgin 3N females; ▨, mated 2N females; ▰, unmated males.

TABLE 2. Host plant choice of hybrids between *R.imitans* and taxon 1. *N*: number of animals tested. Preference given in percentages.

	Host plant		*N*
	F.a.fenas	*A.vinealis*	
F1: ♂♂ and ♀♀	90.8	9.2	130
F2: ♂♂ and ♀♀	79.0	21.0	167

and F2 generations were significantly different ($\chi^2_{(1)}$:7.59**). The results of these choice tests correspond with the classical mendelian ratio. F1 shows the full dominance of the *imitans* genome, while F2 fits the expected ratio of 3:1 ($\chi^2_{(1)}$:1.46 NS).

Breeding experiments: suitability of field host plants

The ability of the *Ribautodelphax* species and taxa to live on twelve different field host plants was tested (Table 3) using three parameters: survival of adults, larval developmental time and reproductive success. To enable comparison of the *Ribautodelphax* species and taxa these parameters were defined relative to the results on their respective field host plants.

Table 3 shows that the shortest larval developmental time and the highest reproduction were always reached on the field host plant of each population. None of the *Ribautodelphax* species or undescribed taxa were shown to be polyphagous. Two species, *R.imitans* and *R.pallens*, had a very narrow host plant range.

Most of the plant species were suitable as host plants for only one *Ribautodelphax* species or taxon. *F.a.fenas* was the only plant species on which almost all *Ribautodelphax* species and new taxa could be reared with various degrees of success; the only exception was *R.pallens*, a species which does not belong to the *collinus* complex. The data on *R.pallens* (Table 3) are contradictory: survival of the adults was high, but only a few larvae were produced and none of them reached the adult stage. These tests were done with a *pallens* population reared for fourteen generations on *F.ovina* in the laboratory and perhaps inbreeding influenced these results. Both populations of taxon 4 were reared successfully on *F.a.fenas* (Table 3). Population B was kept for four generations on *F.a.fenas* prior to testing while population A was reared continuously on the field host plant. Rearing on

different plant species did not change the response of taxon 4 to its field host plant.

No obvious differences were found between the breeding results of *R.pungens* and its triploid form nor between taxon 4 and its associated triploid (Table 3).

Plant species, taxonomically related to the field host plants, are suitable breeding plants for some *Ribautodelphax* species. *A.vinealis*, the field host plant of taxon 1, was until recently considered a subspecies of *A.canina* L. (Tutin *et al.*, 1980). Taxon 1 could be successfully reared on *A.canina* and the production of offspring was only slightly lower than on *A.vinealis*. *R.imitans* is only known from the mediterranean subspecies *F.arundinarea fenas* (Table 1). No difference was found between the breeding results on ssp. *fenas* and on ssp. *arundinacea* Schreber, a subspecies with a distribution throughout the range of this plant species (Tutin *et al.*, 1980).

Breeding experiments: suitability of other plant species

There are only a few published references of host plants of *Ribautodelphax* species that are different from the field host plants already given (Table 1). *Anthraxon hispidus* (Thumb.) has been reported as the possible food plant of *R.collinus* in Japan (Mochida & Okada, 1971), but I doubt whether identification of the planthopper was correct.

Abdul-Nour (1971) collected and reared *R.imitans* on *Brachypodium retusum* (Pers.) Beauv. and *B.phoenicoides* in southern France. His data probably concern the morphologically related taxon 4. I never found *R.imitans* on *Brachypodium* on many field trips to this area. Also *R.imitans* could not be reared on three *Brachypodium* species tested (Table 3). Taxon 4 is quite common in southern France and reproduced on all the tested *Brachypodium* species (Table 3), as well as on *B.retusum*.

Carex arenaria L. was explicitly stated as a host plant of *R.collinus* (=*concinna* Fieb.) in the north of the Federal Republic of Germany (Wagner, 1935). Two males of this material (now in collections of the North Carolina State University) were re-examined and their identification confirmed. However, this host plant record is still in doubt because it was not possible to breed a Dutch *R.collinus* population on *C.arenaria*.

TABLE 3. Breeding results of *Ribautodelphax*. Breeding parameters are S: survival of adults, for 3N survival of ♀♀ only; L: larval developmental time; R: reproduction. The data given are means of two experiments. The values have been defined with the performance on the field host plant as standard. Survival 5: >80%; 4: 60–80; 3: 40–60; 2: 20–40; 1: 5–20 and 0 no survival. Larval developmental time 5: as on field host plant and up to 2 days longer, 4: 2–7, 3: 7–14, 2: 14–21, 1: >21 days longer than on field host plant. Reproduction 5: >75%, 4: 50–75, 3: 20–50, 2: 5–20, 1: <5% and 0: no progeny. Field host plant boxed in solid lines.

	collinus			Taxon 1			*angulosus*			*fanari*			Taxon 2		
	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R
A.capillaris	[5	5	5]	0	4	3	0	–	0	0	–	0	0	–	0
A.vinealis	1	3	2	[5	5	5]	0	–	0	0	3	1	0	–	0
A.odoratum	0	–	0	0	–	0	[5	5	5]	0	0	0	0	2	1
B.pinnatum	2	3	2	0	–	0	1	2	1	1	4	2	0	3	2
B.sylvaticum	1	–	0	0	–	0	0	–	0	0	–	0	0	1	1
B.phoenicoides	0	–	0	0	–	0	0	–	0	1	4	1	0	4	1
E.hispidus	0	3	1	1	4	2	4	1	1	[5	5	5]	1	4	1
F.r.rubra	0	–	0	0	–	0	0	–	0	0	–	0	4	3	1
F.a.fenas	5	4	4	3	4	2	5	3	3	4	4	3	4	3	2
F.ovina	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0
A.elatius	3	4	2	5	4	3	0	4	1	0	4	1	[5	5	5]
P.pratensis	4	2	2	3	3	1	4	3	1	0	4	1	0	–	0

	pungens, 2N population A			*pungens*, 3N population A			*pungens*, 2N population B			*pungens*, 2N population C			*albostriatus*		
	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R
A.capillaris	0	–	0	0	–	0	0	1	1	0	–	0	0	–	0
A.vinealis	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0
A.odoratum	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0
B.pinnatum	[5	5	5]	[5	5	5]	5	4	5	4	5	4	0	2	1
B.sylvaticum	5	4	4	2	4	3	[5	5	5]	5	4	4	0	–	0
B.phoenicoides	3	5	4	5	4	4	4	5	3	[5	5	5]	0	–	0
E.hispidus	1	3	2	0	1	1	3	2	2	1	2	1	2	2	1
F.r.rubra	0	–	0	0	–	0	0	–	0	1	–	0	0	–	0
F.a.fenas	5	2	3	5	3	3	4	4	4	3	3	3	5	4	2
F.ovina	0	–	0	1	–	0	0	–	0	0	–	0	0	–	0
A.elatius	0	–	0	1	–	0	2	3	1	1	3	1	1	–	0
P.pratensis	0	–	0	3	–	0	0	1	1	4	1	1	[5	5	5]

	imitans			Taxon 4, 2N population A			Taxon 4, 2N population B			Taxon 4, 3N population B			*pallens*		
	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R
A.capillaris	0	–	0	0	2	1	0	–	0	0	–	0	0	–	0
A.vinealis	0	–	0	0	2	1	0	–	0	0	–	0	1	–	0
A.odoratum	0	–	0	0	–	0	0	–	0	1	–	0	0	–	0
B.pinnatum	0	–	0	5	5	5	5	5	5	5	5	5	0	–	0
B.sylvaticum	0	–	0	5	4	3	5	4	3	5	4	4	1	–	0
B.phoenicoides	0	–	0	[5	5	5]	[5	5	5]	[5	5	5]	0	–	0
E.hispidus	0	–	0	3	1	2	3	1	1	2	2	1	0	–	0
F.r.rubra	2	1	1	0	–	0	1	–	0	1	3	1	0	–	0
F.a.fenas	[5	5	5]	5	5	4	5	5	5	5	5	5	5	–	0
F.ovina	0	–	0	0	3	1	0	–	0	0	–	0	[5	5	5]
A.elatius	0	–	0	0	4	1	0	1	1	1	3	2	0	–	0
P.pratensis	0	–	0	3	–	0	3	–	0	1	–	0	1	–	0

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TABLE 4. Breeding experiments with *R.pungens* on *Brachypodium*. Symbols as in Table 3, adults recaptured after 30 days.

	Plant species								
	B.sylvaticum						*B.pinnatum*		
	Netherlands			Greece			Netherlands		
	S	L	R	S	L	R	S	L	R
R.pungens									
Netherlands	5	4	2	5	4	4	5	5	5
Greece	5	5	5	5	5	5	5	5	5

Geographical variation in host plant relations

Geographical variation in host plant relations was found only in *R.pungens* (Table 1). Several Greek *pungens* populations occurred on *B. sylvaticum* whereas it was associated only with *B.pinnatum* in western Europe.

A clear difference was found in reproductive success between two *R.pungens* populations on Dutch *B.sylvaticum* (Table 4). The number of offspring of the Dutch *R.pungens* population was almost 6 times higher on the Greek *B.sylvaticum* than on the Dutch *B.sylvaticum*. These results clearly indicate geographical variation in both the host plant and the planthopper populations.

Adaptation to a new host plant

To test the possibilities of adaptation to a new host plant species a selection experiment was started with *R.collinus* on *B.pinnatum*. In experiment A with a 'high' selection pressure *R.collinus* remained on *B.pinnatum* during its whole life cycle. In experiment B with a 'low' selection pressure females were forced to oviposit in *B.pinnatum* and the third instars were placed back on their field host plant *A.capilaris*. The new adults of each generation were then placed again on *B.pinnatum*. The experiments were made with field samples of two summer generations from the same Dutch locality, each with two cages of ten males and ten females.

In both experiments only a few offspring were produced each generation and their numbers always remained below 10% of the number of offspring produced by *R.collinus* on its field host plant. In experiment A no offspring were produced in the fourth generation nor in the sixth generation of experiment B.

These experiments, although limited, suggest that a switch to a new host plant is not easily made even to a plant species on which the reproductive success of the first generation was not very low.

Discussion

Breeding and choice experiments

Rearing data showed that the potential host plant range of many *Ribautodelphax* species and of the undescribed taxa was wider than the field data and the choice experiments indicated. The planthoppers were forced to live on given plant species in the breeding tests, therefore the planthoppers' physiological potential to utilize the different plant species is tested. Host plant utilization is not easily changed, as is suggested by the selection experiment with *R.collinus*. The different results of the breeding and choice experiments indicate that behavioural aspects of host plant selection dominate markedly over physiological aspects in *Ribautodelphax*, a conclusion that was also made for other insect species (Jermy, 1985).

Most *Ribautodelphax* populations strongly preferred their field host plants. Experiments with taxon 4, however, showed that the host preference could be changed. One population reared on *F.a.fenas* selected this grass species more often compared with another population reared continuously on the field host plant, *B.phoenicoides*. Both diploid and triploid females showed the same preference shift. Since the triploid females were genetically uniform and parthenogenetic these changes must be caused by physiological conditioning to *F.a.fenas* rather than by selection. These behavioural changes may be exceptional and not repeatable in other *Ribautodelphax*, since taxon

4 was the only *Ribautodelphax* that could be reared with almost equal facility on the field host plant and on another grass, *F.a.fenas*, which is taxonomically unrelated to the taxon's field host plant.

Some of the data suggests that both feeding and oviposition preferences might coincide in *Ribautodelphax*. Firstly, the preferred plant species of each population was always its best plant for breeding. Secondly, virgin and mated females of taxon 2 had a similar preference range. Strong feeding and oviposition preferences for specific field host plants reduce the chance of contact between *Ribautodelphax* species in mixed populations and contribute to their reproductive isolation.

Males and females of two *Ribautodelphax* taxa showed an equally strong preference for their field host plants, in contrast to the delphacid, *Nilaparvata lugens*, where males showed more variance in host preference than the females (Sogawa & Pathak, 1970). The population density of *Ribautodelphax* is often low and a strong host preference by both sexes might enhance the chance of contact between the sexes.

Tentative results suggested that host plant choice, in at least two *Ribautodelphax* species, is under control of a single gene or of a group of linked genes. Little is known of the genetics of resource use and preference in phytophagous insects (Futuyma & Peterson, 1985). Data on host plant selection in planthoppers of the genus *Muellerianella* (Drosopoulos, 1977; Booij, 1982b) suggested a genetic system comparable to that in *Ribautodelphax*. Hybrids between *M.fairmairei* (Perris) and *M.brevipennis* (Boheman) strongly preferred the field host plant of the latter species in a choice situation. The suggested simple genetic control of host plant choice in these two delphacid genera contrasts with the polygenic nature of the mechanisms determining the virulence of the 'biotypes' of the planthopper *N.lugens* (Den Hollander & Pathak, 1981; Claridge & Den Hollander, 1982; Claridge *et al.*, 1983). These data are not necessarily contradictory. Behavioural adaptation, such as the host plant choice in *Ribautodelphax* and physiological adaptation, such as the ability to utilize plant fluids in *N.lugens* are not necessarily genetically correlated (Wasserman & Futuyma, 1981; Futuyma & Peterson, 1985).

Taxonomical conclusions

Field observations and experimental results clearly show differences in host plant relations between all members of the *R.collinus* complex. These differences suggest that the four recently discovered taxa and the five already known species of this complex are all true biological species. The differences found between the morphologically similar *R.imitans*/taxon 4 and *R.pungens*/taxon 1 are important in this respect. Taxon 2 and 3 are morphologically indistinguishable, but clear ecological differences were found. Taxon 2 preferred its field host plant and its reproductive success was low on the field host plant of taxon 3. Taxon 3 could not be reared.

Comparison of the host plant relations of the triploid females and their associated bisexual species might give a better insight into the origin of the triploids. Differences between *M.fairmairei* and its associated triploid (Drosopoulos, 1977; Booij, 1982a, b) in host plant range was one of the main arguments for the hypothesis of the hybrid origin of these triploids. Differences between diploid and triploid females were not found in *R.pungens* and taxon 4, either in host choice, or in breeding results. Two possibilities may explain the absence of these differences. Firstly, the triploids might have an autoploid origin being genetically identical to their diploid associates. Secondly, an alloploid or hybrid origin of the triploids is not ruled out by the host plant data, if it is assumed that the associated species contributed two genomes to the triploids which are completely dominant over the genome contribution of the second parent species.

C. F. M. den Bieman

nelore Hoch. F. M. Muller identified most grass species.

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ACOUSTIC DIFFERENTIATION AND VARIATION IN PLANTHOPPERS OF THE GENUS RIBAUTODELPHAX (HOMOPTERA, DELPHACIDAE).

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ABSTRACT

Calling signals of seven species and four recently discovered Taxa of the genus Ribautodelphax were recorded and analysed. Clear differences in calling variables were found between all Ribautodelphax studied which suggests that all taxa investigated are true species. Interspecific differences were distinct in males as well as females.

Variation in calling variables was larger between individuals than within individuals. Interpopulation variation in calling variables of both males and females is described for some species. Aberrant acoustic signals of one population, provisionally included in one of the new Taxa, suggsted that another new species might be involved.

INTRODUCTION

Investigations in recent years on planthopper species (Delphacidae) have demonstrated that their substrate-transmitted acoustic signals show species specific-characteristics (e.g. Booij, 1982; Ichikawa, 1976; Strübing & Hasse, 1975; de Vrijer, 1981; 1986). Therefore analysis of acoustic signals is a very valuable tool for elucidation of taxonomically difficult groups consisting of sibling species (Claridge, 1983).

Studies of planthoppers have demonstrated that some species have a wide repertoire of acoustic signals (Booij, 1982; Ichikawa, 1982) that are produced in different behavioral contexts. Signals that are produced in the first step of the mating behaviour generally show the greatest interspecific differences (Claridge, 1985b; Alexander, 1967). These signals are termed 'calling signals' because they are used to attract the attention of conspecifics. Differences in calling signals between species might lead

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Table I. Localities of _Ribautodelphax_ populations used for acoustic studies.

	Code[a]	Locality	Latitude ($^\circ$N)	Longitude ($^\circ$E)
		R. COLLINUS COMPLEX		
collinus	NL1	Limburg, Plasmolen.	51.44	5.57
(Boheman)	NL2	Utrecht, Maarn.	52.04	5.21
	YU1	Srbija, Trstenik.	43.36	21.00
	BG1	Kyustendil, Rilski Manastir.	42.07	23.19
	GR1	Trikkala, Meg. Panagia (Pindos Mts.).	39.49	21.20
angulosus	NL3	Utrecht, Leersum.	52.01	5.26
(Ribaut)	GR5	Drama, Meg. Panagia (Rodopi Mts.).	41.29	24.16
pungens	NL4	Limburg, Bemelen.	50.50	5.45
(Ribaut)	NL5	Limburg, Kunderberg.	50.53	5.56
	BE1	Namur, Jemelle.	50.10	5.16
	BE2	Namur, Dinant.	50.16	4.55
	BE3	Namur, Nismes.	50.04	4.33
	BE4	Luxembourg, Torgny.	49.31	5.28
	DE1	Nordrhein-Westfalen, Rodderberg.	50.38	7.12
	DE2	Hessen, Amöneberg.	50.48	8.56
	DE3	Rheinland-Pfalz, Ebernburg.	49.47	7.47
	DE4	Baden-Württemberg, Tübingen.	48.32	9.02
	FR1	Ardèche, Coulens.	44.32	4.18
	FR2	Alpes de Haute Provence, Montfuron.	43.54	5.41
	YU2	Slovenija, Postojna.	45.44	14.11
	GR2	Florina, Kotas.	40.42	21.15
	GR3	Fokis, Elaion.	38.33	22.25
	GR4	Fokis, Skaloula.	38.30	22.15
imitans	FR1	Ardèche, Coulens.	44.32	4.18
(Ribaut)	FR3	Pyrenées Orientales, St. Cyprien Plage.	42.42	3.02
	GR3	Fokis, Elaion.	38.33	22.25
fanari	FR4	Bouches du Rhône, Etang de Vaccarès.	43.32	4.38
nom. nud.				
Taxon 1	NL6	Gelderland, Hoge Veluwe.	52.03	5.51
Taxon 2	GR6	Florina, Kalo Nero Mts.	40.44	21.11
Taxon 3	FR5	Vaucluse, Mont Ventoux.	44.09	5.15
Taxon 4	FR6	Vaucluse, St. Estéve.	44.08	5.14
	FR7	Vaucluse, Crillon-le-Brave.	44.07	5.08
	FR8	Vaucluse, Bedoin.	44.07	5.10
	FR9	Bouches du Rhône, Les Baumettes.	43.42	4.53
	FR10	Aude, Tuchan.	42.53	2.43
	ES1	Valencia, Sagunto.	39.42	0.17
		OTHER RIBAUTODELPHAX SPECIES		
pallens	NO1	Sør-Trødelag, Dovrefjell.	62.16	9.32
(Stål)	NO2	Oppland, Hinseter.	61.37	9.00
albostriatus	NL7	Limburg, Wrakelberg.	50.50	5.55
(Fieber)	BE5	Namur, Jemelle.	50.10	5.16
	DE5	Nordrhein-Westfalen, Königswinter.	50.41	7.11
	FR8	Vaucluse, Bedoin.	44.07	5.10
	YU1	Srbija, Trstenik.	43.36	21.00

[a]Belgium (BE), Bulgaria (BG), France (FR), Greece (GR), Netherlands (NL), Norway (NO), Spain (ES), W. Germany (DE) and Yugoslavia (YU).

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to a reduction in time and energy spend contacting other species.

The genus Ribautodelphax comprises nine species in W. Europe, five of these species belong to the R. collinus complex (den Bieman, in prep.). Recently several populations of uncertain status in this complex were discovered. They are provisionally referred by numbers 1 to 4 (den Bieman, 1987). Identification problems in the R. collinus complex are numerous because of slight and variable morphological differences. A careful analysis of acoustic communication in the R. collinus complex may add to recognition of the true limits of biological species and may be critical in determining the status of recently discovered Taxa. Studies on differentiation in other characteristies have also been undertaken, e.g. host-plant relations (den Bieman, 1987), morphology and allozyme patterns (den Bieman, in prep.).

This paper will present information on differentiation and variation in calling songs of bisexual Ribautodelphax species and new Taxa. In populations of R. pungens (Ribaut) and Taxon 4, pseudogamous triploid females occur (den Bieman, 1984). The acoustics of the diploid females of these two taxa and of the associated triploid females will be discussed in a separate paper.

MATERIAL AND METHODS

Planthoppers

Five Ribautodelphax species and four new Taxa belonging to the R. collinus complex were studied (Table 1). Two species not belonging to this complex were also compared. All Ribautodelphax populations were reared in a greenhouse on their respective host plants (20 °C, light phase 18 h (den Bieman, 1987). Each laboratory population was started with at least five males and five females. To reduce the possible loss of variation by inbreeding, only animals from the first four laboratory-bred generations were used. Most populations were reared in two cages and males were transferred to the parallel rearing in each generation. Only some populations of R. pungens were reared in a single cage (Table 1: BE2, BE3, DE1 and FR1).

Males and females were isolated as fifth instars and recorded as

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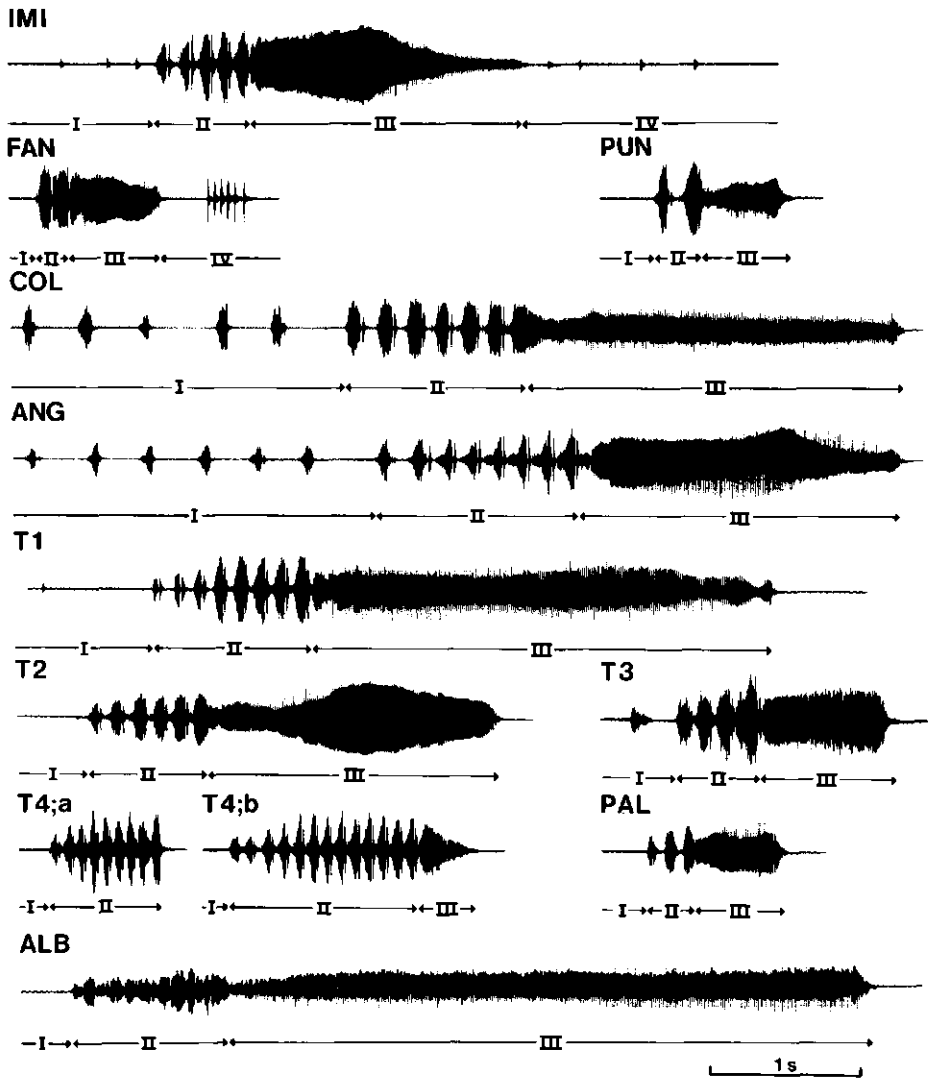
Fig. 1. Male calling signals of <u>Ribautodelphax</u>, I to IV indicate successive Sections. Abbreviations:IMI, <u>imitans</u> (GR3); FAN, <u>fanari</u> (FR4); PUN, <u>pungens</u> (BE4); COL, <u>collinus</u> (BG1); ANG, <u>angulosus</u> (GR5): T1, Taxon 1 (NL6); T2, Taxon 2 (GR6); T3, Taxon 3 (FR5); T4, Taxon 4 (a: FR8 and b: FR10); PAL, <u>pallens</u> (NO1) and ALB, <u>albostriatus</u> (DE5). Codes in brackets represent populations as listed in Table I.

adults of age 7-9 days.

Recording technique

An extensive description of the technique used for the recording of acoustic signals of planthoppers is given by de Vrijer (1984) and only some details are mentioned here. All species were recorded on a single grass species, Agrostis capillaris L. This grass species is the host plant of R. collinus but was highly suitable as recording substrate for other planthoppers. The grass stem was connected with an accelerometer (Bruel & Kjaer 8307) and the amplified signal (amplifier, Bruel & Kjaer 2625) was recorded (recorder, Revox A 77) on tape (Agfa PEM 268, tape speed 19.5 cm/s). Signals were analysed from oscillograms (Siemens Oscillomink; 20 mm corresponding to 1 s). Calling signals were recorded of spontaneously singing males, and females were induced to call by playback with conspecific male signals (de Vrijer 1984; Ichikawa, 1976).

Several experiments showed that temperature has a pronounced effect on acoustic signals in Ribautodelphax. To eliminate this effect, all recordings were made between 19 and 20 °C.

Terminology and analysis

Booij (1982) defined calling signals as sounds produced spontaneously by single males or females. Because spontaneously calling females were rarely observed in most Ribautodelphax populations, this definition was extended to include also female signals produced in response to playback with male calling signals. Other terms follow Alexander (1967), Booij (1982) and de Vrijer (1984).

For quantitative analysis, male calling signals were subdivided into four Sections, but not every Section was found for every individual or species (Fig. 1). This subdivision was based on the two sound elements that can be distinguished, the 'buzz' of Section III and the 'chirps' of the remaining Sections. These sound elements clearly separated the Sections II and IV from Section III, but a division between Section I and II was not always easy.

Chirps in Sections I and IV often consisted of one pulse, though some species frequently produced chirps composed of several pulses. Within some

calling strophes, gradual changes between the two types of chirps were found. The complex structure of the chirps of Section II (Fig. 1) usually distinguished it from the preceding one. A distance between the chirps of Section II of less than one chirp duration was taken as an additional criterion to characterize Section II if composite chirps were found in Section I. The chirps of Section I are more distantly spaced.

The number of chirps in Sections I and IV were only counted when successive calling strophes were clearly separated. Chirps separated from other chirp trains by an arbitrarily chosen interval of longer than three seconds were not counted.

For analysis, five variables were measured of each individual calling strophe: the number of chirps in Sections I, II and IV and the duration of Sections II and III.

Females emitted calls composed of simple 'click' series (Fig. 7). The duration of each female calling signal and the click repetition rate (CRR) at the beginning and end of a calling strophe were measured. For species with a strophe duration longer than three seconds, the duration of ten click intervals was measured 1 s after the beginning and 0.5 s before the end of a strophe (Fig. 7, R. imitans) to avoid irregularities, which often occur at the beginning and end of a strophe as very low click amplitudes and irregular distances between clicks. Hardly any irregularities were found in the calls of species with a strophe duration of less than three seconds. For these species, the length of five click intervals was measured 0.25 s after the beginning and 0.13 s before the end of a strophe (Fig. 7, R. fanari).

Of each individual ten calling signals were analysed and attempts were made to record ten males and ten females of every population to assess variability in calling songs. Whenever necessary, the normal distribution of the data was tested with the Kolmogorov-Smirnov test (Sokal & Rohlf, 1969).

RÉSULTS

MALE CALLING SIGNALS

Calling signals were produced by single males within a few minutes of transfer to the grass stem used for recording. In some species, intervals

between calling strophes usually lasted several seconds, e.g. R. pungens, while intervals of many minutes occurred in other species. Males remaining silent for a prolonged period often started singing again after removal and return on the grass stem.

Distress signals and agressive songs frequently produced in other planthopper species (Booij, 1982; Ichikawa, 1982) were never recorded in Ribautodelphax, either in recordings of single males or in observations of groups of males.

Intraspecific variability

Analysis of ten calling strophes of individual males showed a considerable variation. The number of chirps and duration of Section II, and the duration of Section III was relatively constant for individual males. However Sections I and IV showed a wide variability within individuals and between individuals of the same population. In many populations, some males incorporated Sections I and IV in all ten calling strophes, other males never produced these Sections, and the remaining individuals produced these Sections occasionally. Also the number of chirps of Sections I or IV of each calling strophe was very variable, e.g. 0 to 51 chirps in R. angulosus. Because of this wide variability, Sections I and IV were not analysed in detail. Analysis of variance of one randomly sampled population of every Ribautodelphax species indicated that the within-individual variation is always smaller than the variation between individuals for the three variables studied (Table II, males). This suggests that the variability of calling signals is better assessed by recording more individuals than by studying more strophes per individual. Similar results were found for the planthopper genus Javesella (de Vrijer 1984, 1986).

Differences between populations from different geographic areas could be studied for six Ribautodelphax species and for Taxon 4 (Fig. 2 and 3). In the two species R. angulosus and R. pallens, no significant differences were found in the three variables studied, although the two populations of R. angulosus were obtained from widely separated localities, Greece and the Netherlands. Moreover, extensive field-work, as well as data from the literature and museum collections strongly indicate that R. angulosus is a very rare planthopper.

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Number chirps
Section II

Duration
Section II

Duration
Section III

COL

NL 1 (10)
NL 2 (10)
YU 1 (10)
BG 1 (10)
GR 1 (10)

0 5 10   0 1 2 s   1 2 3 s

ANG

NL 3 (10)
GR 5 (10)

0 5 10   0 1 2 s   1 2 3 s

IMI

FR 1 (10)
FR 3 (10)
GR 3 (10)

0 5 10   0 1 2 s   0 1 2 s

T4

FR 6 (10)
FR 7 (10)
FR 8 (10)
FR 9 (10)
FR 10 (10)
ES 1 (10)

16.0

0 5 10   0 1 2 s

PAL

NO 1 (10)
NO 2 (10)

0 5 10   0 1 2 s   0 1 2 s

ALB

NL 7 (10)
BE 5 (5)
DE 5 (5)
FR 8 (5)
YU 1 (10)

0 1 2 s   3 4 s
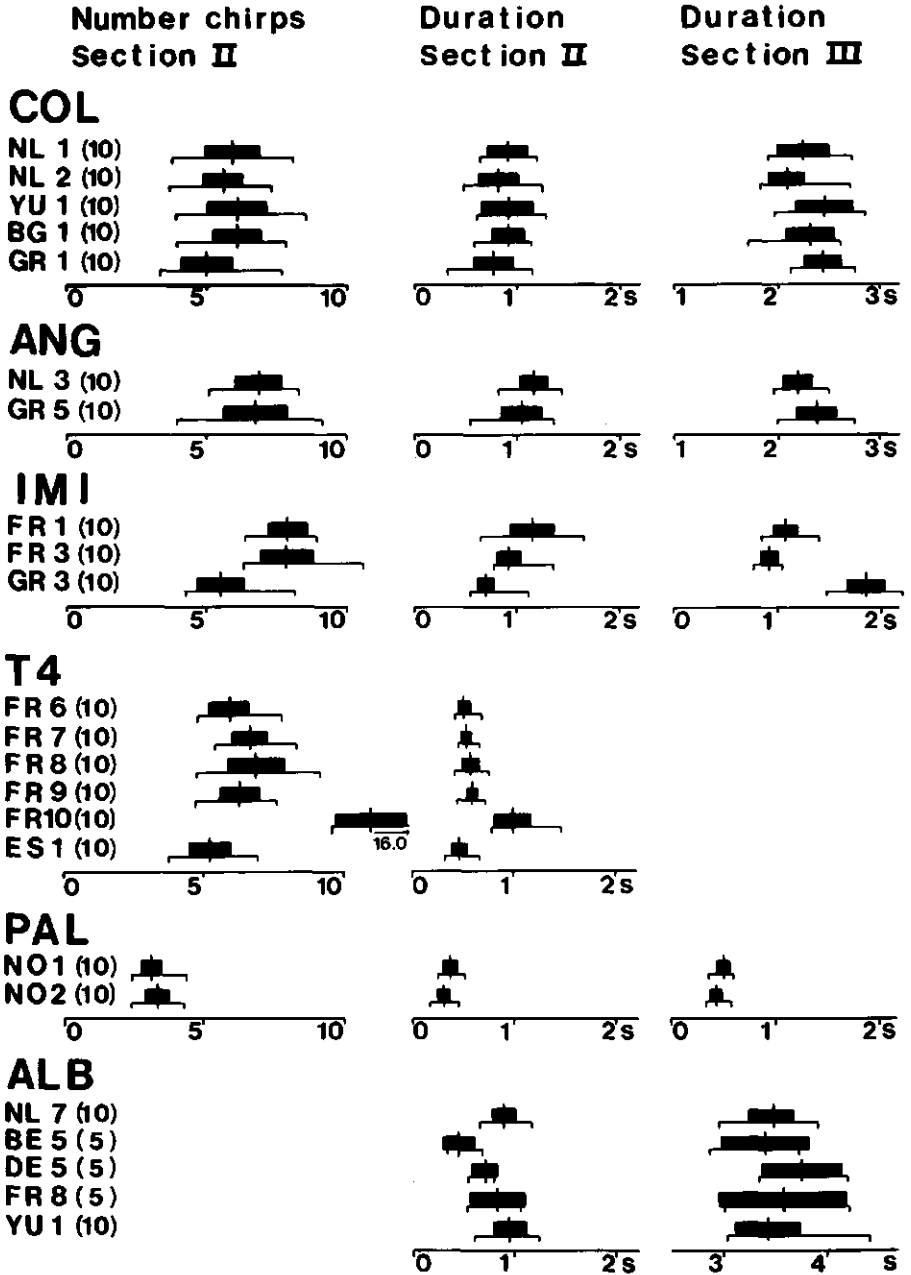
Fig. 2. Intraspecific variability in male calling signals of Ribauto-delphax. Number of chirps in Section II not counted for R. albostria-tus. In brackets, number of individuals studied. For each variable: mean (vertical line); 95% confidence limits (black bar) and range of individual means (horizontal line). Abbreviations as in Fig. 1 and Table I.

Table II. Analysis of variance among individuals and within each individual of one population of each <u>Ribautodelphax</u> species or new Taxon. F values given. * P < 0.10; ** P < 0.01 and ***P < 0.001. Abbreviations as in Table 1 and Fig. 1.

Spe-cies	Popu-lation	MALES			FEMALES		
		Number chirps Section	Duration	Section	Duration strophe	Click Repetition Rate Start signal	End signal
		II	II	III			
COL	NL1	41.12***	29.83***	34.56***	57.54***	22.78***	100.44***
ANG	NL3	21.46***	12.34***	17.56***	37.33***	87.20***	19.90***
PUN	NL5	4.00***	7.02***	17.02***			
IMI	FR3	33.19***	34.00***	7.80***	18.71***	40.14***	109.18***
FAN	FR4	6.70***	8.64***	10.84***	45.68***	31.76***	29.80***
T 1	NL6	19.14***	129.79***	10.67***	10.02***	17.65***	36.77***
T 2	GR6	38.66***	33.10***	8.93***	18.59***	15.34***	13.10***
T 3	FR5	16.71***	13.56***	16.20***	4.35**	3.38**	3.82*
T 4	FR6	24.80***	18.50***	-			
PAL	N01	18.68***	36.67***	29.55***	43.48***	30.20***	57.33***
ALB	NL7	-	7.77***	8.71***	6.52***	23.65***	13.33***
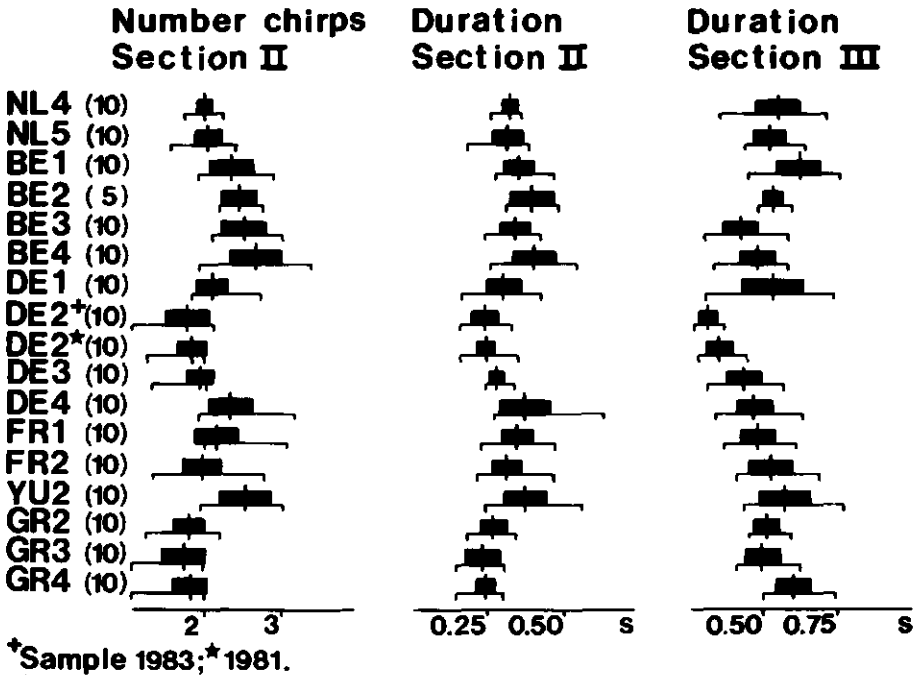
Fig. 3. Intraspecific variation in male calling signals of R. pungens. In brackets number of individuals studied. For abbreviations and statistics see Table I and Fig. 2.

Among some of the populations of R. collinus and R. albostriatus, significant differences were found in one single variable (Fig. 2). R. imitans showed significant differences in all three variables; especially the duration of Section III differed strongly between the Greek and the two French populations. The combined data of the duration of Section III from these three populations are not normally distributed and for this variable only data of the two French populations were used.

Taxon 4 showed much variability in male signals (Fig. 1 and 2). Especially the variation in the duration of Section III, 'buzz length', is striking. Section III is found in each calling strophe of the other ten Ribautodelphax species and new Taxa studied, but no male of the populations FR6 and FR9 of Taxon 4 emitted Section III. Of the remaining populations of Taxon 4, some males of the populations FR7, FR8 and ES1, and all males of population FR10 included Section III in one or more calling strophes. Population FR10 produced a significantly longer Section III than these three populations ($U_{[10,19]}$= 13; P < 0.001; Mann-Whitney test). Population FR10 also differed strongly from all other populations of Taxon 4 in the number of chirps and the duration of Section II (Fig. 2); even the ranges of individual means for these two variables did not overlap. Population FR10 resembled the other populations of Taxon 4 in morphology and host-plant relations (den Bieman, 1987; in prep.). However, these acoustic differences suggest that population FR10 might represent another new species. For this reason it was not used for comparisons of Taxon 4 with the other Ribautodelphax species and new Taxa.

R. pungens is not uncommon in W. and SE. Europe and populations were sampled over a wide geographic range (Fig. 3). Means of three variables of sixteen populations were analysed with Duncan's multiple range test (Fig. 4). Most populations belonged to widely overlapping subsets in all three variables and no complete segregation was found in any variable. No regular geographic pattern of variation could be discerned.

Cultures of R. pungens started with rather small samples of 5-20 males and the same number of females. Extensive field-work during the last four years showed, however, that many R. pungens populations are actually small. Slight differences in calling variables between R. pungens populations could be due to sampling bias. However the absence of any significant difference between two samples from the same locality of population DE2 (Fig. 4) suggests that sample artefacts were minimal for this population.

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NO. CHIRPS
SECTION II

DURATION
SECTION II

DURATION
SECTION III

NO. CHIRPS SECTION II	DURATION SECTION II	DURATION SECTION III
● GR 3	● GR 3	DE 2;83
DE 2;83	DE 2;83	DE 2;81
o GR 2	o GR 4	BE 3
o GR 4	DE 2;81	DE 3
DE 2;81	o GR 2	DE 4
DE 3	DE 3	FR 1
● FR 2	DE 1	BE 4
NL 4	● FR 2	● GR 3
NL 5	NL 5	o GR 2
DE 1	NL 4	NL 5
FR 1	FR 1	DE 1
DE 4	BE 3	● FR 2
BE 1	BE 1	BE 2
BE 2	YU 2	NL 4
BE 3	DE 4	YU 2
YU 2	BE 4	o GR 4
BE 4	BE 2	BE 1
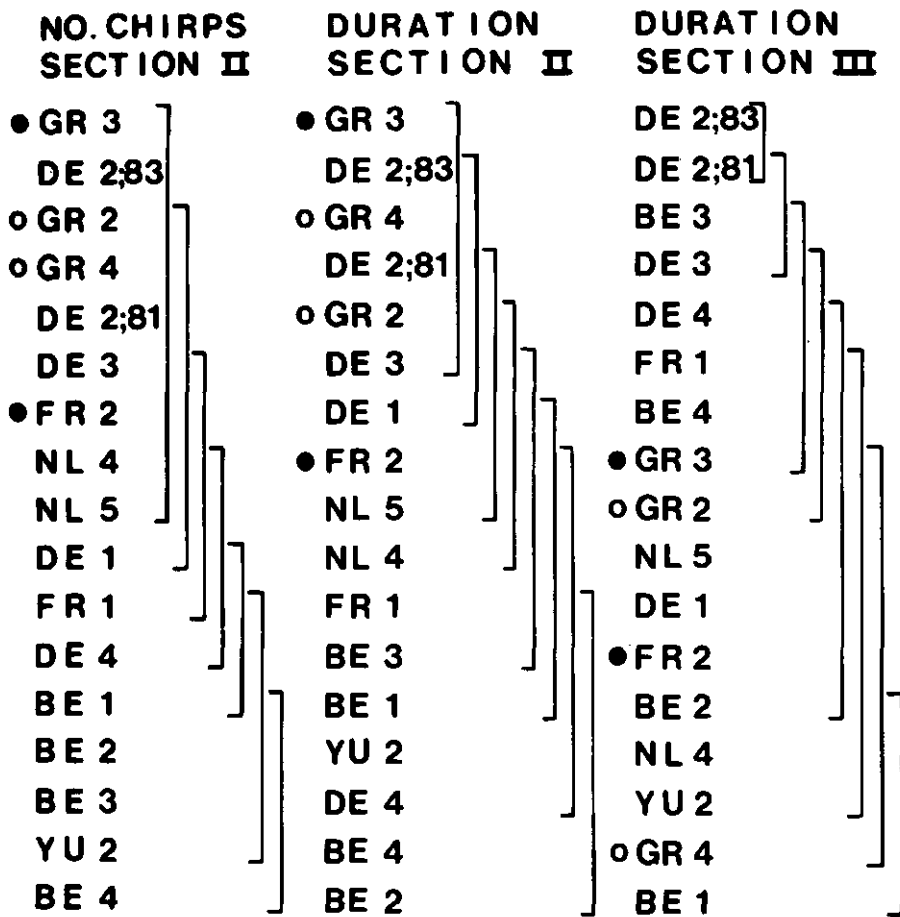
Fig. 4. Statistical analysis of male calling variables of R. pungens with Duncan's multiple range test. Vertical lines link populations with maximum non-significant differences between means at P = 0.05. Means based on ten individuals per population; population BE2, five males only. ● Populations sampled from Brachypodium phoenicoides, o populations from B. sylvaticum, remaining populations sampled from B. pinnatum. For abbreviations see Table I.

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Populations from the same geographic area, e.g. the two Dutch populations (NL4 and 5), often occur in the same subset of sample means. These data indicate that the differences found between the laboratory cultures represent real differences between field populations.

The variability of the populations BE2, BE3, DE1 and FR1 of R. pungens could eventually have been reduced by inbreeding, because each of these populations was reared in a single cage. However, there are no indications (Fig. 3) that these populations exhibited less variability compared to the other populations that were reared in two cages of which males were exchanged in each generation.

Populations of R. pungens were collected from three host plants (den Bieman, 1987). No differences in acoustic signals could be detected between populations collected from different host plants (Fig. 4).

Interspecific differences

Characteristic differences between the calling signals of Ribautodelphax species and new Taxa (Fig. 1) are easily seen and heard. In almost every recording, a similar sound element is found. It consists of a buzz (Section III) preceded by one or more composite chirps (Section II). A buzz was absent from the signals of several populations of Taxon 4.

To characterize the male calling strophes, three variables were analysed (Fig. 5). Only one population was available for recording for some species, whereas up to sixteen populations were analysed for others (Table I). Of R. albostriatus, one of the species not belonging to the R. collinus complex, only two variables could be measured because the chirps of Section II were not distinguishable from each other in most calling strophes.

Distinct differences between many species were found in the duration of Section III, the 'buzz length' (Fig. 5). This variable also distinguished Taxon 4 (not included in Fig.5) from the other Ribautodelphax studied. Most males of Taxon 4 frequently omitted Section III from their calling strophes, whereas it was always present in strophes of the other species. If present, Section III was very short in Taxon 4: 0.15 ± 0.06 s (N=19).

All species and new Taxa differed in at least one variable (Table III). Noteworthy are the clear differences between species that are morphologically close to one another: R. imitans/Taxon 4, R. pungens/Taxon

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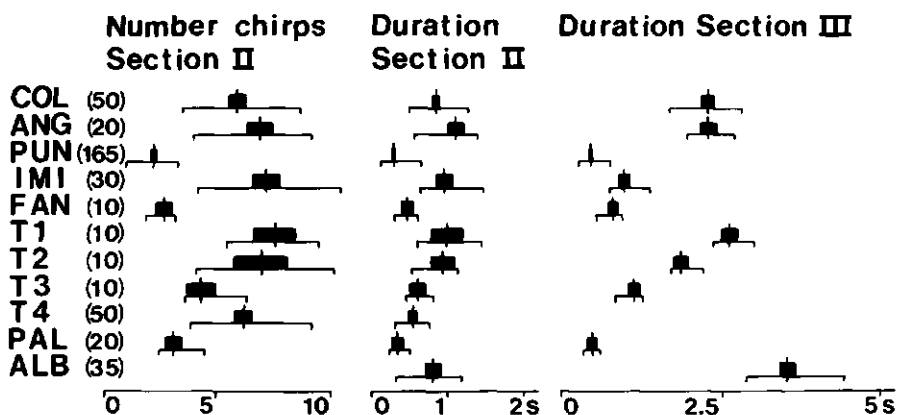
Fig. 5. Interspecific differences in three variables of calling
signals of Ribautodelphax males, of R. albostriatus and Taxon 4 only
two variables. In brackets number of individuals studied. For
abbreviations and statistics see Fig. 1 and Fig. 2.

Table III. Analysis of male calling signals of Ribautodelphax with
Duncan's multiple range test. A common letter in vertical columns
indicates absence of significant differences at P = 0.05. N, number
of individuals. For other abbreviations see Fig. 1.

Ribautodelphax	Number chirps Section	Duration Section		N
	II	II	III	
COL	D	DE	E	50
ANG	E	G	E	20
PUN	A	A	A	165
IMI*	E	EF	C	30
FAN	AB	B	B	10
T 1	E	FG	F	10
T 2	E	EF	D	10
T 3	C	C	C	10
T 4**	D	BC	-	60
PAL	B	A	A	20
ALB	-	D	G	35

* Population GR3 omitted for duration of Section III.
** Population FR10 omitted.

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1, and Taxon 2/Taxon 3.

Some species may be separated by the occurrence and structure of their Sections I or IV. Section IV was found in calling strophes of R. fanari (32%), and in R. imitans (population FR 1, 25%; FR3, 57% and GR3, 42%). However these species differed in the structure of Section IV. The chirps of Section IV were regularly spaced in 92% of the strophes with this Section for R. fanari (Fig. 1), while these chirps were irregularly distributed in R. imitans.

In only the species R. angulosus was Section I found in almost every calling strophe (99.5%). R. pallens and R. pungens included Section I in less than 20% of their calling strophes. The other species and new Taxa were intermediate though there were large differences between populations.

FEMALE CALLING SIGNALS

Females readily produce calling signals after one or two playback stimuli with conspecific male signals. Spontaneously produced signals were excluded from sound analysis.

Intraspecific variability

The variation between individuals and within individuals was analysed for one randomly sampled population of each Ribautodelphax (Table II, females). The results of the female calling signals correspond with those on individual variability in male signals. Variation between individuals is higher than within individuals in all three variables.

Of five Ribautodelphax species, populations from different geographic areas were recorded (Fig. 6). No significant differences were found between populations of R. angulosus, R. pallens and R. albostriatus. Only some populations of R. collinus differed significantly from each other in strophe duration.

Female signals of population FR1 of R. imitans differed significantly from the populations FR3 and GR3 in all variables studied, but their ranges of individual means still showed overlap. Comparison of the CRR at both

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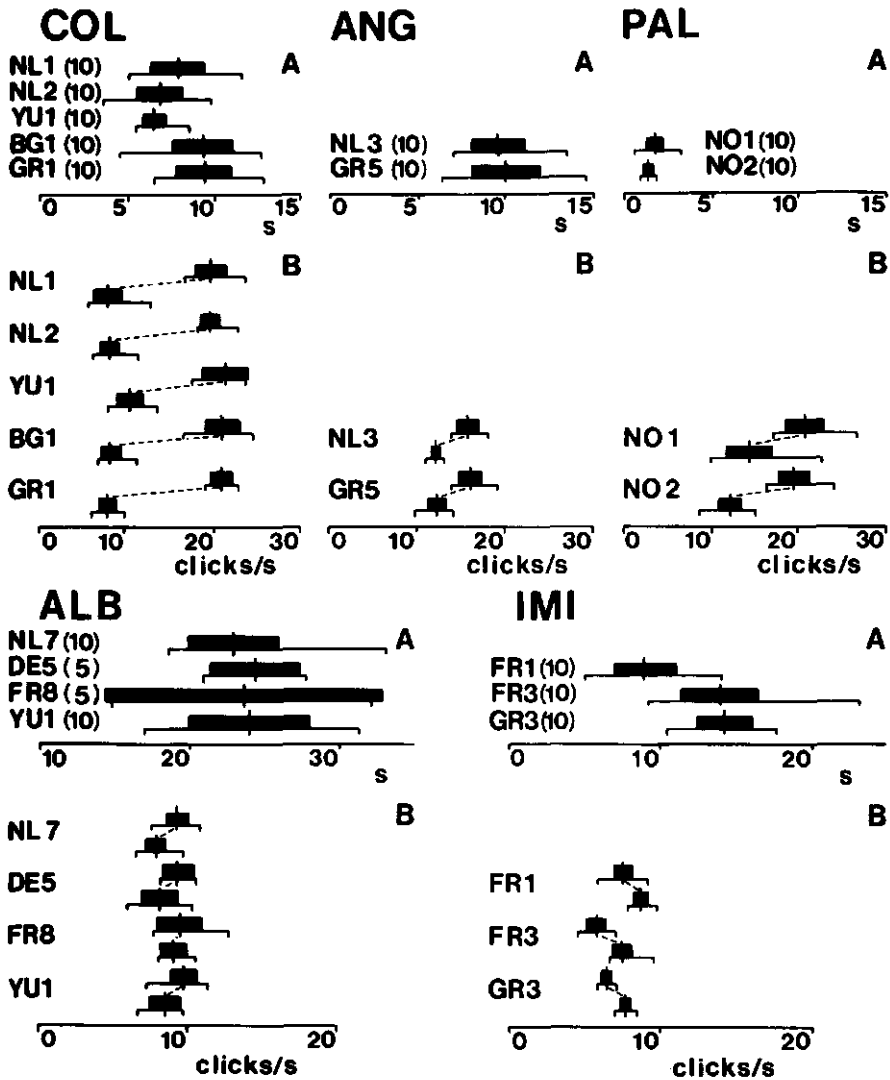
Fig. 6. Intraspecific variation in calling signals of Ribautodelphax females. A. Duration of calling strophes. B. CRR at the beginning (upper bar) and end of a strophe connected by a broken line for each population. Between brackets number of individuals recorded. Abbreviations and statistics as in Fig. 1 and 2.

ends of a calling strophe showed an increase of the CRR in all three populations.

Interspecific differences

A representative calling strophe of each species and each new Taxon is illustrated in Fig. 7. The CRR of species with a strophe duration of more than three seconds was measured in a different way from those of species with a shorter signal. Consequently, comparisons of CRR between species are only valid within each of these two groups.

Interspecific differences in duration of the calling strophes (Fig. 8A) were found between four species and Taxon 1, all with a mean strophe duration longer than six seconds. The duration of the calling strophes of the four Ribautodelphax with a short signal showed overlap, but Taxa 2 and 3 differed significantly.

The CRR (Figure 8B and C) declined at the end of a strophe for five Ribautodelphax species and three of the new Taxa, but increased for R imitans. A considerable·decrease was found for some species, e.g. R. collinus, while this decrease was small for others, e.g. Taxon 2. Calling strophes of R. albostriatus have a marked structure (Fig. 7) starting with a low CRR followed by an increase and a final decrease in CRR. For several long signals, this pattern was produced twice in the same calling strophe.

Female signals of the six Ribautodelphax species and three of the new Taxa differed significantly from one another in at least one calling variable. However no differences were found between R. fanari and R. pallens (Fig. 8). Significant differences in all variables were observed between the morphologically indistinguishable Taxa 2 and 3.

DISCUSSION

Intraspecific variability

Some populations of R. collinus, R. pungens and R. albostriatus differed significantly from one another in at least one calling variable of one or both sexes. These slight differences, however, do not give any reason to assume that there are more species than have been distinguished. No significant differences were observed between populations of R.
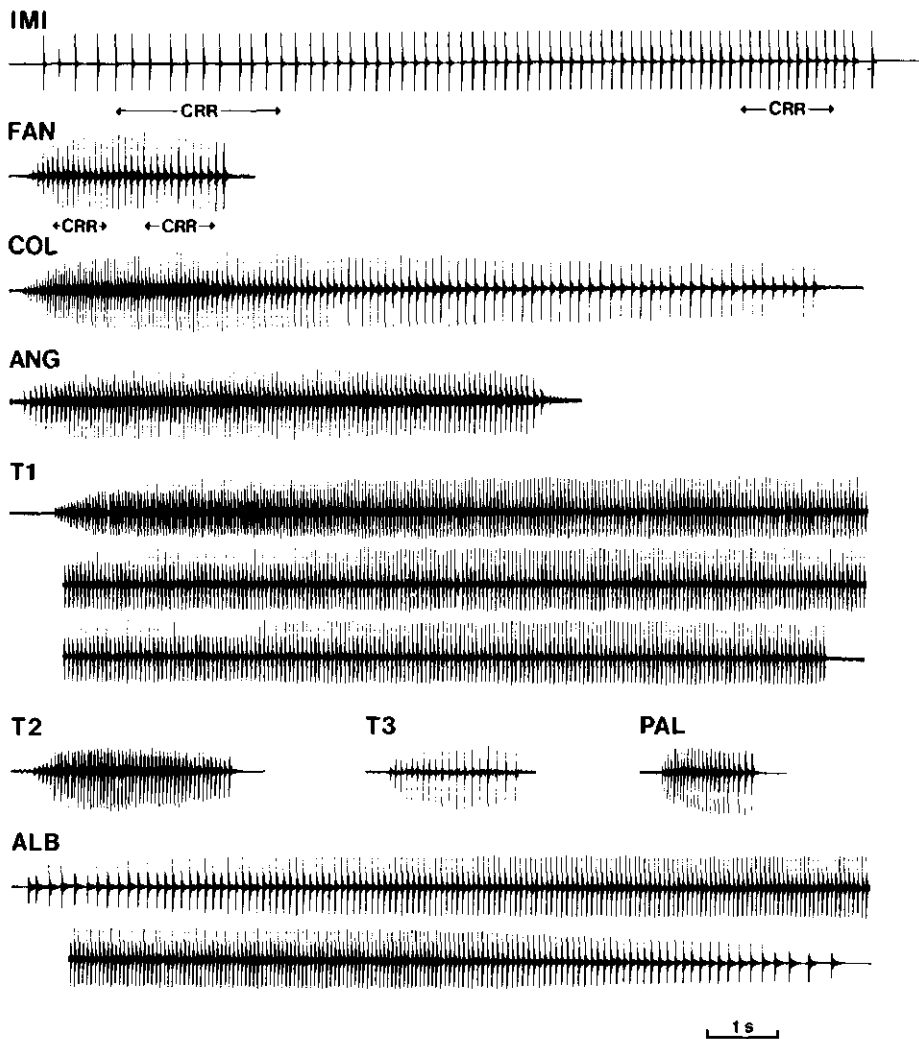
Fig. 7. Calling signals of <u>Ribautodelphax</u> females. The recorded specimens are from the following localities (in brackets): IMI (FR3);FAN (FR4); COL (BG1); ANG (GR5); T 1 (NL6); T 2 (GR6); T 3 (FR5); PAL (N01) and ALB (DE5). For abbreviations see Table I and Fig. 1.
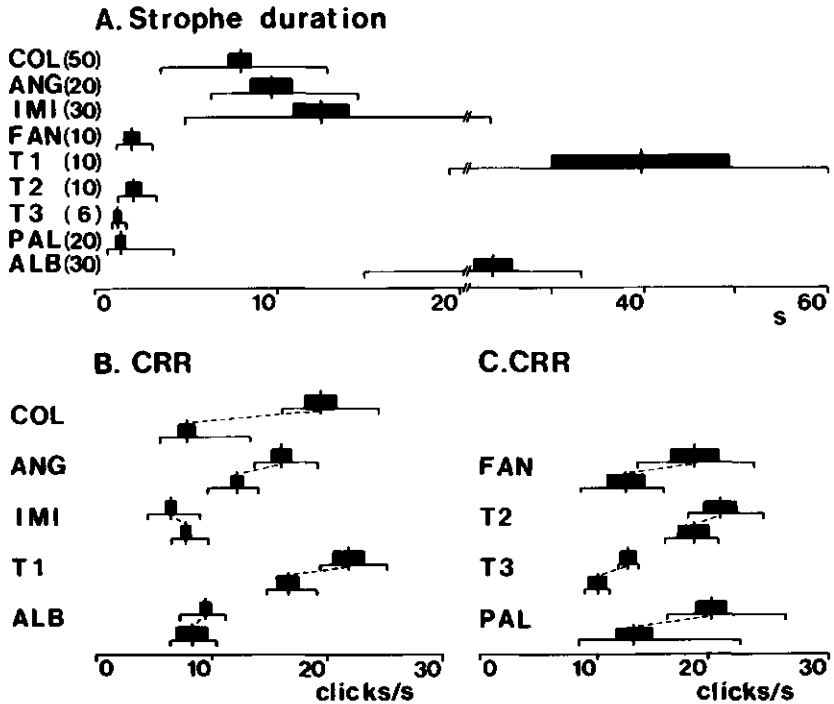
Fig. 8. Interspecific differences in three variables of calling signals of Ribautodelphax females. B and C Click repetition rate of species with long (> 3 s) and short (< 3 s) strophe duration, respectively. CRR at the beginning (upper bar) and end of a strophe (lower bar) are connected with a broken line. In brackets, number of individuals recorded. Abbreviations and statistics as in Fig. 1 and Fig. 2.

angulosus and R. pallens, contrary to the strong differences between populations of R. imitans and Taxon 4.

Analysis of the differences between populations for male and female signals of R. imitans gave conflicting results. Male signals of the two French populations differed significantly from the Greek population in all variables. One of these French populations differed from the other two populations in female songs. Acoustic signals of both sexes had unique characteristics: the occurrence and structure of Section IV of male songs and the increase in CRR of female calls. Both of these characteristics were

found in the three populations studied. These results indicate that all three populations probably belong to a single species despite their strong interpopulation differences. Analysis of the acoustic signals of other populations of R. imitans will be necessary to test this conclusion.

One population (FR10) of Taxon 4 differed strongly from the other populations in all variables of the male signal. These differences strongly suggest that population FR10 represents an unknown Ribautodelphax species. A close relation between Taxon 4 and this population FR10 is indicated by the variability of Section III, which was absent from many calling strophes of males of each population of Taxon 4, including population FR10, but was always present in male calling strophes of the other Ribautodelphax populations studied. Analysis of the acoustic signals of other populations of Taxon 4 especially from the area surrounding population FR10 is necessary to clear the taxonomic status of this population. Provisionally, population FR10 has not been included in Taxon 4.

Only two other studies have been published on variation in male calling signals between geographically separated populations of planthoppers. Female signals, however, had never been studied on a large georgraphic scale. In neither of these studies on male signals (Booij, 1982; Claridge et al., 1985) was any geographic pattern found in variability. Neither was clinal variation found in male or in female signals in any Ribautodelphax species.

Interspecific differences

Differences in male and female calling songs were found between all members of the R. collinus complex studied. These differences suggest that the four recently recognized Taxa and the five known species are probably all true species.

It is not always possible to use acoustic signals of single specimens for species indentification because of the large overlap of the ranges of individual means of the variables. Also combinations of variables are not always species-specific.*

*Note: As discussed by de Vrijer (1986), an analysis of calling signals of the type presented in this paper may not be sufficiently detailed for every objective. So copies of selected original recordings may be obtained by sending a blank audiocassette to the author's address.

Calling signals of more than one species were extensively studied in two other planthopper genera: Muellerianella (Booij, 1982) and Javesella (de Vrijer, 1981; 1986). Calling signals proved to be more differentiated in males than females of both genera. Female calling songs could not be reliably distinguished between the Muellerianella species, contrary to the female songs of most Javesella species. In Ribautodelphax, interspecific differences in male and female calling signals are distinct in most variables. In leafhoppers (Cicadelledae), clear interspecific differences in male and female calling songs were described in the genus Nephotettix (Inoue, 1982, 1983), but female signals of Euscelis were less distinct than male signals (Strübing, 1983).

Signals of Ribautodelphax females are more complex than those of other planthopper genera studied. A temporal change in CRR is distinct in calls of Ribautodelphax females, but such changes were not found in female signals of Nilaparvata (Claridge, 1985a) and Javesella (de Vrijer, 1986). Their female songs consist of sequences of regularly repeated clicks. Female signals of Muellerianella are too short to investigate these changes.

A common pattern in male calling signals is obvious in Ribautodelphax. Such a common pattern is also evident in male signals of the closely related Muellerianella species (Booij, 1982). Judging from morphology and hybridization studies (de Vrijer, 1986; in prep.), Javesella species are more differentiated than Muellerianella or Ribautodelphax species. This conclusion accords with a greater diversity of male calling signals in Javesella.

The importance of the various acoustic variables for mate recognition in planthoppers has only once been investigated experimentally. Claridge et al. (1984, 1985) recently demonstrated the importance of one variable of the male signal for mate recognition in Nilaparvata lugens. Such information is not available for Ribautodelphax. So a discussion on the origin and variability of acoustic signals is not yet possible. Playback studies with different species and with different populations of the same species will provide a tool to test the relevance of specific variables for mate recognition.

Comparative studies on the acoustics of related taxa may provide insights into their phylogenetic relationships. A comparison of the entire acoustic behaviour of the R. collinus complex is not yet possible. Recent

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research on museum collections showed that several unknown species not included in this study belong to this complex. Moreover, only one stage of the acoustic repertoire has yet been studied.

Some conclusions may be drawn about the relationship between the R. collinus complex and the two other species that do not belong to this complex. Morphological data (den Bieman, in prep.) indicate that the R. collinus complex is more related to R. pallens than to R. albostriatus. Acoustic characteristics of both sexes appear to support this view on their interrelationships. The gross structure of the male and female signal of R. pallens resembles that of the R. collinus complex. R. albostriatus differs, however, from this complex in the structure of Section II of the male signal and the pattern of changes in CRR of the female call.

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VARIABILITY IN FEMALE CALLING SIGNALS IN MIXED POPULATIONS OF PSEUDOGAMOUS FORMS AND BISEXUAL <u>RIBAUTODELPHAX</u> SPECIES (HOMOPTERA, DELPHACIDAE).

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ABSTRACT

Female calling signals were studied in two <u>Ribautodelphax</u> species with mixed populations of diploid and pseudogamous triploid females. Significant variation was found between populations and within populations. Only minor differences were found between diploid and triploid females of the same populations. The results are discussed in view of hypotheses on the origin of the pseudogamous triploid females and in relation to coexistence of biparental species and pseudogamous females.

INTRODUCTION

Acoustic signals play an important role in the intraspecific communication of planthoppers (Delphacidae) (reviews in Claridge 1985a, 1985b). Recently, interspecific differentiation has been described in calling signals of male and female planthoppers of the genus <u>Ribautodelphax</u> (den Bieman 1986). Significant differences among conspecific populations were found in several <u>Ribautodelphax</u> species, but these differences were small compared to interspecific divergences. Moreover, only slight acoustic variation was found within populations of all species examined so far.

In populations of two species, <u>R. pungens</u> (Ribaut) and an undescribed species, provisionally indicated as Taxon 4, sexually reproducing diploid females and males occurred together with pseudogamous triploid females (den Bieman, 1984). These pseudogamous females depend on the bisexual species because they need to be inseminated by males to initiate the development of their eggs, although their all-female triploid offspring receives maternal genes only.

Pseudogamous triploid females were also discovered in the planthopper genus <u>Muellerianella</u> (Drosopoulos 1976, Booij 1982a, b). A hybrid origin was suggested for these <u>Muellerianella</u> triploids on the basis of

distribution patterns, hybridization tests and ecological relations (Drosopoulos 1978, Booij 1982b). Acoustic studies, however, revealed no differences between diploid females and associated triploids (Booij, 1982a). I know only of one other example of a comparison between the acoustic signals of triploids and related diploid species. Calls of hybrid triploid male frogs, Rana esculenta L., were intermediate between calls of both parent species (Schneider & Tunner, 1982).

Comparative acoustic studies of bisexual females and pseudogamous females of R. pungens and Taxon 4, and of the female signals of other Ribautodelphax species, might elucidate the origin of the Ribautodelphax triploids. In general, triploids of hybrid, allopolyploid, origin should show characteristics of both parent species, as in R. esculenta, while autopolyploid triploids should be similar to the single parent species.

MATERIAL AND METHODS

Planthoppers

For acoustic studies eighteen populations of R. pungens were sampled (Table 1). Karyological studies (den Bieman, in prep.) revealed that triploid females occurred in most populations of R. pungens. Both diploid and triploid females were studied, however, in only seven populations. Of the remaining populations, either diploid or triploid females were recorded.

Seven populations of Taxon 4 were studied and triploids of two populations were recorded (Table I). The taxonomic status of Taxon 4 is discussed by den Bieman (1986, 1987, and in prep.) and it is treated as a valid species in this paper.

Diploid and pseudogamous triploid females will be indicated with 2N and 3N, respectively.

Planthoppers were reared on their specific host plants (den Bieman, 1987) in a greenhouse (20 °C, light phase 18h). Only material of the first four laboratory bred populations was recorded in order to reduce the chance of loss of variation during continued rearing or due to inbreeding in the diploids (den Bieman, 1986).

Recording technique

The technique used for the registration of substrate-borne acoustic signals of planthoppers was described by de Vrijer (1984). Females were isolated as fifth instars and recorded as adults of 7-10 days. All recordings were made between 19 and 20 °C (den Bieman, 1986).

Most Ribautodelphax females rarely produced spontaneous calls, but they readily responded to playback with recorded conspecific male signals. Like other female planthoppers (Booij, 1982; Claridge, 1985a, b; de Vrijer, 1984, 1986), Ribautodelphax females emitted calls composed of a series of clicks (den Bieman, 1986); each separated series is called a 'strophe'. The majority of Ribautodelphax females responded with only one strophe to a male signal, but several females of R. pungens and Taxon 4 emitted a number of short strophes after one playback stimulus (e.g. Fig. 1, I).

Female calling was induced with a male signal from the same population to which the females belonged. Females of two R. pungens populations from which no males were available, YU3 and GR1, were stimulated with sounds of the Dutch population NL4. Most females readily responded to the first playback stimulus. The next playback signal was not started until five seconds after a female stopped its response calling. Females remaining silent after playback were stimulated again after thirty seconds.

Determination of the end of a female response was difficult for some females that frequently started spontaneous calling and that emitted a number of short strophes in answer to one playback stimulus. For these females, the interval between the end of a female strophe and the beginning of the next playback signal was often only 3 to 4 s. The first strophe emitted after a playback signal was taken as the calling strophe of these females. Several observations of pairs of males and females showed that many females emitted only one strophe in between two male signals, though longer series of strophes were also observed.

Signal analysis

Female signals induced by playback stimuli were considered female calling signals (den Bieman, 1986; de Vrijer, 1984), though these were originally defined as spontaneously produced signals by single females (Booij, 1982a).

Table I. Ribautodelphax populations used for acoustic studies. Karyology of field populations: 2N+3N, both diploid and triploid females present; 2N, diploid females only; ?, no test; * R. pungens associated with 3N of Taxon 4.

Code[a]	Locality	Latitude (^{o}N)	Longitude (^{o}E)	Karyology
	R. PUNGENS (RIBAUT)			
NL4	Limburg, Bemelen.	50.50	5.45	2N+3N
NL5	Limburg, Kunderberg.	50.53	5.56	2N+3N
BE1	Namur, Jemelle.	50.10	5.16	2N+3N
BE2	Namur, Dinant.	50.16	4.55	2N+3N
BE3	Namur, Nismes.	50.04	4.33	2N+3N
BE4	Luxembourg, Torgny.	49.31	5.28	2N+3N
DE1	Nordrhein-Westfalen, Rodderberg.	50.38	7.12	2N+3N
DE2	Hessen, Amöneberg.	50.48	8.56	2N
DE3	Rheinland-Pfalz, Ebernburg.	49.47	7.47	2N+3N
DE4	Baden-Württemberg, Tübingen.	48.32	9.02	2N+3N
FR1	Ardèche, Coulens.	44.32	4.18	2N+3N
FR2	Alpes de Haute Provence, Montfuron.	43.54	5.41	*
YU2	Slovenija, Postojna.	45.44	14.11	2N
YU3	Bosna-Hercegovina, Jajce.	44.16	17.19	2N+3N
GR1	Trikkala, Meg. Panagia (Pindos Mts.).	39.49	21.20	2N+3N
GR2	Florina, Kotas.	40.42	21.15	2N+3N
GR3	Fokis, Elaion.	38.33	22.25	2N+3N
GR4	Fokis, Skaloula.	38.30	22.15	?
	TAXON 4			
FR2	Alpes de Haute Provence, Montfuron.	43.54	5.41	*
FR6	Vaucluse, St. Estéve.	44.08	5.14	2N+3N
FR7	Vaucluse, Crillon-le-Brave.	44.07	5.08	2N
FR8	Vaucluse, Bedoin.	44.07	5.10	2N
FR9	Bouches du Rhône, Les Baumettes.	43.42	4.53	2N+3N
FR10	Aude, Tuchan.	42.53	2.43	?
ES1	Valencia, Sagunto.	39.42	0.17	?

[a]Belgium (BE), France (FR), Greece (GR), Netherlands (NL), Spain (ES), W. Germany (DE) and Yugoslavia (YU).

Table II. One-way analysis of variance of female calling signals of Ribautodelphax comparing variation among individuals and within individuals. One population analysed for each species and triploid form. F values given, *** P<0.001. Abbreviations as in Table I.

	Population	Duration strophe	CRR start	CRR end
R. pungens-2N	NL4	14.85***	34.17***	41.78***
R. pungens-3N	NL4	235.94***	176.11***	78.19***
Taxon 4-2N	FR9	37.50***	26.99***	18.84***
Taxon 4-3N	FR9	97.96***	33.52***	45.69***

It was not possible to analyse all signals of R. pungens equally, because of their variability. The strophe duration could be measured for all signals, but the click repetition rate (CRR) had to be determined in different ways. For short signals, less than 3 s, the CRR was estimated by measuring the length of five click intervals 0.25 s after the beginning and 0.13 s before the end of a signal. The CRR could not be measured directly at the beginning and end of a signal because of low click amplitudes and irregular click intervals. The CRR's of signals shorter than 0.75 s could not be measured. More irregularities were found at the beginning and end of longer signals; their CRR was calculated by measuring the duration of 10 click intervals 1 s after the beginning and 0.5 s before the end of a signal.

Females were scored as 'spontaneous' singers if they started calling without playback stimulation during the first two minutes after placing a female on the grass stem used for recording. As already described, some females emitted more than one strophe in answer to a single male stimulus and they were indicated as 'multiple-calling females'. The time lag between the start of the male playback signal and the beginning of the female answer is called the 'response-delay period'. It is not known which part of the male signal induced a female to respond. Therefore the beginning of the male signal was arbitrarily chosen as the starting point for the response-delay period.

Ten calling signals of each female were analysed and 10 diploid and 10 triploid females of each population were recorded, unless stated otherwise.

RESULTS

Ribautodelphax pungens

Calling classes

Calling signals of R. pungens 2N and 3N females were highly variable. However, the variation between individuals of one randomly sampled population was higher than the within-individual variation for both 2N and 3N (Table II).

In order to arrange the variation between individuals in a compact way the females were grouped into eight 'calling classes'. The mean strophe
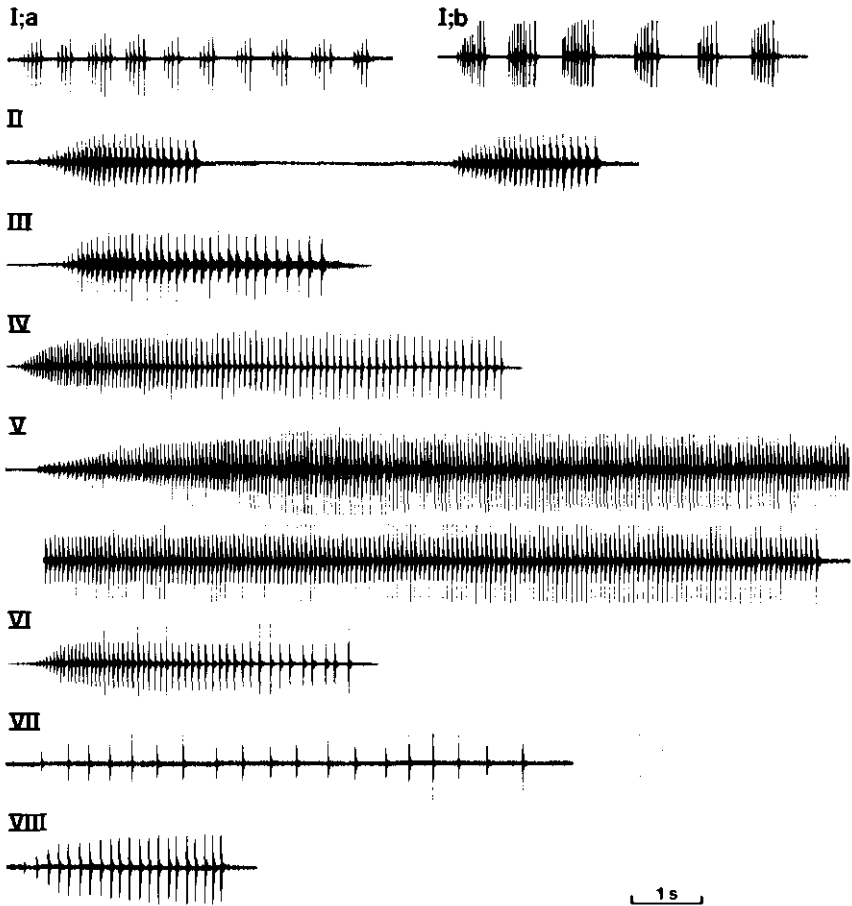
Fig. 1. Variability in calling signals of R. pungens females. Eight calling classes (I-VIII) are given. Codes in brackets represent populations as listed in Table I: Ib (BE1, 3N); Ib (BE3, 3N); II (GR2, 2N); III (GR2, 2N); IV (BE1, 3N); V (BE4, 3N); VI (NL4, 3N); VII (FR, 2N) and VIII (GR1, 3N).

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duration of every individual was used as the grouping criterion for the first five classes. Some females with strongly deviating CRR were grouped into three additional classes. A representative signal of each calling class is presented in Fig. 1, and each class is briefly described.

Class I. Strophe duration of less than 1 s. A highly variable number of strophes, maximally 60 recorded, separated by short intervals were emitted. Each strophe started with a low click amplitude and the CRR at the end was lower than at the beginning (Fig. 1, Ib).

Class II. Strophes with a duration of 1 to 3 s. Often several strophes, 34 maximally found, separated by longer intervals, were produced after one male stimulus.

Class III. Strophe duration of 3 to 5 s with a repetition of one to seven strophes.

Class IV. Strophes lasting from 5 to 15 s, rarely more than one strophe was produced after one single stimulus.

Class V. Strophes of more than 15 s, only one strophe was emitted in response to one male stimulus.

Class VI. CRR at the end of the strophes decreased by a factor of 2.5 to 3.5 compared to the CRR at the beginning. Mean strophe duration between 4 and 6 s.

Class VII. Strophes with an extremely low CRR of 2.7 to 6.5. Mean strophe duration of 3.5 to 6 s.

Class VIII. Strophes with a slight increase in CRR, contrary to the decline in CRR found in signals of all other classes. Mean strophe duration of 3 to 5 s.

As could be expected on the basis of the low within-individual variation, only a few of the signals exceeded the limits of each calling class (Table III). Two out of 250 females, both of population YU2, could not be attributed to one of the eight calling classes, because they produced both very long and short signals. Only a few females of the classes VI to VIII were found, and these data are too limited for comparison with the other classes.

Some remarkable differences were found between several of the first five classes concerning the percentages of spontaneously calling females and of multiple-calling females (Table III). Classes IV and V showed the lowest percentages of both variables. Slight differences in response-delay periods might be due to differences in the duration of the male signals

Table III. Characteristics of female calling classes (I-VIII) of R. pungens and Taxon 4; 2N and 3N are taken together. For details see text.

class	n	A	B	C	D	
R. PUNGENS						
I	83	5.1	72.3	94.0	3.59 ± .37	(62)
II	63	7.8	73.0	77.8	2.68 ± .21	(54)
III	16	15.0	75.0	50.0	3.25 ± .25	(12)
IV	24	7.1	33.3	8.3	9.39 ± 1.00	(22)
V	37	4.3	21.6	0.0	16.57 ± .88	(29)
VI	5	0	-	80.0	6.61 ± .17	(4)
VII	10	0	30.0	40.0	1.93 ± .21	(10)
VIII	10	0	60.0	60.0	3.37 ± .09	(10)
TAXON 4						
I	44	3.6	22.7	86.4	.41 ± .01	(43)
II	16	3.8	6.3	56.3	.67 ± .04	(16)
III	10	21.0	30.0	0.0	.85 ± .05	(10)
IV	9	5.6	0.0	0.0	2.17 ± .13	(9)
V	1	10.0	0.0	0.0	2.91	(1)

n. Number of females
A. Percentage of strophes falling outside limits of calling class. Ten strophes were recorded for each female.
B. Percentage of individuals calling spontaneously.
C. Percentage of multiple-calling females.
D. Response-delay period, mean and standard error given, in brackets number of females for which the response-delay period was measured.

used for playback, if females used some part other than the beginning of the male signal as a trigger for answering. The duration of the male signals used ranged from 0.68 to 1.15 s. These differences in male signals could not account for the differences in response-delay periods between the classes IV and V and the other classes (Table III). The long response-delay periods and the low frequency of spontaneous callings of classes IV and V will limit the 'advertising time' of a female and their long signal duration might give some compensation.

Diploid and triploid females of the same calling classes showed no significant differences in the percentages of spontaneous and multiple-calling females or in the duration of the response-delay periods.

Table IV. Number of females belonging to the various calling classes of <u>R. pungens</u> and Taxon 4. I-VIII: calling classes. Abbreviations as in Table I.

Population + generation	I	II	III	IV	V	VI	VII	I	II	III	IV	V	VI	VIII
R.PUNGENS	DIPLOIDS							TRIPLOIDS						
NL4; F3	7	3	.	.	.	.	.	.	6	.	.	.	4	.
NL5; F4	8	2	.	.	.	.	.	.	.	.	.	.	.	.
BE1; F1	5	5	.	.	.	.	.	2	.	.	6	2	.	.
BE2; F3	.	.	.	.	.	.	.	10	.	.	.	.	.	.
BE3; F2	3	.	.	.	.	.	.	10	.	.	.	.	.	.
BE4; F3	.	.	.	.	10	.	.	.	.	.	.	10	.	.
DE1; F3	2	.	.	4	1	.	.	2	.	.	8	.	.	.
DE2; F1	20	.	.	.	.	.	.	.	.	.	.	.	.	.
DE3; F3	9	1	.	.	.	.	.	1	9	.	.	.	.	.
DE4; F3	.	2	7	1	.	.	.	.	7	3	.	.	.	.
FR1; F2	.	.	.	.	.	.	10	.	.	.	.	.	.	.
FR2; F2	.	8	1	.	.	1	.	.	.	.	.	.	.	.
YU2; F1	.	.	1	2	5	.	.	.	.	.	.	.	.	.
YU3; F2	.	.	.	.	.	.	.	.	.	.	1	9	.	.
GR1; F2	.	.	.	.	.	.	.	.	.	.	.	.	.	10
GR2; F2	.	8	2	.	.	.	.	.	.	.	.	.	.	.
GR3; F2	4	6	.	.	.	.	.	.	.	.	.	.	.	.
GR4; F4	.	6	2	2	.	.	.	.	.	.	.	.	.	.
TAXON 4	DIPLOIDS							TRIPOIDS						
FR2; F2	.	.	.	.	.	.	.	.	.	10	.	.	.	.
FR6; F1	10	.	.	.	.	.	.	.	.	.	.	.	.	.
FR7; F2	9	1	.	.	.	.	.	.	.	.	.	.	.	.
FR8; F2	10	.	.	.	.	.	.	.	.	.	.	.	.	.
FR9; F3	1	9	.	.	.	.	.	9	1	.	.	.	.	.
FR10; F2	.	.	.	9	1	.	.	.	.	.	.	.	.	.
ES1; F3	5	5	.	.	.	.	.	.	.	.	.	.	.	.

Variability within populations

The presence of the various calling classes in every <u>R. pungens</u> population is indicated in Table IV. Although only a small number of females was studied from each population, all variation present in the laboratory populations was probably represented because additional recordings of several laboratory populations revealed no additional variation. One should note that chance effects in rearing and sampling of the ten females used for recording might have influenced the ratio between the number of females of the different calling classes within each population.

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As discussed for male signals (den Bieman, 1986), part of the acoustic variation present in the field populations could have been missed because each rearing started with only 5-20 females and males; on the other hand, many field populations are actually very small. Two populations (DE2 and BE2) were sampled twice in the field. Both samples of population DE2 belonged to class I (Table IV) but the second sample of population BE2 showed that 3N of class V were present while these were not represented in the first sample.

All recorded females of several populations belonged to a single calling class, while two or three classes were found in others (Table IV). No variation was found in one population with only 2N (DE2), and in five populations with both 2N and 3N (BE2, BE3, BE4, FR1 and GR1). These data indicate that this type of variability also occurred in populations without triploids. Comparison of the variation in populations of different geographic areas showed no specific geographic variation pattern.

Differences between 2N and 3N of the same population

Calling signals of both 2N and 3N females of seven populations were analysed (Table IV). Two populations, BE3 and BE4, showed no variation in female calling signals, and both 2N and 3N emitted similar signals. Calling signals of diploid females of three populations, DE1, DE3 and DE4, were more variable than the signals of the triploid females of the same populations, while the reverse was found in population NL4 and BE1. This limited comparison showed that 3N produced the same type of signals as the 2N females of the same population or of populations in the same geographic area. The only exception was class VI of population NL4.

Few females of the classes VI to VIII were found, and moreover class VII contained only 2N, and class VIII only 3N. Therefore these three classes were not analysed further.

No significant differences in CRR were found between the classes I and II and between the classes III, IV and V of the same population and these classes were combined into two groups. The CRR of these two groups was measured in different ways and should be analysed separately. Significant differences in CRR were found between a number of populations (Table V), but no geographic trend was evident. Comparison of the CRR's of 2N and 3N of the same populations revealed a remarkable phenomenon. For every

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Table V. Statistical analysis of CRR of female signals of R. pungens with Duncan's multiple range test. Vertical lines link populations with maximum non-significant differences of means ($\overline{X}$) at P=0.05. For details see text and for abbreviations, Table I.

A. Females of classes I and II

CRR start Population	$\overline{X}$	CRR end Population	$\overline{X}$
GR4-2N	11.1	GR4-2N	9.6
NL4-3N	11.8	NL4-3N	10.0
BE1-2N	13.3	BE1-2N	12.5
NL4-2N	14.3	NL4-2N	13.1
GR2-2N	19.1	GR2-2N	13.4
DE4-2N	22.4	DE4-2N	19.2
DE4-3N	22.6	NL5-2N	19.3
NL5-2N	24.5	DE4-3N	21.1
BE2-3N	28.1	BE2-2N	22.5
BE3-3N	30.1	FR2-2N	23.8
BE3-2N	30.6	BE3-2N	24.4
FR2-2N	31.7	BE3-3N	24.5
DE3-3N	35.0	DE3-3N	29.2
DE3-2N	38.3	DE3-2N	31.2

B. Females of classes III-V

CRR start Population	$\overline{X}$	CRR end Population	$\overline{X}$
DE1-3N	10.4	YU3-3N	5.8
GR4-2N	11.1	DE1-3N	6.8
YU3-3N	11.7	GR4-2N	8.0
DE1-2N	15.5	DE1-2N	8.3
GR2-2N	17.6	GR2-2N	9.4
BE1-3N	17.6	BE1-3N	11.1
YU2-2N	19.3	YU2-2N	12.4
BE4-3N	20.2	BE4-3N	13.9
DE4-2N	23.1	BE4-2N	14.9
BE4-2N	24.1	DE4-2N	20.3
DE4-3N	28.0	DE4-3N	25.6

population, the CRR of 2N and 3N at the beginning and end of a signal belonged to the same sub-unit of sample means. Only in population DE4 was a significant difference found between 2N and 3N in CRR at the end of the signal (Table VB), but their sample means were still close to one another.

Taxon 4

Taxon 4 from SW.Europe could be studied less extensively than R. pungens. Many data on Taxon 4 supported the results of R. pungens.

Female calling signals of 2N and 3N of Taxon 4 showed a considerable variation. The within-individual variation of both 2N and 3N females was, however, small compared to the variability between individuals (Table II).

The classification of calling signals used for R. pungens was also appropriate for Taxon 4, but only the first five classes were represented.
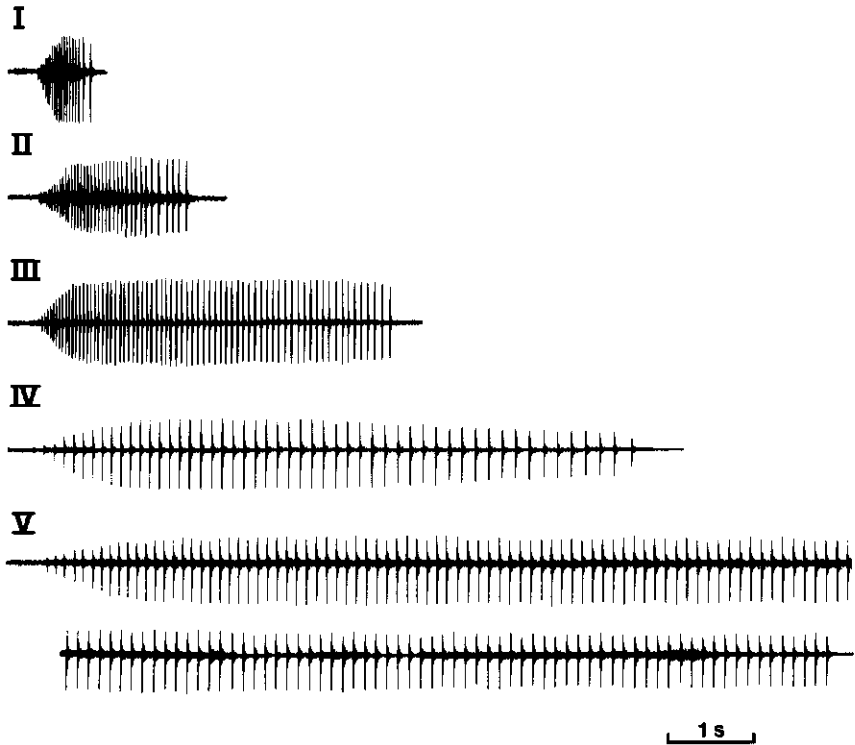
Fig. 2. Variability in calling signals of Taxon 4 females. Five calling classes (I-V) are given. Codes in brackets represent populations as listed in Table I; I (FR8, 2N); II (FR2, 3N); II (FR2, 3N); IV (FR10, 2N) and V (FR10, 2N).

Table VI. Statistical analysis of CRR of female signals of Taxon 4 with Ducan's multiple range test. For statistics see Table V and for abbreviations, Table I.

	CRR start		CRR end	
	Population	$\bar{X}$	Population	$\bar{X}$
	FR10-2N	8.6]	FR10-2N	7.2]
	ES1 -2N	12.4]	ES1 -2N	10.3]
	FR2 -3N	26.0	FR2 -3N	13.0]
	FR9 -2N	27.7]]	FR9 -2N	16.3]
	FR9 -3N	29.6]]	FR6 -2N	23.3
	FR6 -2N	31.6]	FR9 -3N	23.5
	FR7 -2N	32.3]	FR7 -2N	23.7]
	FR8 -2N	38.5]	FR8 -2N	28.6]

A representative signal of every calling class is presented in Fig. 2. As in R. pungens, only a limited number of calling signals of Taxon 4 fell outside the range of each class (Table III). Compared to R. pungens, few females of Taxon 4 started spontaneous calling (Table III). Multiple-calling females were only found in the first two classes and were lacking in the others (Table III). The classes IV and V showed the longest response-delay periods, but the male signal used for playback of these classes was longer (1.19 s) than the ones used for the other classes (ranging from 0.32 to 0.69 s).

All females of three populations belonged to one calling class (Table IV), while two classes were represented in others. Diploid and triploid females of population FR9 belonged to the same two classes.

Females of Taxon 4 produced very regular signals and all signals could be measured in a similar way as the short signals of R. pungens. Significant differences in CRR were found between many populations (Table VI). Diploids and triploids of population FR9 showed no difference in CRR at the beginning of the signal, but did show differences at the end.

Analysis of male signals (den Bieman, 1986) raised doubts as to the taxonomic status of population FR10; it might represent an unknown Ribautodelphax species. Clear differences in female signals between population FR10 and the other populations seem to support this conclusion. However, these differences are not exceptional in view of the differences between the other populations of Taxon 4. Moreover, female signals of R. pungens, the only other Ribautodelphax species that is associated with 3N, were also highly variable. Consequently, conclusions as to the taxonomic status of populations can not be based on interpopulation differences in female signals of these two species.

Taxon 4, 3N in association with R. pungens

R. pungens males served as sperm donors for 3N of Taxon 4 in population FR2. These triploid females responded less readily to playback with male signals of R. pungens, population FR2, than to those of Taxon 4, population FR6. The response-delay period showed significant differences, with 0.85 ± 0.05 s when stimulated with males of Taxon 4 and 1.98 ± 0.35 s after stimulation with R. pungens; the duration of the male signal was 0.69 and 0.87 s, respectively ($U_{(10,6)} = 60$, $P < 0.001$; Mann-Whitney test);

differences in other variables were not significant.

The association found in population FR2 suggests that triploid females may use different Ribautodelphax species as sperm donors. Despite extensive searching, no associations between 3N and other Ribautodelphax species were found. In the planthopper genus Muellerianella comparable situations occurred. Muellerianella triploids were often associated with M. fairmairei (Perris), but at several localities these triploids lived together with another species, M. brevipennis (Boheman), as sperm donor (Booij, 1982b). Unfortunately, the acoustic signals of these associations have not been studied.

DISCUSSION

Calling signals of 2N and 3N females of R. pungens and Taxon 4 showed a wide variability within as well as between populations. This variability was much wider than the variability found in calling signals of females of nine other Ribautodelphax species which were not associated with pseudogamous 3N (den Bieman 1986). These differences between species with and without 3N offer important information for a discussion on the origin of the 3N and on the coexistence of biparental species and pseudogamous forms. Because more information is available on R. pungens attention will be focused on this species.

The discussion has to remain partly speculative because information on two important aspects is not yet available. Firstly, not much is known of the genetic basis of female songs in planthoppers. Analyses of the acoustic signals of hybrids of the planthopper genus Nilaparvata suggest a polygenic system of inheritance of female songs (Claridge et al., 1985), however, other genera have not yet been studied. Secondly, one may wonder whether the differences found in female signals, CRR and strophe duration, are indeed important for intraspecific communication. For the following discussion this positive assumption is made, but further experiments are needed to investigate this aspect.

Origin of triploid females

Many pseudogamous animals are known to be polyploids, usually triploids, and pseudogamous fishes (Schultz, 1980), salamanders (Uzzell,

1964; Uzzell & Goldblatt, 1967) and Muellerianella planthoppers (Drosopoulos, 1978) are said to be of hybrid, allopolyploid origin. An autopolyploid origin of polyploid animals is less often assumed (Lokki & Saura, 1980; Schultz, 1980).

As described before, comparisons between pseudogamous triploids and related diploid species might give clues to the origin of the 3N. Unfortunately, the high variability of the female signals of R. pungens, Taxon 4 and their triploids renders a comparison with the other Ribautodelphax species problematic. Comparison of the signals of 2N and 3N, however, offers information on the origin of triploids. The variability in calling signals of the triploids suggests that triploids evolved not once but on several occasions, because parthenogenetic animals such as these pseudogamous 3N probably will not change rapidly. Calling signals of 2N and 3N of the same R. pungens populations resembled one another in CRR, and all calling classes found in the 3N occurred also in the 2N of the same population or in populations from the same geographic area. The only exception, class VI of population NL4, might be attributable to the small field sample taken. These similarities suggest that the 3N probably evolved separately in every population from the local bisexual R. pungens.

Despite extensive searching, only a single Ribautodelphax species, i.e. R. pungens, was found at each of the seven localities from which both 2N and 3N were studied. The occurrence of other Ribautodelphax species at these localities in the past can never be excluded, but seems very unlikely since three of these populations were sampled from road sides and were probably recently established. This information, together with the close similarity in calling signals of both syntopic female forms, makes an allopolyploid origin of the 3N less probable.

Taxon 4 was studied less extensively, and for only one population could 2N be compared with 3N. These data do not contradict the conclusions for R. pungens 2N and 3N.

Coexistence of 2N and 3N

Pseudogamous triploid females depend on the sperm of a bisexual male to initiate reproduction. If a male is not present in a population the triploids cannot reproduce. Instability of mixed populations of pseudogamous forms and bisexual species might result from the twofold

reproductive advantage of the pseudogamous triploids, because they produce female offspring only. This advantage is only realized if differences in other reproductive factors are absent, such as the number of eggs produced or the likelyhood of being inseminated. In two well-studied groups, Poeciliopsis fishes (Moore, 1976; McKay, 1971) and Amystoma salamanders (Uzzell, 1964, 1969), mate discrimination proved to be an important mechanism to stabilize populations of sexual and pseudogamous forms (see also Stenseth et al., 1985). Field tests with Muellerianella planthoppers indicated differential insemination of the two female forms. However, no mate discrimination by males was found in some choice experiments (Booij & Guldemond, 1984).

Acoustic signals, and especially the calling signals produced during the first step of sexual communication, are important in pair formation in planthoppers (Claridge, 1985a, b; de Vrijer, 1984). The variability in female calling signals of R. pungens and Taxon 4 offers males an opportunity for mate discrimination. Mate choice experiments will demonstrate whether mate discrimination indeed functions as a mechanism to stabilize mixed populations of pseudogamous and bisexual forms in Ribautodelphax.

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HYBRIDIZATION STUDIES IN THE PLANTHOPPER GENUS RIBAUTODELPHAX (HOMOPTERA, DELPHACIDAE).

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ABSTRACT

Seven known Ribautodelphax species and four recently discovered taxa were crossed. Forty-three percent of the interspecific crosses were successful. Interspecific crosses generally resulted in low numbers of hybrids. In some combinations prolonged larval development of the hybrid males was observed. Backcrosses showed that both male and female hybrids were fertile. Reproductive isolation was mainly maintained by premating barriers. Insemination tests revealed that prolonged confinement of males and females lowered mating thresholds. The taxonomic status of the known species and new taxa was shown to be in need of revision. No indications for a hybrid origin of the pseudogamous triploid Ribautodelphax females were found.

Key words: biosystematics, Delphacidae, hybridization, planthoppers, reproductive isolation, Ribautodelphax.

INTRODUCTION

Several planthoppers (Delphacidae) of the Ribautodelphax collinus complex show slight and to some extent variable morphological differences raising doubt as to their taxonomic status. In Europe nine Ribautodelphax species occur (Nast, 1972; Asche et al., 1986) of which five belong to the R. collinus complex. Recently, several new populations of this complex were discovered; they are provisionally indicated as 'Taxon 1 to 4'. Studies on host-plant relations and acoustic signals have shown differences among the

seven Ribautodelphax species and four new Taxa studied (den Bieman, 1986, 1987a, b).

Hybridization studies may be helpful to clear the taxonomic status of the known species and of the new Taxa. However, the limited value of hybridization studies for the solution of taxonomic problems has been generally recognized (e.g. Rosen, 1979; Ross, 1974; Wiley, 1981), because the results may be strongly dependent on experimental conditions. Nevertheless, crossing experiments and mate-choice tests may reveal the existence and efficiency of behavioral barriers to mating. Moreover, hybridization experiments offer the only possible way to investigate post-mating barriers. Post-mating barriers were strongly developed in the planthopper genera Javesella (Strübing & Hasse, 1975; de Vrijer, 1981), and Muellerianella (Booij, 1982a; Drosopoulos, 1977), while hybrids in Nilaparvata were fully fertile (Claridge et al., 1985). Experiments revealed pre-mating barriers in all three genera.

There is, however, an additional reason to study hybridization in Ribautodelphax. In populations of R. pungens (Ribaut) and of Taxon 4, pseudogamous triploid females occur. These triploid females depend on the bisexual diploid species, because their triploid eggs need to be activated by sperm, though their all-female triploid offspring receive maternal genes only. Hybridization tests are needed to infer the mode of origin of these triploids. A hybrid origin is suggested for many polyploid populations (Bullini, 1985; White 1973, 1978) including the triploid forms in populations of the planthopper Muellerianella fairmairei (Perris) (Drosopoulos, 1976).

MATERIAL AND METHODS

For convenience, all new Taxa and known Ribautodelphax species are provisionally referred to as species in this paper. Their taxonomic treatment is in progress and will conclude a series of papers on biological differentiation within the R. collinus complex. Triploid females are indicated by the name of their sperm-donor species.

In addition to the nine species of the R. collinus complex, two outgroup species, R. pallens and R. albostriatus, were studied (Table 1). For R. pungens, population NL4 was used for most experiments unless otherwise stated.

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Table 1. Origin of <u>Ribautodelphax</u> populations used for hybridization tests.
A, number of offspring produced in cages with ten pairs of males and females
B, larval developmental period in days. Mean and S.E. given; in brackets
number of cages observed.

Ribautodelphax	Code[a]	Locality	A	B
		R. COLLINUS COMPLEX		
collinus	NL1	Limburg, Plasmolen.	814+120 (10)	30.8+0.9 (6)
(Boheman)				
angulosus	NL3	Utrecht, Leersum.	738+100 (10)	29.8+0.8 (6)
(Ribaut)				
pungens	NL4	Limburg, Bemelen, $2N^b$.	649+58 (10)	32.6+1.0 (5)
(Ribaut)	NL4	Limburg, Bemelen, $3N^b$.	796+91 (10)	32.4+1.3 (5)
	DE2	Hessen, Amöneburg.		
	GR3	Fokis, Elaion.		
	GR4	Fokis, Skaloula.		
imitans	FR3	Pyrenées Orientales,	695+51 (10)	30.7+0.8 (5)
(Ribaut)		St. Cyprien.		
fanari	FR4	Bouches du Rhône, Etang.	953+102 (10)	36.5+1.3 (4)
Asche, Drosopoulos		de Vaccarès.		
& Hoch				
Taxon 1	NL4	Gelderland, Hoge Veluwe	744+82 (10)	29.5+0.8 (5)
Taxon 2	GR6	Florina, Kalo Nero Mts	407+98 (10)	32.0+2.6 (3)
Taxon 3	FR5	Vaucluse, Mt. Ventoux		
Taxon 4	FR9	Bouches du Rhône,		
		les Baumettes, $2N^b$.	645+70 (10)	35.8+0.7 (6)
	FR9	Bouches du Rhône,		
		les Baumettes, $3N^b$.	693+67 (7)	37.5+1.0 (6)
		OTHER RIBAUTODELPHAX SPECIES		
pallens	NO1	Sør-Trødelag, Dovrefjell.	592+102 (6)	34.5+1.5 (2)
(Stål)				
albostriatus	NL7	Limburg, Wrakelberg	991+190 (4)	24.0+0.0 (2)
(Fieber)				

[a]France (FR), Greece (GR), Netherlands (NL), Norway (NO) and W. Germany (DE).
[b]2N and 3N, diploid and triploid females, respectively.

    Stock cultures of planthoppers were kept on their specific host plants
(den Bieman, 1987a) in a greenhouse (20 °C; light phase 18h) and
experiments were carried out under similar conditons. Chances of inbreeding
were reduced by exchange of males between parallel rearings at each
generation. Diapause problems hampered mass rearing of Taxon 3, therefore
this species was not available for most experiments. The other species
could be reared without any problem.

    Hybridization tests were carried out in cages with the host plants of
the two species to be tested. Males and females were separated as fifth-
instars and experiments were started with adults of age 7 to 9 days. Males
and females became sexually mature between 5 and 7 days after emergence.

Ten pairs of males and females were confined in one cage for 30 days. These cages were inspected daily for hatched larvae and new adults. The period between hatching of the first larva and emergence of the first adult was taken as a measure of the time for larval development. About two weeks after the emergence of the first adult, the number of offspring was counted. When available, the sex ratio was assessed on basis of a sample of at least one hunderd adults and last instars. Each hybridization experiment was replicated once. Backcrosses and crosses between F1's and F2's were conducted in a similar way but were not replicated.

Insemination tests were made in the same way as the hybridization experiments. Insemination was tested by examination of the spermatheca in physiological saline. It was observed that sperm remained active 35 days after insemination. Fertility of hybrid and backcross males was deduced from the development of the testes and the presence of mobile sperm. At least 10 males were examined for each successful combination.

To study the karyotypes of hybrid and backcross females chromosome preparations were made of ovarian eggs. The chorion of mature eggs was removed after a 30-minute treatment with 50% propionic acid and the eggs were stained overnight with lacto-acetic orcein. A detailed description of this method is given by Booij (1982b). Squash preparations of testes were made after fixation in Carnoy (6:3:1) and were stained with 0.4% cristal violet (Gut, 1976).

RESULTS

Interspecific hybridization

Many of the Ribautodelphax species hybridized successfully in no-choice situations (Table 2). Four variables were assessed: the percentage of inseminated females, the larval developmental time, the number of offspring and their sex ratio. To enable comparison of different combinations of species, two variables, number of offspring and larval developmental time, were defined relative to the mean values for the two parent species (Table 1). The differences in larval developmental time between the hybrids and their parents were usually less than five days and only stronger deviations are indicated (Table 2). Insemination percentages

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Table 2. Results of interspecific crosses between Ribautodelphax species. The values of the number of offspring have been defined using the mean values of homogamic crosses of both parent species as standard. A, number of offspring: 0, no offspring; 1, ≤ 10%; 2, 10-20%; 3, 20-40%; 4, 40-70% and 5, >70%. B, percentage of insemination:*, less than 70% of females recollected; 0, no insemination; 1, ≤ 10% 2, 10-20%; 3, 20-40%; 4, 40-70% and 5, >65%. C, sex ratio: % of females given: 1, 45-55%; 2, 35-45%; 3,<35%; 4, 55-65% and 5, >65%.

MALES

FEMALES	col			ang			pun			imi			fan			T 1			T 2			T 4			pal			alb		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
collinus	-	-	-	1	*	1	1	*	1a	2	*	1	2	1	1	1	*	4	1	1	3b	0	*	-	0	0	-	0	0	-
angulosus	1	1	1	-	-	-	0	0	-a	1	*	1	2	*	4	0	0	-	0	*	-	0	0	-	0	0	-	0	*	-
pungens, 2N	2	1	1a	3	*	1a	-	-	-	3	*	1	3	2	4	0	0	-a	0	*	-	3	*	1	2	1	1	0	*	-
pungens, 3N	3	2		2	*		-	-	-	3	3		3	2	1	0	0	-				4	5		0	0	-	0	0	-
imitans	2	*	1	1	*	1	1	1	1	-	-	-	0	0	-	1	*	5b	0	0	-b	5	5	1	0	*	-	0	0	-
fanari	5	4	1	2	2	1	3	1	1	0	0	-	-	-	-	0	0	-	1	1	1b	2	2	1	0	*	-	0	0	-
Taxon 1	1	*	1	1	*	5b	0	0	-a	3	3	1	1	1	2	-	-	-	0	*	-	1	*	1	0	0	-	0	*	-
Taxon 2	1	*	4	0	0	-	0	0	-	1	1	5bc	0	0	-	0	0	-	-	-	-	0	0	-	0	*	-	0	0	-
Taxon 4, 2N	2	1	4de	1	*	4d	3	*	4de	4	5	1	2	1	4de	0	0	-	2	1	4d	-	-	-	0	0	-	0	0	-
Taxon 4, 3N	2	1		2	1		4	4		5	5		1	1		0	0	-	0	0		-	-	-	0	0	-	0	0	-
pallens	0	*	-	1	*	3bf	2	*	4	0	0	-	0	1	-	0	*	-	0	*	-	0	0	-	-	-	-	0	0	-
albostriatus	0	0	-	0	*	-	0	*	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-	-	-	-

a Four cages with ten pairs of males and females.
b Less than 50 adults and last instars.
c Hybrid males sterile, six males available.
d Larval developmental time of males and females different.
e Larval developmental time of females 7 to 8 days shorter than in parent species.
f Larval developmental time of females 35 days longer than in parent species.

	MALES							
FEMALES	col		ang		pun		T 1	
collinus	98		15	(35)	5	(10)	0	(5)
angulosus	0	(10)	100		0	(0)	0	(0)
pungens, 2N	5	(5)	10	(10)	95		0	(0)
pungens, 3N	8	(15)	10	(15)	95		0	(0)
Taxon 1	5	(32[b])	3[a]	(11[b])	0	(5)	95	

[a] Only 19, and [b] 39 females recollected.

of cages in which less than 70% of the females could be re-collected were discarded.

Fourty-three percent of the interspecific crosses resulted in production of offspring, but the numbers of offspring were low compared to the results of homogamic crosses. However, many hybrids were produced between R. imitans and Taxon 4 and in crosses between R. fanari (♀♀) and R. collinus. This last species hybridized successfully in both directions with all other species of the R. collinus complex, except with females of Taxon 4. Only five of the eighteen crosses involving Taxon 2 produced offspring; the hybridization success of the other species of the R. collinus complex varied between these extremes.

The fraction of inseminated females in interspecific crosses was usually low even after 30 days of confinement, while homogamic crosses showed 90 to 100% insemination after only 3 days. The close relation between the numbers of hybrids produced and the insemination percentages (Table 2) suggests that reduced mating success might be the main cause of the low numbers of offspring produced. Significant larval mortality was never observed, and the correlation between the number of inseminated females and the number of offspring indicates that the mortality of hybrid eggs and larvae was not higher than in homogamic crosses. In one combination, R. pallens (♀♀) with R. fanari, one inseminated female was found, but no hybrids were produced. Possibly this female was inseminated just before the adults were re-collected, or the hybrids died in the embryonal stage.

In several crosses the first larva hatched after a long period and one explanation might be that heterospecific mating partners were only accepted after prolonged confinement with no choice of mates. Insemination experiments with four Ribautodelphax species from the Netherlands confirmed this suggestion (Table 3). Homogamic crosses showed high insemination levels after 3 days in contrast to heterogamic crosses. Prolonged confinement increased the insemination percentages in most combinations, even in some combinations where no insemination was found in the first three days. Significant differences between the results of the hybridization and insemination tests were only observed in one combination. One inseminated female was detected in a cross between Taxon 1 (♀♀) and R. pungens (Table 3), but inseminated females of this combination were never found in the hybridization tests (Table 2).

Striking differences between reciprocal combinations were observed in the insemination and hybridization tests, suggesting that conspecific males and females showed different thresholds to interspecific mating.

In a number of crosses with females of Taxon 4, marked differences were noted between the larval developmental periods of males and females (Table 2). Normally, the first male emerged one or two days before the first female in homogamic and heterogamic crosses. However, in crosses between Taxon 4 (♀♀) and R. collinus the first male appeared 26 days after the first female. These differences were 21 days in crosses with males of R. angulosus, 15 days with R. pungens and R. fanari and even 134 days in crosses between Taxon 4 (♀♀) and Taxon 2. The larval developmental periods of female hybrids of three of these combinations were shorter than those of their parents (Table 2). Differential larval development was never observed in crosses between Taxon 4 (♀♀) and R. imitans, nor in any cross with males of Taxon 4.

Taxon 3 was only crossed with its morphologically close relative Taxon 2. One out of 10 females of Taxon 2 was inseminated after 30 days of confinement with 3 males of Taxon 3 and 22 female and 16 male hybrids were produced.

Morphologic and acoustic data (den Bieman, 1986, and in prep.) suggest that R. pallens is more similar to the R. collinus complex than R. albostriatus. Hybridization tests indicate a similar relationship (Table 2). No hybrids were produced in crosses with R. albostriatus, while R. pallens successfully hybridized with R. pungens and produced one single

Table 4. Results of hybrid crosses and backcrosses in Ribautodelphax. A, number of offspring; B, percentage of insemination; C, sex ratio. Scales of A, B and C as in Table 2. $B_{1,1}$: backcross with males; $B_{1,2}$: with females of the mother species; $B_{1,3}$: backcross with males; $B_{1,4}$: with females of the father species. Every combination refers to one cage with ten pairs of males and females.

hybrid with R. angulosus.

Nearly equal numbers of males and females were produced in most heterospecific crosses (Table 2). Several combinations showed slightly male or female-biased sex ratios, while strongly biased ratios were only found in some crosses with a low number of offspring . For most hybrid males the testes were fully developed and contained mobile sperm. However, males of the cross between females of Taxon 2 and R. imitans were sterile though early stages of spermatid development were found in squash preparations of their underdeveloped testes.

Males of several species could function as sperm-donors for the two triploid forms (Table 2). The diploid and triploid females of R. pungens were largely similar in their mating relations to other Ribautodelphax species. The same accounts for the diploids and triploids of Taxon 4. However, the two triploid forms showed differences, e.g. 100% of the triploids of Taxon 4 were inseminated by males of R. imitans compared to only 35% of the triploids of R. pungens.

Hybrid crosses and backcrosses.

All backcrosses and crosses between F1's were successful (Table 4). The number of offspring in the majority of these 108 crosses was at least equal to the number of offspring in the first hybrid generation. In several combinations, e.g. R. pungens (♀♀) with Taxon 4, Taxon 4 (♀♀) with R. collinus and R. imitans with Taxon 4, the number of offspring equaled the production in homogamic crosses, indicating that the fertility of the hybrids was at normal levels.
The number of offspring of seven crosses between F2's was at least equal to the results of the corresponding crosses between F1's (Table 4) and no sign of hybrid breakdown was noted. One cross between F2's of the combination R. pungens (♀♀) with R. imitans produced less offspring than the cross between the F1's. The low numbers of offspring of several crosses correspond to a

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PARENTS OF HYBRIDS	F1xF1			$B_{1,1}$			$B_{1,2}$			$B_{1,3}$			$B_{1,4}$			F2xF2		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
collinus ♀♀ x																		
<u>angulosus</u>	4	4	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<u>pungens</u>	3	*	1[a]	4	5	1	.	.	.	.	.	.	.	.	.	.	.	.
<u>imitans</u>	2	*	1	.	.	.	.	.	.	.	.	.	.	.	.	2	*	1
<u>fanari</u>	3	*	1	4	*	5[b]	3	*	1	4	*	3[b]	5	5	1[b]	.	.	.
Taxon 1	2	*	1	4	4	5[b]	4	5	1	1	1	1	3	3	1	4	*	4
Taxon 2	3	4	1	.	.	.	.	.	.	.	.	.	4	*	1	.	.	.
angulosus ♀♀ x																		
<u>collinus</u>	1	*	1	.	.	.	5	5	1	4	5	1	4	5	1	.	.	.
<u>imitans</u>	2	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<u>fanari</u>	1	*	5[b]	4	*	1	.	.	.	4	5	1	.	.	.	.	.	.
pungens ♀♀ x																		
<u>collinus</u>	2	*	1[a]	4	5	1[a]	5	5	1	4	*	1	5	4	1	.	.	.
<u>angulosus</u>	3	2	1[a]	4	4	1[a]	4	*	4[a,c]	4	*	1[a]	5	4	1[a]	3	*	1
<u>imitans</u>	4	5	1	.	.	.	.	.	.	.	.	.	.	.	.	1	*	1
<u>fanari</u>	3	*	1	5	*	1	5	*	1	4	*	1[b]	4	*	1[b]	.	.	.
Taxon 4	5	5	1	5	5	1	5	5	1	5	5	1	5	5	1	.	.	.
<u>pallens</u>	3	*	1	3	*	1	.	.	.	.	.	.	.	.	.	.	.	.
imitans ♀♀ x																		
<u>collinus</u>	2	3	1	.	.	.	.	.	.	.	.	.	.	.	.	3	3	1
<u>angulosus</u>	3	*	1	.	.	.	.	.	.	.	.	.	.	.	.	3	3	1
<u>pungens</u>	3	*	1	3	*	1	1	*	1	.	.	.	.	.	.	.	.	.
Taxon 4	5	5	1[a]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
fanari ♀♀ x																		
<u>collinus</u>	3	*	1	.	.	.	5	*	1[b]	.	.	.	.	.	.	.	.	.
<u>angulosus</u>	3	4	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<u>pungens</u>	1	*	1	2	*	1	5	5	1	5	5	1	5	5	1	.	.	.
Taxon 4	3	5	1	3	5	4	3	5	1	4	5	1	3	4	1	.	.	.
Taxon 1 ♀♀ x																		
<u>collinus</u>	5	5	1	5	5	1	5	5	1	4	5	2	5	4	1	5	*	1
<u>angulosus</u>	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<u>imitans</u>	3	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<u>fanari</u>	1	1	1	3	3	1	1	2	1	.	.	.	1	*	3[b]	.	.	.
Taxon 4	3	2	1	3	4	1	4	4	1	3	4	1	3	2	4	.	.	.
Taxon 2 ♀♀ x																		
<u>collinus</u>	1	1	2[b]	3	*	1	4	*	2	5	5	1	4	*	1	.	.	.
<u>imitans</u>	.	.	.	5	*	4[d]	.	.	.	.	.	.	.	.	.	.	.	.
Taxon 3	3	5	1	5	5	1[d]	.	.	.	.	.	.	.	.	.	.	.	.
Taxon 4 ♀♀ x																		
<u>collinus</u>	.	.	.	3	4	2	.	.	.	.	.	.	.	.	.	.	.	.
<u>angulosus</u>	4	4	2[d]	3	3	1	.	.	.	3	3	1	.	.	.	.	.	.
<u>pungens</u>	5	5	2	5	4	1	.	.	.	5	5	1	.	.	.	.	.	.
<u>imitans</u>	5	5	1[a]	.	.	.	.	.	.	.	.	.	.	.	.	5	5	1
<u>fanari</u>	4	5	2	4	4	1	4	5	2	4	*	1	3	4	1	.	.	.
Taxon 2	.	.	.	4	5	1	.	.	.	5	5	1	.	.	.	.	.	.
pallens ♀♀ x																		
<u>pungens</u>	4	4	1	1	1	5[a,b]	.	.	.	5	5	1	.	.	.	.	.	.

[a] Two cages with ten pairs of males and females.
[b] Less than 50 adults and last instars.
[c] 20% of males sterile.
[d] Cage with five pairs of males and females only.

Table 5. Meiosis in female F1 hybrids of <u>Ribautodelphax</u>. Given are the number of females with a regular (A) and an abnormal meiosis (B).

	col		ang		pun		imi		fan		T1		T2		T4		pal	
FEMALES	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
<u>collinus</u>	.	.	2	0	9	1[a]	5	0	5	0	10	0	5	0	.	.	.	.
<u>angulosus</u>	5	0	.	.	.	.	10	0	6	0	.	.	.	.	.	.	.	.
<u>pungens</u>	35	9[ab]	9	1[b]	.	.	10	0	10	0	.	.	.	.	10	0	10	0
<u>imitans</u>	9	1[b]	10	0	16	0	.	.	.	.	5	0	.	.	10	0	.	.
<u>fanari</u>	10	0	6	0	14	0	.	.	.	.	.	.	5	0	8	0	.	.
Taxon 1	10	0	.	.	.	.	18	3[b]	5	0	.	.	.	.	5	0	.	.
Taxon 2	10	5[ab]	.	.	.	.	8	2[b]	.	.	.	.	.	.	.	.	.	.
Taxon 4	10	0	7	0	14	1[b]	10	0	9	0	.	.	9	1[b]	.	.	.	.
<u>pallens</u>	.	.	.	.	16	1[c]	.	.	.	.	.	.	.	.	.	.	.	.

[a] Methaphase with chromosomes in two groups.
[b] Methaphase with extra univalents or with trivalents.
[c] Methaphase with 30 univalents, another with 17 chromosomal units.

low fraction of inseminated females (Table 4), suggesting that barriers to mating also contributed to reproductive isolation in the second and third hybrid generation.

Five males of every backcross and cross between F1's and F2's were examined and all showed normally developed testes with mobile sperm. Only two out of ten males of one backcross (Table 4) possessed undeveloped testes without sperm development.

Strongly female-biased sex ratios were not observed in backcrosses or crosses between hybrids apart from some deviations in crosses with few adult offspring (Table 4). Prolonged larval development of males was not noted.

Meiosis in female hybrids.

In <u>Ribautodelphax</u> diploid females usually have a chromosome number of $2n = 30$; however, in field-samples some females occurred with $2n = 31$ or 32 (den Bieman, in prep.). Deviations from these numbers in hybrids were recorded as meiotic disturbances.

Only a few meiotic abnormalities were found in female hybrids (Fig. 1; Table 5: 6.4%). In the eggs of nine hybrids (39 meiotic plates) chromosomes were arranged in two separate groups (Fig. 1c). The exact number of chromosomes in each group could not be counted but is near to 15. For one egg, the meiotic plate even suggested that some chromosomes were in fact
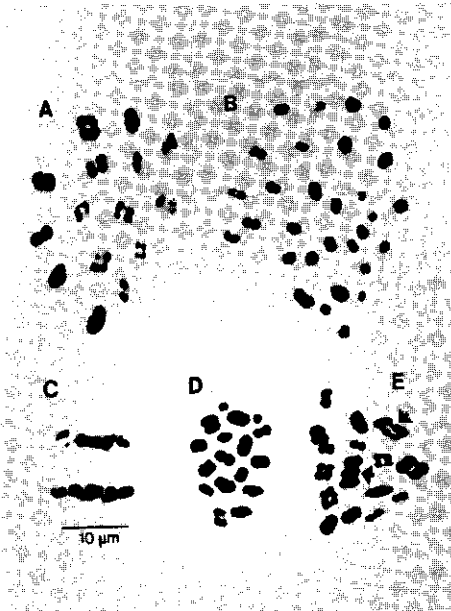
Figure 1. Meiotic configurations in ovarian eggs of Ribautodelphax hybrids (A) Normal meiosis with 15 bivalents, bivalents partly visible, R. pungens (♀♀) x R. pallens; (B) metaphase with 30 univalents, R. pallens (♀♀) x R. pungens; (C) anaphase with two groups of chromosomes, side view, R. pungens (♀♀) x R. collinus; (D) metaphase with additional univalents, Taxon 4 (♀♀) x R. collinus; (E) metaphase with trivalents (indicated by arrows), R. pungens (♀♀) x R. collinus. (1C by A.J. de Winter).

bivalents and consequently this egg could be tetraploid resulting from an endoduplication. Provisionally, however, it is assumed that these bivalents consisted of two neighbouring univalents. Probably these meiotic plates represent anaphases, though this phase was never observed in ovarian eggs in an extensive karyologic analysis of Ribautodelphax (den Bieman, in prep.). The eggs of fifteen females (78 meiotic plates) showed metaphases with additional univalents (Fig. 1d) or with trivalents (Fig. 1c). One female from a cross between R. pallens (♀♀) and R. pungens possessed an egg with 30 univalents (Fig. 1b) and another with 17 chromosomal units.

In the backcrosses and crosses between F1's and F2's even less meiotic irregularities were observed than in the first hybrid generation (Table 6: 0.5%), suggesting that females with irregular meiotic plates contributed less to the next generation.

Special attention was given to hybrid combinations with R. pungens and Taxon 4, both sperm-donor species for triploid females. Many disturbed nuclei were found in hybrids between R. pungens (♀♀) and R. collinus (Table 5), but these disturbances were not observed in their backcrosses (Table 6). No indications were found that hybridizing and backcrossing of R. pungens or Taxon 4 with any of the other Ribautodelphax species might result in the synthesis of triploids.

Table 6. Meiosis in females of hybrid crosses and backcrosses of Ribautodelphax Given are the number of females with regular (A) and abnormal meiosis (B). For abbreviations, see Table 4.

Parents ♀♀	♂♂	F1xF1 A B	$B_{1,1}$ A B	$B_{1,2}$ A B	$B_{1,3}$ A B	$B_{1,4}$ A B
collinus x	pungens	18 0	. .	. .	. .	. .
	imitans	5 0	. .	. .	. .	. .
	fanari	15 0	. .	. .	5 0	. .
	Taxon 1	9 0	. .	5 0	. .	5 0
	Taxon 2	4 0	. .	. .	. .	4 0
angulosus x	imitans	6 0	. .	. .	. .	. .
	fanari	10 0	6 0	. .	. .	. .
pungens x	collinus	30 0	23 0	10 0	10 0	11 0
	angulosus	17 0	16 0	12 0	16 0	10 0
	imitans	5 0	. .	. .	. .	. .
	fanari	10 0	10 0	. .	. .	. .
	Taxon 4	5 0	10 0	. .	5 0	5 0
imitans x	collinus	10 0	. .	. .	. .	. .
	angulosus	5 0	. .	. .	. .	. .
	pungens	10 0	15 0	2 0	. .	. .
	Taxon 4	12 0	. .	. .	. .	. .
fanari x	collinus	8 0	. .	. .	. .	. .
	angulosus	6 0	. .	. .	. .	. .
	pungens	9 1^a	5 0	. .	6 0	. .
	Taxon 4	5 0	5 0	. .	5 0	5 0
Taxon 1 x	imitans	4 0	. .	. .	. .	. .
	fanari	5 0	. .	. .	. .	. .
	Taxon 4	6 0	3 0	2 0	3 0	2 0
Taxon 2 x	collinus	10 0	9 1^a	5 0	10 0	. .
	imitans	. .	9 1^a	. .	. .	. .
	Taxon 3	5 0	. .	. .	. .	. .
Taxon 4 x	collinus	. .	5 0	. .	. .	. .
	angulosus	10 0	5 0	. .	6 0	. .
	pungens	10 0	5 0	. .	5 0	. .
	imitans	12 0	. .	. .	. .	. .
	fanari	5 0	5 0	2 0	. .	2 0
	Taxon 2	. .	5 0	. .	3 0	. .
pallens x	pungens	18 0	4 0	. .	. .	. .

[a] Methaphase with additional univalents.

Insemination experiments with R. imitans and Taxon 4.

Crosses between R. imitans and Taxon 4 resulted in many hybrids and high insemination levels were found after 30 days of confinement (Table 2), suggesting that barriers to mating were ineffective between them. These data resulted, however, from no-choice experiments and to investigate whether mate discrimination was really absent additional experiments were carried out (Table 7). Homogamic crosses resulted in 90-100% insemination

Table 7. Results of insemination experiments with R. imitans and Taxon 4. Each cage with 5 males and 10 (no-choice) or 20 females (choice experiments). Each combination once repeated. A, total number of females. B, percentage of insemination.

MALES	FEMALES imitans A	imitans B	Taxon 4 A	Taxon 4 B	MALES	FEMALES imitans A	imitans B	Taxon 4 A	Taxon 4 B	Duration days
imitans	20	100	0		Taxon 4	0		20	90	3
	20	100	20	0		20	0	20	90	3
	0		20	15		20	10	0		3
	20	100	20	80		20	5	20	100	7
	0		20	70		20	25	0		7
	0		20	100		20	60	20	100	15
						20	80	0		15

within three days, but in this period only few females were inseminated in heterogamic crosses and in the choice tests not even a single female was inseminated by a male from the other species. The percentages of heterospecifically inseminated females increased during prolongend confinement with clear differences between reciprocal combinations. Females become unreceptive to new mating attempts immediately after insemination, and consequently after insemination of the conspecific females in the choice tests males can no longer choose between mating partners. These tests clearly indicate that barriers to mating existed between R. imitans and Taxon 4, but these barriers break down after prolonged confinement.

In choice experiments, the wing length was used as a marker to distinguish between the females of R. imitans and Taxon 4. Of Taxon 4 only brachypterous females were available and no control experiments could be made to assess the influence of wing length on mate choice. However, choice experiments with R. pungens revealed no discrimination between wing forms by males.

Intraspecific insemination in R. pungens.

On the basis of slight morphological differences, Drosopoulos (1982) suggested that some Greek R. pungens populations, e.g. GR4, represent a new species. Insemination experiments with four populations of R. pungens (Table 8) showed high insemination levels in homogamic crosses after three days, in contrast to some heterogamic crosses e.g. with males of DE2.

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	MALES							
	NL4		DE2		GR3		GR4	
FEMALES								
NL4, 2N	95		25	(90)	38	(80)	78	(100)
NL4, 3N	95		23	(55)	88	(100)	98	(100)
DE2	78	(80)	98		48	(85)	65	(90)
GR3	88	(90)	40	(85)	95		98	
GR4	35	(95[a])	15	(40)	88		100	

[a] Only 19 females recollected.

Prolonged confinement increased the insemination levels in most combinations to 80 or 100%. Again marked differences were observed between reciprocal combinations. These experiments did not show differences between population GR4 and the remaining populations and failed to support the suggested distinct status of this population.

Mating capacity.

Males of three Ribautodelphax species were able to inseminate about one female every three days (Table 9). This insemination capacity seems low compared to the few data available for other planthoppers. Booij & Guldemond (1984) found an insemination capacity of 2.2 females per day for males of Muellerianella fairmairei (Perris), one male of Sogatodes orizicola (Muir) could inseminate three females in eight hours (McMillian, 1963) and Javesella males were able to inseminate several females per day (de Vrijer, pers. comm.).

Planthopper females often mate only once or twice (Oh, 1979). One mating of Ribautodelphax suffices for the production of fertilized eggs during more than 35 days, probably a longer period than the average life span of females under field conditions.

Table 9. Insemination capacity of Ribautodelphax males. One male confined with 15 conspecific females for 15 days. Given: $\bar{X}$, mean number of inseminated females /day. N, number investigated.

	N	$\bar{X}$	S.E	Range
R. collinus	6	0.37	0.03	0.27-0.47
R. angulosus	10	0.36	0.05	0.20-0.60
R. pungens	10	0.33	0.03	0.20-0.47

Natural hybrids.

Because most species of the R. collinus complex are morphologically very similar to one another, it is difficult to detect hybrids in the field. Moreover, most Ribautodelphax species are rare planthoppers and only few localities are known where species that hybridize under laboratory conditions are living syntopically. At one locality near Wageningen, the Netherlands, a mixed population of R. albostriatus, R. collinus and R. angulosus was sampled in the years 1954-1957 by R.H. Cobben. Only the last two species hybridized successfully in the laboratory. Eighty males appeared to belong to R. collinus and 198 to R. angulosus; two males (0.7%) possessed intermediate characters resembling laboratory hybrids between R. angulosus (♀♀) and R. collinus. Near Vébron (Lozère, France; leg. R.H. Cobben) a single male was collected resembling laboratory hybrids between R. collinus and R. pungens. Unfortunately, this male was the only Ribautodelphax sampled from this locality, and it is not known whether the two purported parent species occurred in this locality. If it is not a hybrid, this male could represent an unknown Ribautodelphax species.

DISCUSSION

Reproductive isolation

Premating barriers are strongly developed in Ribautodelphax resulting in low levels of insemination in interspecific crosses. Such strong premating barriers are most adaptive when the parental investment in mating is high (Bernard, 1983; Hieber & Cohen, 1983) as in both sexes of Ribautodelphax. Data on interspecific insemination and mating capacity are available only of one other planthopper genus. Males of Muellerianella have

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a five-fold insemination capacity compared to Ribautodelphax and, judging from the many interspecific matings, premating barriers were less developed (Booij, 1982a,b; Drosopoulos, 1977).

Acoustic signals play an important role in intraspecific communication in planthoppers (review in Claridge, 1985). All Ribautodelphax species and new Taxa differ in male and female acoustic signals (den Bieman, 1986); these differences might be important in preventing interspecific mating. However, experimental evidence on the functional importance of these acoustic differences in reproductive isolation is lacking.

Post-mating barriers play only a minor role in reducing introgression in Ribautodelphax. A low percentage of eggs showed abnormal meiosis probably resulting in reduced viability. Retarded development of male hybrids in several crosses with females of Taxon 4 also adds to reproductive isolation. Post-mating barriers were much more strongly developed in the planthopper genera Javesella (Strübing & Hasse, 1975; de Vrijer, 1981) and Muellerianella (Booij, 1982a,b; Drosopoulos, 1977).

It should be noted that the hybridization experiments were carried out with one arbitrarily chosen population of each Ribautodelphax species or Taxon. Intraspecific crosses in R. pungens indicated, that populations may show considerable differences in acceptance of heterogamic partners. Consequently, interspecific crosses with other populations of each species might give variable results.

The extent of crossability of Ribautodelphax species under laboratory conditions suggests that introgression is possible. However, the importance of hybridization under field conditions remains questionable, even though some putative natural hybrids were found. Primarily, hybridization chances are reduced because the rarity of many Ribautodelphax species limits the number of mixed populations. Crossing experiments indicate that premating barriers exist between all species, and insemination experiments with R. imitans and Taxon 4 and with populations of R. pungens indicate that young adults are even more critical in mate choice than older ones. In field populations most females will be inseminated in these first days, especially because the first males are ready to mate when the first females become receptive, because of the slightly faster development of the former. Phenological differences (den Bieman, in prep.) and particularly differences in host plants (den Bieman, 1987) are further important to maintain premating isolation.

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About two thirds of the crosses between the members of the R. collinus complex hybrids were successful, but generally only few females were inseminated heterospecifically due to premating barriers. The existence of these premating barriers suggests that the known species and new Taxa of the R. collinus complex might be true species, despite their potential hybridization capacity. In this respect, data on the three pairs of morphologically closely related Ribautodelphax are important. Hybridization between R. pungens and Taxon 1 was unsuccessful and crosses between Taxon 2 and Taxon 3 resulted in low insemination levels. R. imitans and Taxon 4 mated readily, but insemination tests suggest that mating barriers existed between them, especially between young adults. These premating barriers were more stongly developed than between conspecific populations of R. pungens. However, to assess the reproductive isolation between R. imitans and Taxon 4 other populations should also be studied.

The success of the crossing experiments suggests that the members of the R. collinus complex are closely related. However, hypotheses on phylogenetic patterns may not be deduced from hybridization data, because the ability to hybridize is often considered to be a plesiomorphic character (Funk, 1985; Rosen, 1979; Wiley, 1981).

Origin of triploid females.

Generally it is assumed that most polyploid animals are of hybrid origin (Bullini, 1985; White, 1973, 1978), though an autopolyploid origin is not excluded in some groups (Lokki & Saura, 1980). Drosopoulos (1978) found that triploids of the planthopper genus Muellerianella could be produced by hybridization. The failing of attempts to repeat these results in later extensive experiments questioned the true allopolyploid nature of these triploids (Booij, 1982a). Neither karyologic investigations of F2 and backcross females nor deviations in sex ratios of the hybrid offspring indicate that triploids may result from hybridization between Ribautodelphax species. Also the absence of differences in host-plant relations, acoustics (den Bieman, 1987a, b) and in mating relations to other Ribautodelphax species between diploids and triploids of R. pungens and of Taxon 4 do not support a hybrid origin of the Ribautodelphax triploids.

A hybrid origin of the Ribautodelphax triploids is, however, not

completely ruled out by these crossing experiments. Several hybrids possessed eggs with irregular meiotic plates, one female possessed an egg with 30 univalents while in others, eggs with two groups of about 15 chromosomes, apparently precocious anaphase I, were found. These meiotic irregularities were rarely found in later hybrid generations. On the assumption that the tendency to meiotic irregularity is a dominant genetical condition characterictic, this suggests that females with irregular meiosis may not have produced progeny. However, not all F1-females were used for backcrossing and females with irregular meiosis were perhaps not taken for further crossings. Normal meiotic divisions are impossible in eggs with 30 univalents, but could be replaced by a mitotic division as in triploid eggs (Drosopoulos, 1977). Fusion of diploid eggs with haploid sperm could then give rise to new triploids. The combination of events needed, viability of abnormal eggs, meiosis with first division restitution and fertilization of unreduced eggs, could be very rare and might explain why the crosses in Ribautodelphax and the majority of crosses in Muellerianella failed to produce triploids.

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KARYOTYPIC VARIATION IN BISEXUAL SPECIES AND PSEUDOGAMOUS FORMS OF THE PLANTHOPPER GENUS RIBAUTODELPHAX (HOMOPTERA, DELPHACIDAE).

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ABSTRACT

Chromosome numbers of Ribautodelphax collinus, R. angulosus, R. pungens, R. imitans, R. fanari, R. pallens, R. albostriatus and of four undescribed species, provisionally indicated as Taxon 1 to 4, were determined. The chromosome number of all species was found to be 2n=30 (14II+XX-XO); however, in several species some females with 2n=31 or 32 also occurred. One female of R. collinus and one of Taxon 1 possessed eggs with 16 bivalents and one egg with 32 bivalents. One female of R. pungens contained only tetraploid eggs. Pseudogamous triploid females occurred in populations of R. pungens and Taxon 4. Their chromosome numbers ranged from 3n=40 to 46 in R.pungens and 3n=41 to 45 in Taxon 4. In several pseudogamous females from two R pungens populations, eggs with 45 univalents and with various numbers of univalents, bivalents and trivalents were found. The mode of origin of pseudogamous triploid females is discussed and it is concluded that karyotypic data are consistent with an autopolyploid origin.

INTRODUCTION

The genus Ribautodelphax comprises nine planthopper species (Delphacidae) in Europe. Within this genus a complex of five sibling species may be distinguished. Recently, several new populations of this R. collinus complex were discovered and they are provisionally indicated as 'Taxon 1 to 4'. The taxonomic status, ecology and acoustic behaviour of the members of this complex is discussed elsewhere (den Bieman, 1986, 1987a, b, in prep).

In several Ribautodelphax populations, female-biased sex ratios were

noticed. Rearing experiments and karyologic investigations showed that these populations consisted of mixtures of the bisexual species and triploid pseudogamous females. For their reproduction, these pseudogamous triploid females must mate with males, because insemination is necessary for the development of the eggs, but their all-female triploid offspring receive maternal genes only. As a consequence the triploid females behave as 'sexual parasites' of the bisexual species.

In planthoppers, pseudogamous triploid females also occur in the genus Muellerianella (Drosopoulos, 1976). Extensive analyses revealed that these triploids consisted of clones with differences in chromosome numbers and host-plant relations (Booij, 1982a, c).

Karyologic studies were undertaken to trace mixed populations of triploid females and the various Ribautodelphax species and to assess variation in chromosome numbers. Moreover, karyologic data might provide clues to the mode of origin of the triploid females.

MATERIAL AND METHODS

For convenience, all known Ribautodelphax species and the four new Taxa are provisionally treated as species in this paper. The triploid forms are indicated by the name of the bisexual species on which they depend for sperm in the field.

From 1981-1984, samples for karyologic studies were taken throughout Europe. Nine species of the R. collinus complex were studied: R. collinus (Boheman), R. angulosus (Ribaut), R. pungens (Ribaut), R. imitans (Ribaut), R. fanari Asche, Drosopoulos & Hoch, and the four new Taxa. For comparison two outgroup species were investigated: R. pallens (Stål) and R. albostriatus (Fieber). Unfortunately, most Ribautodelphax species are rare and their populations are often small resulting in limited sample sizes. Field collected males and females were stored in a mixture of absolute methyl alcohol and glacial acetic acid (3:1) (Drosopoulos, pers. comm.; Kuznetsova, 1982).

Chromosome studies were focused on females because of our interest in the distribution and origin of the all-female triploid forms. Only ten young laboratory-bred or field-collected males of each species were studied to assess the type of sex-determination system. The techniques employed for the preparation of ovarian eggs and of testes have been described by Booij

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(1982c) and den Bieman (1987c).

No attempt was made to study the meiotic processes in detail and only the number of chromosomes was counted. Well-spread meiotic metaphase I preparations of diploid eggs were studied. Chromosomes may have different orientations (Halkka, 1959) making a distinction between bivalents and univalents not always easy, but, since all available eggs of each female were studied, the chromosome number of most females could be counted. Very small chromosomes were considered as univalents. No distinction could be made between the individual holokinetic chromosomes. Chromosomes of triploids were counted using camera lucida drawings.

RESULTS

Chromosome numbers in diploid females.

Females of all Ribautodelphax species investigated possessed a modal diploid number of 2n=30 arranged as 15 bivalents in the metaphase I plates (Tables 1, 2 and 3; Fig. 1). In the meiotic plates of females, the sex chromosomes and autosomes could not be distinguished.

Some females, mainly of R. pungens and Taxon 1, possessed aberrant numbers of chromosomes of 2n=31 or 32 in all their eggs (Fig. 1B, E, F and J, Table 1 and 2). Females with 2n=31 possessed 15 bivalents and a small supernumerary chromosome. These small chromosomes might be small B-chromosomes as has been reported for several planthoppers (Booij, 1982c; Halkka, 1959).

Four females, three of R. pungens (Table 2) and one of Taxon 4 (Table 3), possessed eggs with mixtures of univalents and bivalents. These females might represent diploids with several additional chromosomes or triploids with some bivalent formation. The high number of chromosomes of one of the R. pungens females, which had about 25 univalents and bivalents, and the one of Taxon 4, which had 25 to 28 univalents and bivalents, suggest that they represented such triploids.

Among diploid eggs (2n=32) of one female of R. collinus and one of Taxon 4, one egg with 32 bivalents (4n=64) was found (Table 1, Fig. 1K). Eggs with a tetraploid number of chromosomes were also found in a female of R. pungens, population FR 18 (Table 2). Unfortunately, only one female of this population was karyotyped. The tetraploid eggs may result from a

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Table 1. Chromosome numbers in field populations of <u>Ribautodelphax</u> species. For each locality the number of females with a certain diploid karyotype is given. Repeated samples of the same locality are combined.

	Code[a]	Locality	Chromosome numbers		
			30	31	32
<u>collinus</u>	NL1	Limburg, Plasmolen[b].	18	1	4
	NL2	Utrecht, Maarn.	6	1	.
	YU1	Srbija, Trstenik.	5	.	.
<u>angulosus</u>	NL3	Utrecht, Leersum.	43	1	.
	GR5	Drama, Meg. Panagia (Rodopi Mts.).	8	1	.
<u>imitans</u>	FR3	Pyrenées Orientales, St. Cyprien.	19	.	.
	GR3	Fokis, Elaion.	8	.	.
<u>fanari</u>	FR4	Bouches du Rhône, Etang de Vaccarès.	5	.	1
	GR7	Rodopi, Fanari.	5	.	.
	GR8	Xanthi, Porto Lagos.	5	.	.
Taxon 1	NL6	Gelderland, Hoge Veluwe[c].	53	5	7
Taxon 2	GR6	Florina, Kalo Nero Mts.	5	1	.
Taxon 3	FR5	Vaucluse, Mont Ventoux.	15	.	.
<u>pallens</u>	NO1	Sør-Trødelag, Dovrefjell.	14	.	.
<u>albostriatus</u>	NL7	Limburg, Wrakelberg.	13	.	.
	FR11	Jura, Pont d'Hery.	7	.	.

[a] Belgium (BE), France (FR), Greece (GR), Netherlands (NL), Norway (NO), W. Germany (DE) and Yugoslavia (YU).
[b] One female with two nuclei with 16 bivalents and one nucleus with 32 bivalents, and another female with two nuclei with 16 bivalents and one univalent.
[c] One female with three nuclei with 16 bivalents and one nucleus with 32 bivalents.

premeiotic endoduplication. Quadrivalents were not observed, so homologous bivalents showed no synapsis. Reductional division of tetraploid eggs should result in gametes with 30 (or 32) chromosomes, and when these are fertilized by normal haploid sperm they might give rise to new triploids. However, it remains uncertain whether these tetraploid eggs are viable because triploids were never discovered in populations of <u>R. collinus</u> or Taxon 1.

Meiosis in males.

All eleven <u>Ribautodelphax</u> species had a XX-XO sex determination system, which is consistent with the observations of Halkka (1959) on <u>R. pallens</u> and <u>R. albostriatus</u>. The course of meiosis is, in general terms, similar in all planthoppers with an XO system and details have been reported for several species (Barrion & Saxena, 1985; Den Hollander, 1982; Halkka, 1959; Kuznetsova, 1982). During late diakinesis the sex chromosome

Table 2. Chromosome numbers in field populations of R. pungens. Most populations were a mixture of triploid females and the diploid species. For each locality the number of females with the various diploid or triploid number of chromosomes is given. Repeated samples of each locality are combined. For codes see Table 1.

Code	Locality	Chromosome numbers									
		DIPLOIDS			TRIPLOIDS						
		30	31	32	40	41	42	43	44	45	46
NL4	Limburg, Bemelen.	75	2	4	.	1	5	31	58	31	4
NL5	Limburg, Kunderberg.	45	.	1	.	.	.	.	.	3	.
NL7	Limburg, Wrakelberg.	8	.	.	.	.	.	1	6	10	3
NL8	Limburg, Maastricht.	5	.	.	.	.	.	1	.	1	.
NL9	Limburg, Wahlwiller.	21	.	.	.	.	.	.	1	1	.
BE1	Namur, Jemelle.	12	.	.	.	.	.	1	3	1	.
BE2	Namur, Dinant.	5	.	.	.	.	.	.	2	5	.
BE3	Namur, Nismes[a].	8	.	.	.	.	.	1	1	11	2
BE4	Luxembourg, Torgny.	3	.	.	.	.	.	.	.	4	.
BE6	Namur, Han sur Lesse.	3	.	.	.	.	.	.	.	12	.
BE7	Namur, Rochefort.	6	.	.	.	.	.	.	1	8	.
DE2	Hessen, Amöneburg.	30	.	.	.	.	.	.	.	.	.
DE4	Baden-Württemberg, Tübingen[b].	4	.	.	.	1	.	.	1	7	.
DE6	Baden-Württemberg, Hayingen.	1	.	.	.	.	.	.	3	4	.
DE7	Baden-Württemberg, Hundersingen.	1	.	.	.	.	.	.	2	2	.
FR1	Ardèche, Coulens.	6	.	.	.	.	.	.	.	.	.
FR2	Alpes de Haute Provence, Montfuron[c].	15	.	.	.	.	.	.	.	.	.
FR11	Jura, Pont d'Hery.	4	1	.	.	.	1	.	.	14	.
FR17	Gard, Lasalle[d].	1	.	.	.	.	.	.	1	1	.
FR18	Jura, Chemilla[d].	.	.	.	.	.	.	.	.	.	.
FR19	Ardèche, Asperjac.	5	.	.	.	.	.	1	.	4	.
FR20	Ain, Treffort[e].	3	.	.	1	2	1	.	6	5	.
FR21	Jura, Salins-les-Bains.	13	.	.	.	.	.	2	.	3	.
YU2	Slovenija, Postojna.	10	.	.	.	.	.	.	.	.	.
YU3	Bosni-Hercegovina, Jajce[f].	3	.	1	.	.	.	1	1	11	1
YU4	Croatia, Zdihovo.	1	.	.	.	.	.	.	1	3	.
GR2	Florina, Kotas.	5	1	.	.	.	.	.	1	3	.
GR3	Fokis, Elaion.	3	.	1	.	.	.	.	.	1	.
GR4	Fokis, Skaloula.	7	.	.	.	.	.	.	.	.	.
GR9	Kozani, Vourinos Mts.	.	1	2	.	.	.	.	.	4	1
	No of individuals	303	5	10	1	4	7	39	88	149	11
	No of localities	28	4	6	1	3	3	8	15	24	5

[a] One female with a mixture of 19 bivalents and univalents.
[b] Five females with nuclei with 45 univalents and with various numbers of univalents, bivalents and trivalents.
[c] One female with a mixture of about 25 bivalents and univalents.
[d] One female with seven eggs each with 30 or close to 30 bivalents.
[e] One female with a mixture of 21 bivalents and univalents.
[f] Three females with nuclei with about 45 univalents and with various numbers
· of univalents, bivalents and trivalents.

Table 3. Chromosome numbers in field samples of Taxon 4. Pseudogamous triploid females occurred in four populations. For each locality the number of females with the various diploid or triploid number of chromosomes is given. Repeated samples of the same locality are combined. For codes see Table 1.

Code	Locality	DIPLOIDS		TRIPLOIDS		
		30	32	41	44	45
FR2	Alpes de Haute Provence, Montfuron[a].	·	·	·	2	·
FR6	Vaucluse, St. Estéve[b].	35	1	·	2	·
FR7	Vaucluse, Crillon-le-Brave.	15	·	·	·	·
FR8	Vaucluse, Bedoin.	6	·	·	·	·
FR9	Bouches du Rhône, les Baumettes.	5	·	·	1	3
FR12	Var, Rians.	4	·	·	·	·
FR13	Vaucluse, Col de Murs.	1	·	1	1	1

[a] R. pungens functions as sperm donor
[b] One female with a mixture of 25-28 bivalents and univalents
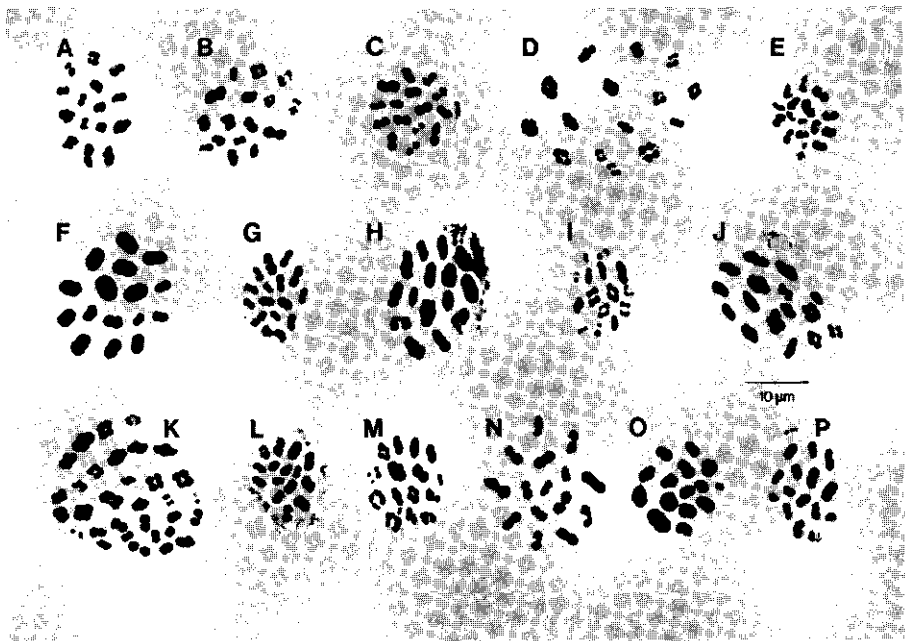
Fig.1. Meiotic metaphase I of ovarian eggs of Ribautodelphax (A) R. collinus, NL1, 2n=30; (B) R. collinus, NL2, 2n=31; (C) R. angulosus, NL3, 2n=30; (D) R. pungens, DE2, 2n=30; (E) R. pungens, GR2, 2n=31; (F) R. pungens, NL4, 2n=32; (G) R. imitans, FR3, 2n=30; (H) R. fanari, GR7, 2n=30; (I) Taxon 1, NL6, 2n=30; (J) Taxon 1, NL6, 2n=32; (K) Taxon 1, NL6, 4n=64; (L) Taxon 2, GR6, 2n=30; (M) Taxon 3, FR5, 2n=30; (N) Taxon 4, FR6, 2n=30; (0) R. pallens, NO1, 2n=30; and (P) R. albostriatus, NL7, 2n=30. Codes indicate populations, see Tables 1, 2 and 3. Univalents arrowed.

Fig. 2. Meiosis in males of R. collinus, population NL1. (A) Metaphase I with sex chromosome arrowed; (B) anaphase I; (C+D) metaphase II showing 14 and 15 chromosomes, respectively.
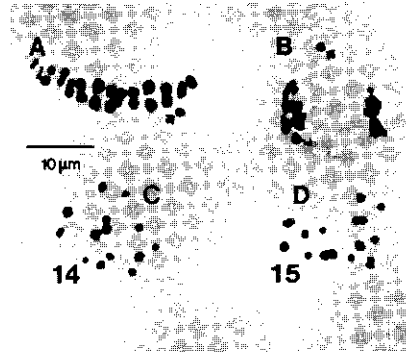
becomes clearly isolated from the group of bivalent autosomes (Fig. 2A). Its isolated position and its univalent condition makes it easy to identify. During anaphase the sex chromosome always moves behind the autosomes(Fig. 2B). The first meiotic division is a reductional division resulting in two types of secondary spermatocytes one with and one without the sex chromosome (Fig. 2C and D).

Chromosome numbers in triploids.

Triploid females were discovered in populations of R. pungens (Table 2) and of Taxon 4 (Table 3). The first were studied extensively, but only a few triploids of Taxon 4 were investigated because they were only recently dicovered.

The majority of the triploids of Ribautodelphax possessed eggs with univalents only (Fig.3) suggesting that the first meiotic division is suppressed as in triploids of the planthopper genus Muellerianella (Drosopoulos, 1976). In populations of Taxon 4, triploids with 41, 44 and 45 chromosomes were found (Table 3). A wider variation was noted in triploids of R. pungens with chromosome numbers from 3n=40 to 46 (Table 2, Fig. 3); karyotypes with 44 and 45 univalents prevailed. Karyotypic differences were not observed among the eggs of individual females and also not among the offspring of three individual females with 3n=45 and of two females with 3n=44. These data suggests that the variation in chromosome numbers of the triploids is primarily clonal variation.

The frequency of a karyotype is negatively correlated with its degree of aneuploidy (Table 2). Karyotypes closer to 3n=45 were more common than strongly deviating karyotypes. A similar relation was found in triploids of
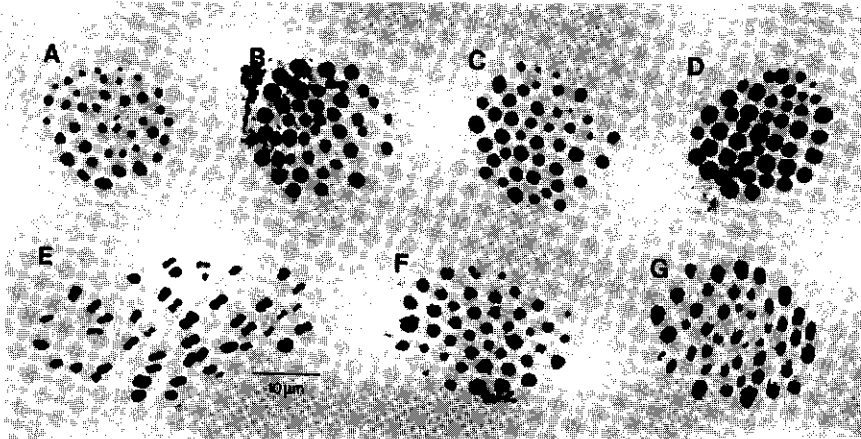
Fig. 3. Metaphase configurations of ovarian eggs of Ribautodelphax triploids. E, lateral view; others, polar view. (A) R. pungens, FR20, 3n=40; (B) R. pungens, NL4, 3n=43; (C) R. pungens, NL4, 3n=44; (D) R. pungens, DE4, 3n=45; (E) R. pungens, BE3, 3n=46; (F) Taxon 4, FR6, 3n=44; and (G) Taxon 4, FR9, 3n=45. Codes indicate populations, see Tables 2 and 3.

the genus Muellerianella (Booij, 1982c). In several populations of R. pungens only one karyotype was found, but in others, two to six different karyotypes occurred. None of the more frequent karyotypes was restricted to a single geographic area.

Several triploids from the populations DE4 and YU3 of R. pungens (Table 2) possessed eggs with different numbers of chromosomal units (Fig. 4). In each of these eight females, eggs with 45 univalents and eggs with different numbers of univalents, bivalents and trivalents were found. In rearing experiments these aberrant females of population YU3 appeared to reproduce by pseudogamy. Of the 2th, 4th, 6th and 8th generation 20 to 40 females were karyotyped. After eight generations some metaphase disturbances were still found but their frequency was greatly reduced and 90% (N=30) of the females of the eighth generation possessed eggs with 45 univalents only.

Triploid females of 14 populations of R. pungens and of two populations of Taxon 4 were reared and appeared to have a pseudogamous mode of reproduction. Despite many tests none of these triploids could produce offspring in the absence of males.

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Fig. 4. Chromosome complements of R. pungens, population DE4, ♀1. (A) Nucleus with 45 univalents; (B-E) nuclei with a variable number of univalents, bivalents and trivalents.
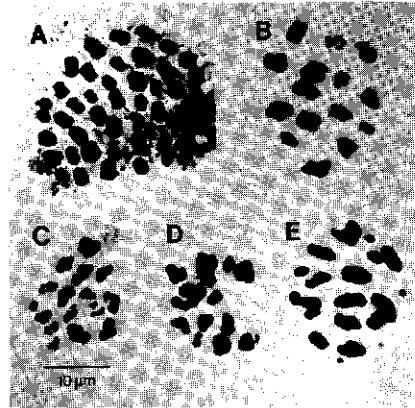
Males in triploid rearings.

In rearings of triploids of the populations NL4 and YU3 of R. pungens, five males were produced among several thousands of triploid females. These males appeared to be sterile and no sign of spermatid development was found; their karyotype could not be determined. All five males were morphologically identical to R. pungens and resembled this species in acoustic signals.

Several mechanisms are known, which may account for the origin of these five males. In the diploid parthenogenetic stick insect Carausius morosus Br. loss of a sex chromosome resulted in the occurrence of males (Pijnacker, 1966). Occasional fertilization of parthenogenetic triploid Cnemidophorus lizards produced tetraploids, some of them being males (Lowe et al., 1970; Cole, 1979). A third pathway has been reported in the pseudogamous triploid fish Poeciliopsis monacha - 2 lucida, in which the loss of one complete lucida genome resulted in a sterile diploid male (Ciminio & Schultz, 1970). No karyotypic indications for the last two alternatives have been found in triploids of Ribautodelphax, because neither tetraploid nor diploid females have ever been found in triploid rearings. In triploids of taxa such as Ribautodelphax with an XX-XO system, loss of one or more sex chromosomes seems a logical option for the origin of males.

Spontaneous origin of triploids.

Occasional checks of rearings of diploids of R. pungens population
NL4, revealed that in the ninth generation some females contained eggs with
45 univalents. Two females possessed eggs with mixtures of univalents,
bivalents and perhaps trivalents, while a third female showed 30
univalents.

In the glasshouse with the rearings of diploid R. pungens also cages
with triploids of R. pungens and rearings of other Ribautodelphax species
were present. Though all conceivable measures were taken to avoid
contamination of rearings, an accident can never be excluded completely.
For this reason some doubts were raised as to the autopolyploid origin of
the triploid females in the rearing of R. pungens diploids. However, the
occurrence of females with meiotic irregularities ruled out that the
diploid rearing was simply contaminated with triploid females.
Hybridization between R. pungens and another Ribautodelphax species also
seems improbable. The differences in host plants between most
Ribautodelphax species reduces the chance of contamination of rearings.
Moreover, hybridization experiments never resulted in the synthesis of
triploids (den Bieman, 1987c).

To check the autopolyploid origin of the triploids, an experiment was
started with four lines of diploid R. pungens, population NL4, each
originating from one field-collected female. These lines were completely
isolated from all other Ribautodelphax rearings and their offspring were
karyotyped during six generations. In one line, karyologic abnormalities
were noticed in the fourth generation; six of the 20 females investigated
possessed only eggs with 32 univalents. Fusion of these eggs with sperm
could give rise to new triploids. However, no abnormal meiotic
configurations were found in the next generation and the 50 females
investigated possessed only eggs with 16 bivalents indicating that the eggs
with 32 univalents were probably not viable. In the sixth generation of
this line again one female was found with 32 univalents.

DISCUSSION.

The diploid karyotype.

Including the results of this study, the karyotypes of 57 planthopper species are known (Barrion & Saxena, 1985; Bhattacharya & Manna, 1973; den Bieman & de Vrijer, 1987; Booij, 1982c; Den Hollander, 1982; Drosopoulos, 1976, 1982; Halkka, 1959, 1962; Halkka & Heinonen, 1964; Hoch & Remane, 1983; Kuznetsova, 1982). The diploid number of 30 chromosomes found in Ribautodelphax was also observed in 61% of the other planthoppers, whereas it ranges from 24 to 36 in the remaining species. A XX-XO system has been found in 85% of the species and a XX-XY system in the others. Karyotypic differences between congeners have only been observed within one of the twelve genera of which more than one species was studied. These data suggest that karyotypic evolution in planthoppers is rather conservative.

Planthoppers have holocentric chromosomes and therefore simple chromosome breakages or fusions can provide a basis for changes in chromosome numbers (Halkka, 1959; Den Hollander, 1982). The diffuse centromere makes it possible for chromosome fragments to function as autonomous units. Despite these theoretical possibilities, planthoppers seem to remain remarkably conservative for variation within species. However, in most species only a few individuals have been studied. Within the better studied genera Muellerianella and Ribautodelphax some variation in the numbers of autosomes has been noticed in several species. Polymorphism of the sex chromosomes has been reported for Nilaparvata lugens (Stål) (Den Hollander, 1982) and Dicranotropis hamata (Boheman) (Halkka, 1959).

Clonal variation in pseudogamous triploids.

Pseudogamous triploid planthoppers have been discovered in populations of Muellerianella fairmairei (Perris) (Drosopoulos, 1976, 1977; Booij, 1982c), in populations of Ribautodelphax pungens and Taxon 4. Recently, a true parthenogenetic population of triploid females of the genus Delphacodes has been reported from NW Greece (den Bieman & de Vrijer, 1987). Triploids of the first two genera have been studied extensively revealing a wide variation in the number of chromosomes, while only minor

variation has been found in their congeneric diploid bisexual species.

The variation in chromosome numbers might be caused by chromosome fission and fusion, but also by chromosome loss. The ability to tolerate such a loss should be greater in triploids than is their diploid congeners. In the case of Ribautodelphax, some variation in the number of chromosomes could be caused by the two types of sperm. Fusion of an unreduced egg (2n=30) with a sperm with 15 chromosomes would give rise to a triploid with 45 chromosomes, while 44 chromosomes would result from a fusion with a sperm with 14 chromosomes. Variation in the diploid numbers of the unreduced eggs (2n=31 or 32) would result in triploids with 45 or more chromosomes.

Different levels of karyotypic variability in sexual and parthenogenetic forms have also been found in aphids (Aphididae), another homopteran group (Blackman, 1980). Karyotypic variation within aphid species was commonly found when different parthenogenetic, anholocyclic populations of the same species were studied. However, very few cases of karyotypic variation have been found within obligatory sexually reproducing species. It seems that in species with holocentric chromosomes most karyotypic variation is conserved during the reproduction of the parthenogenetic forms, but is selected against in normal meiotic processes.

Recent observations showed that genetic variation within and between parthenogenetic populations is much wider than often assumed (Vrijenhoek, 1984). A common characteristic of the variation patterns of parthenogenetic forms is that one or a few clones predominate in most populations, while the other clones occur only locally or in low frequencies (Bell, 1982; Booij, 1982c; Lokki & Saura, 1980; Harshman & Futuyma, 1985). In the triploids of Ribautodelphax a similar pattern is found with a prevalence of clones with 44 or 45 univalents in all populations.

Origin of pseudogamous triploid Ribautodelphax.

Two models have been described for the origin of triploid forms. (1) As a result of hybridization females may produce highly abnormal meiotic plates resulting in unreduced eggs giving rise to triploids after fusion with sperm (Drosopoulos, 1977; Ojima et al., 1975). (2) Unreduced eggs are occasionally produced in many species (Bell, 1982; Bogart, 1980; White, 1973) and fertilization with sperm of the same species would lead to

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autopolyploid triploids. Under both hypotheses an intermediate stage of a diploid parthenogenetic form in advance of polyploidization has been suggested (Bell, 1982; Cole, 1979; Lowe et al., 1970; Lokki & Saura, 1980). For many polyploid parthenogenetic forms a hybrid origin has been assumed (Bell, 1982; Bullini, 1985; White, 1973, 1978), though some polyploids may be autopolyploids (Lokki & Saura, 1980; Went, 1984; Morelli et al., 1983).

Results of previous investigations do not support a hybrid origin of the Ribautodelphax triploids. Hybridization attempts failed to synthesize triploids, though many hybrids could be produced (den Bieman, 1987c). All Ribautodelphax species showed obvious differences in acoustics and host-plant relations. However, no differences have been observed between pseudogamous triploids and bisexual diploids of R. pungens and between triploids and diploids of Taxon 4 (den Bieman, 1986, 1987a, b). Moreover, the pattern of acoustic variation of diploids and triploids of R. pungens suggests an autopolyploid origin of these triploids. Five males originated from triploids of R. pungens and they showed characteristics of this species only. This similarity once more does not support a hybrid origin of the triploids. For the mode of origin of the triploids discovered in rearings of R. pungens an autopolyploid origin seems again the most parsimonious explanation.

Several karyotypic data concur with an autopolyploid origin of the Ribautodelphax triploids. The main prerequisite of autopolyploidy is met in Ribautodelphax, i.e. the spontaneous production of unreduced eggs. Eggs with 32 univalents were observed in an isolated rearing of bisexual R. pungens. In field samples of three species, R. pungens, R. collinus and Taxon 1, one female was found with one or more tetraploid eggs. Normal meiosis would result in eggs with 2n univalents. These unreduced eggs would give rise to triploids after fusion with haploid sperm. All females with tetraploid eggs originated from populations of one Ribautodelphax species only, ruling out that hybridization was the cause of the chromosome duplication. These data indicate that the intermediate stage of a diploid parthenogenetic form is not necessary and no indications for such a form were found.

In triploid planthopper females the first meiotic division is suppressed (Drosopoulos, 1976). White (1978) suggests that the suppression of meiotic events by a single mitotic division seems to be an all-or-none type that could only arise by some kind of macromutation. However,

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observations on Ribautodelphax suggests that another mechanism might be involved. Several females of two R. pungens populations possessed eggs with 45 univalents and eggs with various degrees of bivalent and trivalent formation. Continued rearing showed a decrease in the fraction of females with residual meiotic synapsis. A recent triploidization in both populations seems a logical explanation for these observations. In newly arosen triploids, meiotic synapsis might not yet have been suppressed completely. Partial pairing of chromosomes is probably bound to lead to duplication-deficiency eggs and hence to inviability. This natural selection would contribute to a gradual development of a mechanism to prevent chromosome synapsis.

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ALLOZYME POLYMORPHISM IN PLANTHOPPERS OF THE GENUS RIBAUTODELPHAX (HOMOPTERA, DELPHACIDAE), AND THE ORIGIN OF THE PSEUDOGAMOUS TRIPLOID FORMS.

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ABSTRACT

European populations of the planthopper genus Ribautodelphax, comprising 5 known species and 3 undescribed Taxa of the R. collinus complex and two related species, were screened electrophoretically in order to clarify their taxonomic status and phylogenetic relationships. Pseudogamous triploid females occurring in populations of two species were investigated to assess their mode of origin. Allelic variation was scored at 10 presumed genetic loci. Nine loci proved to be polymorphic, one locus (Ldh) was expressed only in males and one locus (Pgi) was linked to the sex chromosome. Data on intraspecific and interspecific variability are presented. Genetic distance estimates were used to construct UPGMA and distance-Wagner trees. The species of the R. collinus complex clustered into one group with a subgroup consisting of R. imitans and Taxon 4 and the remaining species in the other subgroup. The two outgroup species are loosely appended to this R. collinus complex. Electrophoretic data of the pseudogamous triploid females are consistent with an autopolyploid polyphyletic origin.

INTRODUCTION.

Electrophoresis and other biochemical techniques have become widely used tools in taxonomic research. Electrophoretic analysis of proteins has been applied successfully to clarify the taxonomic status of species as well as the phylogenetic relationships of populations, species and sometimes even higher taxa (Avise, 1975; Ayala, 1983; Berlocher, 1984; Buth, 1984). Moreover, electrophoretic techniques have become powerful tools in elucidating the mode of origin of parthenogenetic forms and in

determining their ancestral species (Lokki, 1983).

In planthoppers (Delphacidae) electrophoresis has been applied so far only in one species, the notorious rice brown planthopper Nilaparvata lugens (Stål) (Claridge, 1980; Claridge et al., 1983; Sogawa, 1978).

Within the planthopper genus Ribautodelphax Wagner, a complex of sibling species around R. collinus (Boheman) may be distinguished to which 5 European species belong. Also included in this complex is a number of recently discovered populations, provisionally indicated as 'Taxon 1 to 4' (den Bieman, 1986, 1987b, c). The morphological differences among the members of the R. collinus complex are slight and somewhat variable, raising doubts as to their taxonomic status.

Karyologic analysis and rearing experiments demonstrated that many populations of R. pungens and Taxon 4 consisted of mixtures of the diploid bisexual species and triploid pseudogamous females (den Bieman, 1987d). These triploid females reproduce parthenogenetically, but insemination by males of a related species is necessary to activate the development of the eggs. The males, however, do not contribute genetically to the all-female triploid offspring of these triploid females.

Allozyme studies were undertaken to determine whether biochemical data could help to characterize the species of the R. collinus complex and to elucidate their phylogenetic relationships. In addition, these investigations were performed to clarify the mode of origin of the pseudogamous triploid Ribautodelphax females.

MATERIAL AND METHODS.

Material. For convenience, all known Ribautodelphax species and the four new Taxa are provisionally indicated as species in this paper. The definitive taxonomic analysis is planned to conclude a series of papers on biological differentiation in this genus. The triploid forms are indicated by the name of the bisexual species on which they depend for sperm in the field.

In total 567 diploid planthoppers representing 18 populations of eight species of the R. collinus complex (Table 1) were assayed by electrophoresis. For comparison, 4 populations of 2 outgroup species, R. pallens (Stål) and R. albostriatus (Fieber), were included. Triploid females of 6 populations of R. pungens (Ribaut) and one population of Taxon

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Table 1. Origin of Ribautodelphax material used for allozyme studies.

SPECIES	ABREVIATION	LOCALITY	CODE[a]
		R. COLLINUS COMPLEX	
R. collinus	COL	Limburg, Plasmolen.	NL1
R. angulosus	ANG	Utrecht, Leersum.	NL3
R. pungens	PUN	Limburg, Bemelen.	NL4
		Limburg, Kunderberg.	NL5
		Limburg, Maastricht.	NL8
		Namur, Jemelle.	BE1
		Namur, Dinant.	BE2
		Nordrhein-Westfalen, Rodderberg.	DE1
		Baden-Württemberg, Tübingen.	DE4
		Trikkala, Meg. Panagia (Pindos Mts.).	GR1
		Fokis, Elaion.	GR3
		Fokis, Skaloula.	GR4
R. imitans	IMI	Pyrenées Orientales, St. Cyprien Plage.	FR3
R. fanari	FAN	Bouches du Rhône, Etang de Vaccarès	FR4
R. Taxon 1	T1	Gelderland, Hoge Veluwe.	NL6
R. Taxon 2	T2	Florina, Kalo Nero Mts.	GR6
R. Taxon 4	T4	Vaucluse, St. Estève.	FR6
		Bouches du Rhône, Les Baumettes.	FR9
		Aude, Tuchan.	FR10
		Valencia, Sagunto.	ES1
		OTHER RIBAUTODELPHAX SPECIES	
R. pallens	PAL	Sør-Trødelag, Dovrefjell	NO1
		Oppland, Hinseter.	NO2
R. albostriatus	ALB	Srbija, Trstenik	YU1
		Namur, Jemelle.	BE5

[a] Belgium (BE); France (FR); Greece (GR); Netherlands (NL); Norway (NO); Spain (ES); W. Germany (DE) and Yugoslavia (YU).

4 were analysed. It was intended to study only field-collected material, but laboratory-bred material had to be included also, because many Ribautodelphax species are rare. To reduce the possible loss of genetic variation from inbreeding, only individuals of the first five laboratory-bred generations were assayed. Parallel rearings were maintained for each population and males were exchanged each generation (den Bieman, 1987b). Triploid and diploid females of R. pungens and Taxon 4 differ only in karyotype and consequently only laboratory-bred females of both species could be analysed. Some hybrids of the reciprocal crosses between R. pungens (population NL4) and R. pallens (population NO1) were included to check the allelic status of the allozymes.

Methods. Enzyme banding patterns were obtained from single individuals, using horizontal starch gel electrophoresis of extracts of whole specimens (males, females, last instars) squashed on Whatman paper. Standard electrophoretic procedures as described by Ayala et al. (1972) were employed and the following buffer system was used: electrode buffer, 0.135 M tris and 0.045 M citric acid, pH 7.1; gel buffer, electrode buffer, 1 : 16, diluted with water. Electrophoresis was carried out at 4°C.

The following enzymes were stained and yielded ten presumed genetic loci : α-Gpdh (alpha-Glycerophosphate dehydrogenase, EC 1.1.1.8); Pgi (Phoshoglucose isomerase, EC 5.3.1.9); Pgm (Phosphoglucomutase, EC 2.7.5.1); Mdh-1, Mdh-2 (Malate dehydrogenase, EC 1.1.1.37); Me (Malic enzyme, EC 1.1.1.40); 6-Pgdh (6-Phosphogluconate dehydrogenase, EC 1.1.1.43); Ldh (Lactate dehydrogenase, EC 1.1.1.28); Idh-NADP (Isocitrate dehydrogenase, NADP dependent, EC 1.1.1.42) and Idh-NAD (Isocitrate dehydrogenase, NAD dependent, EC 1.1.1.41). Staining according to Shaw and Prasad (1970) was done either in a Tris-HCl buffer, pH 8.0 for Me and Mdh or in a Tris-HCl buffer, pH 7.5 for the other enzymes. For staining of Idh-NAD NADP was replaced by NAD.

Mobibity of alleles was assessed either relative to the mobility of an allele of one genotype of the aphid Sitobion avenae F. (Pgi, Pgm and Mdh-1) or relative to an allele of a triploid clone of R. pungens, population NL4 (remaining enzymes). Differences in mobility of rather similar allozymes were confirmed by running these allozymes side-by-side on the same gel. Allozymes were lettered in order of decreasing anodal migration.

In recent years many methods for the phylogenetic analysis of electrophoretic data have been published (Farris, 1981; Felsenstein, 1984; Hedges, 1986; Mickevich & Mitter 1981, 1983; Patton & Avise, 1983; Rogers, 1986). One of the most significant arguments concerns the use of genetic distance estimates vs. coding as discrete character states. However, at this moment there is not a single 'correct' way to analyse electrophoretic data (Berlocher, 1984). Many Ribautodelphax populations and species differ only in the frequency of the various alleles and therefore their phylogenetic analysis must make use of allele frequencies.

Allozyme frequencies for each population were derived from the electrophoretic results and converted to genetic-distance estimates: D_N (Nei, 1972) and D_R (Rogers, 1972). The UPGMA procedure was used to summarize overall electrophoretic similarity, CLUSTAN of D. Wishart.

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Table 2. Allozyme variation for 10 diploid <u>Ribautodelphax</u> species. A letter indicates the presence of that allele. For details see text.

SPECIES	CODE[a]	N1[b]	N2[b]	α-Gpdh	Pgi	Pgm	Mdh-1	Mdh-2	Me	6-Pgdh	Ldh	Idh-NADP
							LOCI					
COL	NL1	31	26	CG	BE	BDFH	E	A	B	B	B	ABE
ANG	NL3	53	19	CF	EG	FH	E	A	B	B	B	A
PUN	NL4	12	23	DF	G	DFH	AE	A	B	B	B	A
	NL5	16	19	DF	G	DFHI	ACE	A	B	AB	B	A
	NL8	-	13	DF	G	DFH	E	A	B	B	-	A
	DE4	-	15	D	G	ADFH	AE	-	-	B	-	A
	BE1	15	-	DF	G	DFH	AE	-	-	-	-	A
	BE2	15	-	DF	G	DFH	AE	-	-	B	-	A
	GR3	-	9	D	DG	DFH	E	A	B	B	-	A
	GR4	-	24	DF	D	FHI	E	A	B	B	-	AD
IMI	FR3	-	39	C	GH	DF	F	A	B	B	B	A
FAN	FR4	-	46	C	G	DF	E	A	B	B	B	A
T1	NL6	17	16	CDG	E	EHI	E	A	B	B	B	AB
T2	GR6	-	34	C	E	DF	E	A	B	B	B	A
T4	FR6	25	32	C	ACEH	BDFG	F	A	B	-	B	A
	FR9	-	23	C	AEH	BDF	F	A	B	B	-	A
	FR10	-	29	CF	ACH	DFG	F	A	B	B	B	A
	ES1	-	16	CF	AGH	BDF	F	A	-	-	-	A
PAL	NO1	-	27	C	A	CD	DFG	B	B	B	-	A
	NO2	-	18	ABC	A	CDJ	D	-	B	B	A	A
ALB	YU1	-	21	CE	I	H	B	A	A	BC	A	A
	BE5	11	-	C	FHI	FH	B	A	-	-	-	AF

[a] Codes of populations listed in Table 1.
[b] N1, N2: number of field-collected and laboratory-bred individuals, respectively.

Because Nei's D is non-metric, only Rogers' D was subjected to a distance-Wagner analysis using the WAGNER 78 computer package of J.S. Farris.

RESULTS

The diploid species.

No obvious differences in allozyme variability were observed between field-collected and laboratory-bred material from the same populations. Therefore, the data are combined (Table 2). Data derived from males, females and last instars are also combined.

Only one, of the 10 loci sampled, Idh-NAD, appeared to be monomorphic across all populations (Table 2). The banding patterns of the heterozygotes showed that 5 enzymes are dimeric: α-Gpdh, Pgi, Idh-NADP, Mdh-1 and 6-

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Table 3. Nei's standard genetic distance for 22 Ribautodelphax populations based on allozyme frequencies of 5 loci (α-Gpdh, Pgm, Pgi, Mdh-1 and Idh-NADP). Abbreviations listed in Table 1.

POPULATIONS		1	2	3	4	5	6	7	8	9	10	11
1	COL NL1	-										
2	ANG NL3	0.255	-									
3	PUN NL4	0.917	0.538	-								
4	NL5	0.876	0.564	0.016	-							
5	NL8	0.799	0.501	0.022	0.025	-						
6	DE4	1.039	0.600	0.009	0.026	0.051	-					
7	BE1	0.837	0.577	0.012	0.002	0.015	0.028	-				
8	BE2	0.918	0.556	0.008	0.029	0.028	0.015	0.027	-			
9	GR3	0.921	0.502	0.030	0.057	0.069	0.036	0.052	0.045	-		
10	GR4	1.062	0.472	0.075	0.098	0.077	0.101	0.081	0.123	0.092	-	
11	IMI FR3	0.861	0.662	0.963	0.954	0.983	0.958	0.978	0.903	0.979	1.300	-
12	FAN FR4	0.448	0.268	0.235	0.267	0.226	0.266	0.258	0.212	0.312	0.397	0.489
13	T1 NL6	0.223	0.044	0.655	0.723	0.608	0.726	0.694	0.773	0.537	0.530	0.888
14	T2 GR6	0.134	0.098	0.594	0.621	0.397	0.642	0.602	0.564	0.572	0.789	0.515
15	T4 FR6	0.516	0.491	1.131	1.028	1.056	1.174	1.047	1.101	1.147	1.441	0.120
16	FR9	0.532	0.488	1.147	1.053	1.078	1.186	1.072	1.117	1.158	1.414	0.163
17	FR10	0.787	0.832	1.249	1.070	1.038	1.363	1.050	1.231	1.312	1.438	0.204
18	ES1	0.803	0.689	0.953	0.874	0.870	0.994	0.889	0.914	1.016	1.191	0.079
19	PAL NO1	0.689	0.790	1.340	1.135	1.145	1.455	1.131	1.355	1.400	1.529	0.603
20	NO2	1.261	1.244	1.333	1.153	1.171	1.420	1.165	1.343	1.370	1.486	1.085
21	ALB YU1	1.175	0.544	1.354	1.430	1.277	1.440	1.385	1.461	1.257	0.997	0.761
22	BE5	1.062	0.554	1.467	1.574	1.407	1.556	1.524	1.574	1.360	1.120	0.751

		12	13	14	15	16	17	18	19	20	21
12	FAN FR4	-									
13	T1 NL6	0.456	-								
14	T2 GR6	0.253	0.128	-							
15	T4 FR6	0.562	0.492	0.355	-						
16	FR9	0.574	0.569	0.361	0.008	-					
17	FR10	0.753	0.950	0.685	0.088	0.094	-				
18	ES1	0.517	0.877	0.584	0.070	0.098	0.077	-			
19	PAL NO1	0.709	0.906	0.636	0.425	0.306	0.237	0.421	-		
20	NO2	1.145	1.340	1.063	0.739	0.682	0.528	0.807	0.176	-	
21	ALB YU1	0.886	0.675	0.874	0.793	0.793	0.918	0.844	0.871	1.322	-
22	BE5	0.877	0.700	0.872	0.778	0.796	0.925	0.790	0.900	1.471	0.027

Pgdh. One enzyme, Pgm, appeared to be monomeric. No conclusion could be drawn on the polymeric status of the remaining enzymes. Analysis of the hybrids between R. pungens and R. pallens revealed that Pgi is sex-chromosome linked. Ribautodelphax species possess an XX-XO sex-determination system (den Bieman, 1987d). Females have two sex chromosomes and the hybrid females consistently showed the three-banded heterozygote configuration, while the hybrid males showed only one band. Ldh appeared to be sex linked, i.e. present in males and absent in females. Sex linkage of Ldh has also been observed for the planthopper Nilaparvata lugens (Stål) (Claridge, 1980).

Only the five most variable loci were scored for all populations: α - Gpdh, Pgi, Pgm, Mdh-1 and Idh-NADP. Nei's genetic distance is listed in Table 3 and an UPGMA dendrogram was derived from these data (Fig. 1). For four species, two or more populations were analysed. Most conspecific populations showed only minor differences and they clustered together. Differences found among many species of the R. collinus complex are of the same order of magnitude as differences found among conspecific populations.

Eight populations of R. pungens were analyzed in order to estimate the amount of intraspecific allozyme variation on a large geographic scale. The two Greek populations, GR3 and GR4, are geographically separated from the remaining populations and proved to be the most distinct genetically (Fig. 1). However, the two Greek populations differ very much from each other as well. Within the W.European populations no geographic pattern of genetic differences was found.

Within Taxon 4, clear differences in male acoustic signals were observed between population FR10 and the remaining populations suggesting that FR10 might represent another unknown species (den Bieman, 1986). However, our electrophoretic data do not support such a separation (Fig. 1).

For further analysis, data of conspecific populations were pooled. The genetic distance indices for the 10 Ribautodelphax species were calculated based on all loci (Table 4). An average D_N of 0.184 $\pm$ 0.093 (S.D.) was calculated within the R. collinus complex contrasting to considerably greater distance estimates among the R. collinus complex and the two outgroup species: 0.636 $\pm$ 0.093 and 0.692 $\pm$ 0.096 for R. pallens and R. albostriatus, respectively. Several species pairs of the R. collinus complex showed a very low D_N value, for example R. angulosus (Ribaut) and
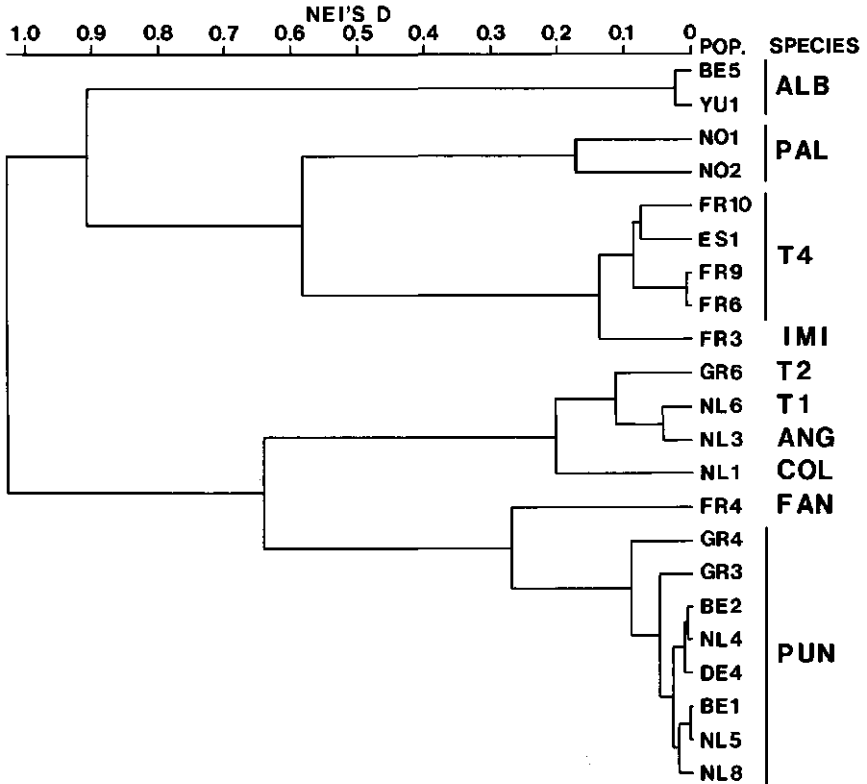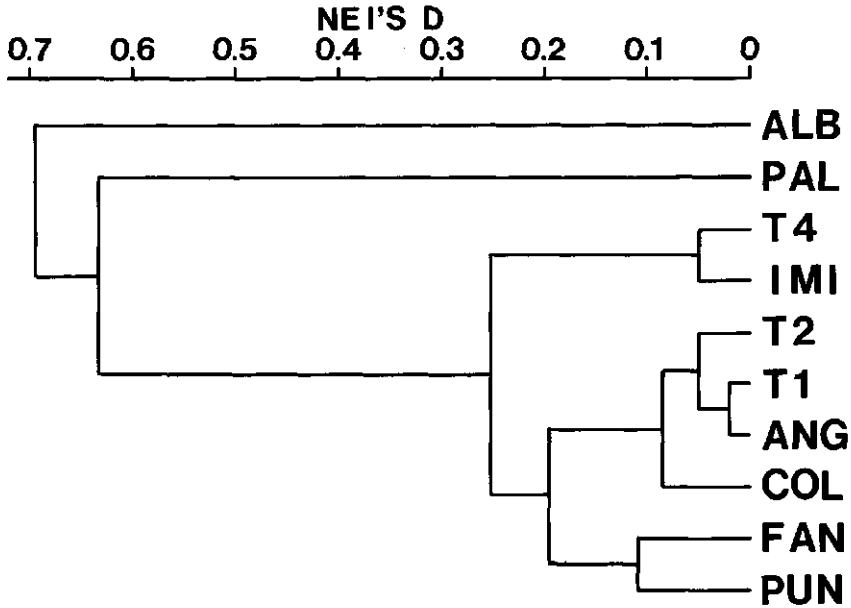
Fig. 1. Dendrogram showing the relationships of 22 Ribautodelphax populations based on Nei's D over 5 loci (UPGMA cluster analysis). Key to abbreviations in Table 1.
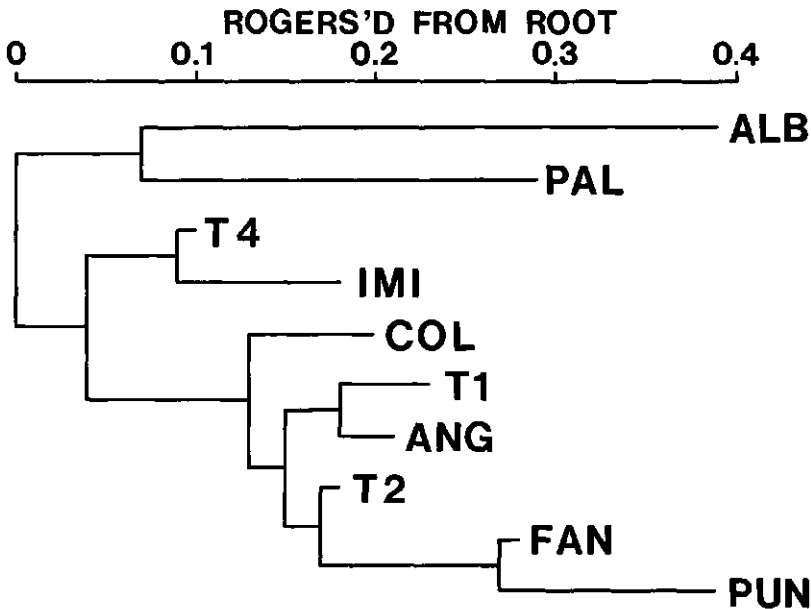
Taxon 1. Species specific alleles were rarely observed within the R. collinus complex (Table 2), whereas the outgroup species R. pallens and R. albostriatus have unique alleles for Mdh-2 and Me, respectively.

The UPGMA dendrogram based on D_R is similar to the one for D_N and only the latter is presented (Fig. 2). All species of the R. collinus complex clustered around the species R. imitans (Ribaut) and Taxon 4, and were well separated from the outgroup species R. pallens and R. albostriatus. A similar result was obtained by constructing the distance-Wagner tree based on D_R (Fig. 3). Only Taxon 2 is placed somewhat differently but still within the same group of species.

Fig. 2. Dendrogram showing the relationships of 10 Ribautodelphax species based on Nei's D over 10 loci (UPGMA cluster analysis). Key to abbrviations in Table 1.

Fig. 3. Distance Wagner Tree (midpoint rooting) for 10 Ribautodelphax species over 10 electrophoretic loci. Key to abbreviations in Table 1.

Table 4. Nei's standard genetic distance (upper right) and Rogers' genetic distance (lower left) for 10 Ribautodelphax species based on the allele frequencies of 10 loci. Abbreviations listed in Table 1.

SPECIES		1	2	3	4	5	6	7	8	9	10
1	COL	-	0.106	0.300	0.300	0.180	0.090	0.059	0.212	0.639	0.789
2	ANG	0.153	-	0.209	0.253	0.117	0.020	0.045	0.216	0.667	0.550
3	PUN	0.299	0.224	-	0.336	0.110	0.236	0.235	0.342	0.776	0.858
4	IMI	0.300	0.178	0.300	-	0.202	0.309	0.223	0.049	0.603	0.668
5	FAN	0.191	0.146	0.127	0.191	-	0.185	0.113	0.223	0.640	0.700
6	T1	0.148	0.080	0.248	0.294	0.203	-	0.057	0.240	0.701	0.613
7	T2	0.119	0.082	0.222	0.285	0.115	0.099	-	0.177	0.610	0.695
8	T4	0.229	0.225	0.310	0.099	0.231	0.257	0.200	-	0.450	0.660
9	PAL	0.489	0.493	0.538	0.475	0.490	0.505	0.475	0.392	-	0.739
10	ALB	0.558	0.450	0.587	0.506	0.519	0.484	0.516	0.498	0.542	-

Table 5. Genotypes of triploid pseudogamous Ribautodelphax females. A letter indicates the presence of that allele, two or three letters indicate heterozygote conditions. Codes of populations listed in Table 1. N, number of females.

CODE	N	α-Gpdh	Pgi	Pgm	Mdh-1	Mdh-2	Me	Idh-NADP
R. pungens populations.								
NL4	14	D	G	F	AE	A	B	A
NL4	10	D	G	DF	E	A	B	A
NL5	8	D	G	DF	E	A	B	AC
NL5	7	D	G	DF	E	A	B	A
NL8	20	D	G	F	AE	A	B	A
DE1	12	D	-	HF	-	A	B	A
DE4	10	D	G	AF	E	A	B	A
GR1	11	D	G	DFI	-	A	B	A
Taxon 4 populations								
FR9	15	C	AH	D	F	A	B	A
FR9	10	C	AH	BD	F	A	B	A

Pseudogamous triploid females.

The genotypes of the pseudogamous triploid females found in populations of R. pungens and Taxon 4 are given in Table 5. A calculation of gene frequencies is not presented because gen-dosis effects could not be interpreted unequivocally in heterozygotes expressing two different alleles. Consequently, we could not prove directly that all three alleles are expressed for each enzyme locus in the triploid females. Fortunately, one genotype in population GR1 of R. pungens expressed three different alleles (D, F, I) in triple heterozygote condition, proving that, at least in Pgm, all three alleles can be expressed.

At several localities two different genotypes were found (Table 5) though only few triploids could be screened from most populations. Interpopulation differences in triploid genotypes were obvious. Ten females of each of the offspring of 7 individual triploid females of R. pungens (population NL4) were examined to prove clonal propagation. The absence of any electrophoretic variation within the offspring of these females and their fixed heterozygosities suggest that the variation in electrophoretic patterns of the triploids is primarily clonal variation and that recombination is absent or rare.

Data on syntopic triploids and diploids from 5 localities can be compared (Table 2 and 5). Unfortunately, diploids of the populations GR1 and DE1 could not be studied. All alleles detected in the triploids of R. pungens and Taxon 4 also occurred in the diploids of the same or nearby populations. The only exception concerns the C-allele of Idh-NADP which occurred in some of the triploids of R. pungens, population NL5. This C-allele was not detected in any of the diploid Ribautodelphax species. Alleles with a high frequency in diploids were always present in the syntopic triploids. The assumed close relationship between triploids and diploids of the same population is further supported by the discovery of the unique A-allele of Pgm in triploids and diploids of R. pungens, population DE4.

Triploids of hybrid origin should show alleles of both parent species provided that the three genomes are expressed as was observed in a Greek genotype for Pgm. Comparisons of the triploid genotypes with the allelic patterns of the various Ribautodelphax species shows obvious differences with many of the non-sperm-donor species (Table 2 and 5). The most

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information is available for the R. pungens triploids. The strongest resemblance in allozyme patterns is found with R. angulosus and R. fanari. However, the triploids of R. pungens show the D-allele of α-Gpdh uniformly, whereas R. angulosus and R. fanari possess the alleles C and F (Table 2). R. imitans, the only potential second parent species for the triploids of Taxon 4, however, does not differ sufficiently from the diploid Taxon 4 genotypes to allow for any information from allozyme data on the mode of origin of these triploids.

DISCUSSION

The diploid species.

The electrophoretic differences among the species of the R. collinus complex are rather small and are of the same order of magnitude as the differences found among conspecific Ribautodelphax populations, e.g. R. pungens populations. Thorpe (1983) found that only 0.5% of the 900 estimates of Nei's D for congeneric species was below 0.105. This figure is, however, 21% for the R. collinus complex. From these data and from the absence of species specific alleles it may be concluded that electrophoretic data do not add much information with respect to the definition of species within the R. collinus complex, at least not without consideration of more loci.

The comparatively low D_N values for the Ribautodelphax species could result from the electrophoretic conditions used. Changes in these conditions or the application of other electrophoretic methods might reveal hidden variation. Unfortunately, the D_N values could not be compared with those from other planthopper genera because they have not been studied electrophoretically.

Our electrophoretic data are more informative from a phylogenetic point of view. However, one should be aware that not all species of the R. collinus complex were included in this study (den Bieman, in prep.) and that only few loci were studied, therefore only tentative conclusions can be drawn. The results of both the UPGMA clustering and the Wagner-distance analysis suggest that the species of the R. collinus complex are more strongly interrelated than they are with either R. pallens or R. albostriatus. Morphologic, acoustic and hybridization data (den Bieman,

1986, 1987c, in prep). suggest that R. pallens is more similar to the R. collinus complex than R. albostriatus. Our electrophoretic data do not contradict this relationship (Fig. 2).

Morphologic, host-plant and hybridization data (den Bieman, 1987b, c, in prep) suggest a close similarity between R. imitans and Taxon 4. Our electrophoretic data support this relationship. R. imitans and Taxon 4 show only minor differences and possess both the F-allele of Mdh-1 which was found only at low frequency in R. pallens. UPGMA and Wagner trees indicate the existence of two sister groups within the R. collinus complex, one of which is formed by R. imitans and Taxon 4.

R. pungens and Taxon 1 resemble each other morphologically, but our electrophoretic data and also acoustic, host-plant and hybridization data contradict a close relationship (den Bieman, 1986, 1987b, c, in prep).

Origin of pseudogamous triploid females.

The discussion on the origin of the pseudogamous triploid Ribautodelphax females will focus on the triploids associated with R. pungens because the most information is available for these. Allozyme data clearly indicate that the triploids of R. pungens and of Taxon 4 represent different forms; compare for example the results on α-Gpdh and Mdh-1 (Table 5).

Triploids of hybrid origin should possess characteristics of both parent species if the three genomes are expressed. Our data on Pgm suggest that the three genomes are indeed expressed in Ribautodelphax. Electrophoretic data on many other triploid animals also demonstrate that all genomes are expressed in triploid genotypes (Christensen et al., 1978; Harshman et al., 1985; Parker et al., 1977; Turner et al., 1983; Uzzell & Goldblatt, 1967; De Almeida Toledo et al., 1985; Vrijenhoek, 1984). A comparison of the electrophoretic data of the triploids with the results obtained for diploid genotypes shows that virtually every allele found in the triploid genotypes is also found in their sperm-donor species. The only allele found in one triploid genotype but not found in any diploid genotype may either result from a mutation after the origin of this triploid clone or is rare in diploids and therefore was not found in the syntopic diploid sperm-donor species because the sample size was too small. The triploids

differed in at least one locus from the non sperm-donor Ribautodelphax species. These results make an allopolyploid origin of the triploid females unlikely and thus an autopolyploid origin might be a more logical explanation.

There are other results from prior investigations which also do not support an allopolyploid origin either. Triploids and diploids of R. pungens showed no obvious differences in host-plant relations and acoustics despite clear differences in these respects between the bisexual Ribautodelphax species (den Bieman, 1986, 1987b). Though many hybrids were produced, no triploids resulted from an extensive crossing program (den Bieman, 1987c). Similar observations were made for the triploids and diploids of Taxon 4.

Karyologic observations suggest a possible mechanism for the origin of the triploids. Unreduced eggs with a diploid number of chromosomes were detected in several Ribautodelphax species (den Bieman,1987d) and would give rise to triploids after fusion with haploid sperm. A triple banding pattern of a monomeric enzyme as found for Pgm in the Greek triploid genotype may originate if the unreduced egg is produced by a heterozygote female and then fertilized by sperm bearing a different allele. Of course mutation of a di-heterozygote triploid may also produce a tri-heterozygote triploid.

The great variability in genotypes of the triploids within and among populations together with the genetic similarity with the local population of the sperm-donor species suggest that the triploid clones originated independently from different diploids and consequently arose polyphyletically. The most frequent alleles of the diploids were also found in the syntopic triploids suggesting that most triploids originated from the local diploid sperm-donor species. This hypothesis is strongly supported by the discovery of an unique allele of Idh-NADP in both the diploids and triploids of R. pungens, population DE4.

A similar conclusion was drawn from the analysis of the acoustic variation pattern of the triploid and diploid females of R. pungens and Taxon 4 (den Bieman, 1987a). Clear differences in acoustic signals were observed between many populations. However, the song patterns of syntopic triploid and diploid females showed many common characteristics. This observation again suggests a multiple origin of the triploids with triploids mainly originating from the local bisexual population.

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An alternative explanation for the electrophoretic and acoustic patterns would be a monophyletic origin of the triploids. However, after this event numerous changes in the parthenogenetic triploids parelleling the variation in the bisexual diploids would have been required making this mode of origin less probable.

Autopolyploidy appears to be an uncommon mode of origin of polyploid parthenogenetic animals as suggested by the many parthenogens of allopolyploid, hybrid origin (reviews in Bullini, 1985; White, 1978).Only a few examples of documented autopolyploid origin of parthenogenetic animals have been published (Lokki & Saura, 1980). In Delphacids, pseudogamous triploid females have also been discovered in the genus Muellerianella. Several investigations suggested an allopolyploid origin of these triploids (Drosopoulos, 1978; Booij 1981, 1982a, but see also Booij, 1982b). Further research, especially electrophoretic analyses, is necessary to investigate whether the mode of origin of the triploid females of the genera Ribautodelphax and Muellerianella has been truely different.

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COEXISTENCE OF PSEUDOGAMOUS AND BISEXUAL PLANTHOPPERS OF THE GENUS RIBAUTODELPHAX (HOMOPTERA, DELPHACIDAE).

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ABSTRACT

The occurrence of pseudogamous triploid females in populations of the diploid species Ribautodelphax pungens (Ribaut) was studied throughout Europe. Considerable differences in triploid frequencies were found between populations but no geographic pattern was discerned. Triploid frequencies proved to be stable from generation to generation. The twofold reproductive advantage of the triploid females is counterbalanced by active mate discrimination by diploid males against triploids, resulting in low insemination ratios of triploid females in field populations with high triploid frequencies. Differences in female acoustic signals may be important in this respect. Winter mortality of diploid larvae was higher than that of triploid larvae. No differences in phenology were found between triploid and diploid females

INTRODUCTION

An intriguing problem in the population ecology of pseudogamous forms concerns the permant coexistence of these forms with their sexual "host" species (Stenseth et al., 1985). Pseudogamy is a special form of parthenogenetic reproduction in which females do not produce offspring unless they are inseminated. However, the sperm does not contribute any genetic material to the all-female progeny of the pseudogamous females. This relationship forces the pseudogamous females to coexist with a sexual species and to compete for sperm with the sexual females.

Theoretically, pseudogamous females have a "twofold" reproductive advantage over the sexual females because they save the cost of producing males. If this potential were fully realized, the ratio of pseudogamous to

sexual females would become increasingly biased and more and more pseudogamous females would have to mate with a diminishing number of males. Mixed populations would become extinct when finally, by sampling error, the remaining males inseminated only pseudogamous forms (Moore, 1984; Stenseth et al., 1985; Wilbur, 1971). However, in field populations the ratios of pseudogamous females to sexual females proved to be rather stable from year to year in planthoppers (Booij & Guldemond, 1984), salamanders (Wilbur, 1971) and moths (Mitter et al., 1979). Several theoretical models have been developed to analyse the competitive relationships between pseudogamous forms and their sperm-donor species (Kiester et al., 1981; Moore, 1979; Stenseth et al., 1985), but relatively few complexes have been analyzed experimentally.

Several mechanisms have been suggested that might contribute to stable coexistence of pseudogamous and sexual forms, including mate preference (McKay, 1971; Uzzell & Goldblatt, 1967), reduced reproduction capacity of pseudogamous forms compared to their host species (Uzzell, 1964, 1969), competitive interactions between both types of larvae (Wilbur, 1971) and differentiation in phenology resulting in differential insemination (Uzzell, 1969).

A second characteristic of many complexes with pseudogamous forms is the variability of the ratio of pseudogamous to sexual females within a species' range of distribution (Booij & Guldemond, 1984; Wilbur, 1971; Mitter et al., 1979).

Pseudogamous females have been found in populations of two planthopper species (Delphacidae) of the genus Ribautodelphax Wagner. These pseudogamous females appeared to be triploid (den Bieman, 1987d). Karyotypic and electrophoretic studies revealed that the offspring of the pseudogamous triploid Ribautodelphax females are genetically identical to their mothers. Ecologic, karyologic, acoustic, elecrophoretic and hybridization studies (den Bieman, 1987a, b, c, d; den Bieman & Eggers-Schumacher, 1987) strongly suggested an autopolyploid origin of these pseudogamous triploid females.

The present study focuses on the association between pseudogamous triploid females and the diploid sexuals of R. pungens (Ribaut). This planthopper species mainly occurs in dry grasslands and lives on several Brachypodium species (den Bieman, 1987b). R. pungens is bivoltine but may have more generations in SE Europe. The occurrence of pseudogamous

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triploids in populations of R. pungens was studied throughout Europe and the dynamics of mixed populations were investigated. Field and laboratory experiments were undertaken to analyse mechanisms that might contribute to a stabilization of mixed populations.

MATERIAL AND METHODS

Samples of R. pungens were taken at 27 localities in different parts of Europe (Table 1). To discriminate between pseudogamous triploid females and sexual diploid R. pungens females chromosome preparations were made (den Bieman, 1987d). The two female types are indicated in this paper as triploids and diploids, respectively. The triploid (asexual) frequency is defined here as the ratio of triploids to diploids + triploids (Booij & Guldemond, 1984).

Laboratory experiments were carried out with the Dutch population NL4 in a greenhouse at 20°C and long-day conditions (LD 18:6). Males and females were isolated as fifth instars and the resulting adults, 7 to 9 days old, were used for experiments. To assess the reproductive capacity of females, single pairs of males and females were confined in a cage for 30 days and their adult offspring was counted. Mate preference experiments were carried out in cages with one male and three triploid and three diploid females. After three days the insemination was checked by examining the spermatheca of the females for the presence of mobile sperm. The wing length, macropterous or brachypterous, was used as a marker to distinguish between diploid and triploid females; only brachypterous males were tested.

Acoustic communication during mate selection was observed in the following experiment (see also Claridge et al., 1984). Three stems of Brachypodium pinnatum were placed in series with the leaves of the outer stems just touching the central stem. A male was placed on the central stem, a diploid female on one of the outside stems and a triploid female on the other. The substrate-borne acoustic signals were recorded from the outside stems (den Bieman, 1986). The signals from each of the outside stems could be heard at a different side of a headphone.

Table 1. Triploid frequencies in mixed populations of R. pungens and triploid pseudogamous forms throughout Europe. Repeated samples of the same locality are pooled.

	Code[a]	Locality	Sample date	Sample size	Triploid frequency
1	NL5	Limburg, Kunderberg.	1981/1985	80	0.05
2	NL7	Limburg, Wrakelberg.	1983/1985	73	0.81
3	NL9	Limburg, Wahlwiller.	20.VIII.1983	31	0.16
4	NL8	Limburg, Maastricht.	18.IV.1981	18	0.22
5	NL4	Limburg, Bemelen.	1980/1985	607	0.80
6	BE2	Namur, Dinant.	20.VII.1982	16	0.69
7	BE3	Namur, Nismes.	21.VII.1982	42	0.71
8	BE6	Namur, Han sur Lesse.	19.VII.1982	20	0.15
9	BE7	Namur, Rochefort.	19.VII.1982	32	0.75
10	BE1	Namur, Jemelle.	18.VII.1982	18	0.33
11	BE4	Luxembourg, Torgny.	22.VII.1982	27	0.81
12	FR21	Jura, Salins-les-Bains.	27.IV.1984	20	0.30
13	FR11	Jura, Pont d'Hery.	27.IV.1984	24	0.79
14	FR20	Ain, Treffort.	26.IV.1984	38	0.89
15	FR19	Ardèche, Asperjac.	16.IV.1984	17	0.53
16	FR1	Ardèche, Coulens.	29.IV.1983	8	0.00
17	FR2	Alpes de Hte Provence, Montfuron.	1983/1984	20	0.00
18	DE2	Hessen, Amöneburg.	1979/1982	29	0.00
19	DE7	Baden-Württemberg, Hundersingen.	17.VIII.1982	10	0.80
20	DE4	Baden-Württemberg, Tübingen.	16.VIII.1982	17	0.71
21	DE6	Baden-Württemberg, Hayingen.	17.VIII.1982	10	0.90
22	YU2	Slovenija, Postojna.	4.VII.1983	10	0.00
23	YU4	Croatia, Zdhihovo.	5.VII.1983	10	0.80
24	YU3	Bosni-Hercegovina, Jajce.	8.VII.1983	33	0.88
25	GR2	Florina, Kotas.	21.VII.1983	15	0.33
26	GR9	Kozani, Vourinos Mts	18.VIII.1983	13	0.77
27	GR3	Fokis, Elaion.	23.VII.1983	11	0.09

[a] Belgium (BE), France (FR), Greece (GR), Netherlands (NL), W. Germany (DE) and Yugoslavia (YU).

RESULTS

Triploid frequencies throughout Europe

Triploid females occurred in most populations of R. pungens (Fig. 1). Distributional data of R. pungens were obtained from museum collections, R. Remane (pers. comm.), Nast (1976), Ossiannilsson (1978) and personal observations. Due to varying sample sizes the estimated triploid frequencies are not equally accurate for each population (Table 1). The triploids were absent in only 4 of the 27 populations and reached
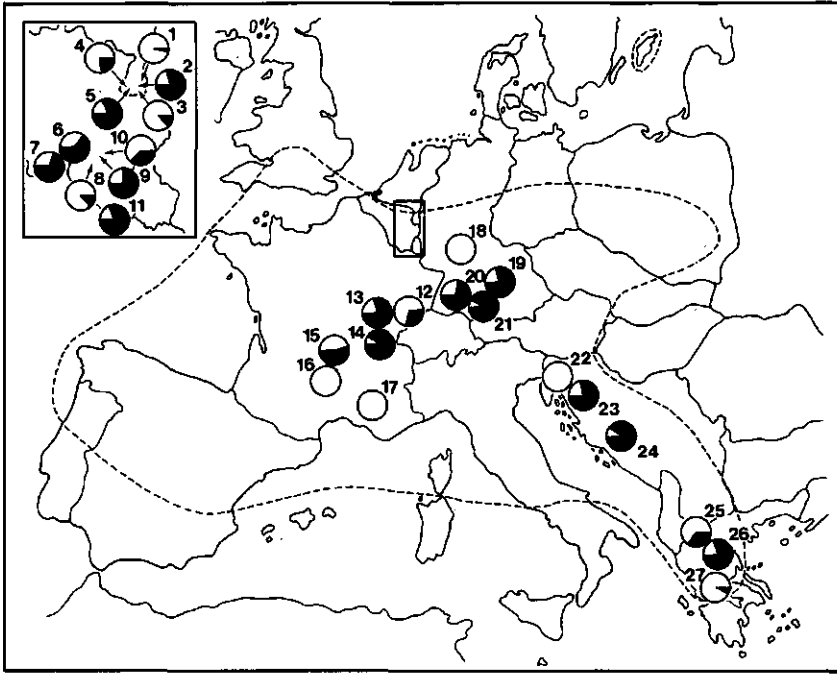
Fig. 1. Frequency of pseudogamous triploid females in populations of the sexual diploid species R. pungens in different parts of Europe. White: diploid females, black: triploid females. The broken line indicates the range of distribution of R. pungens.

frequencies of 0.90 in others. Considerable differences were observed between nearby populations with no obvious differences in vegetation or exposition; e.g. the Dutch populations NL5 and NL7 are only three km apart but have triploid frequencies of 0.05 and 0.81, respectively.

No geographic pattern could be discovered for the triploid frequencies. Triploids appeared to occur throughout the area of R. pungens. Highly female biased sex ratios of some recently sampled Spanish populations suggest that triploids also occurred at the SW border of the area of R. pungens.

Stability of triploid frequencies in mixed populations.

Three Dutch populations of R. pungens were regularly sampled to study changes in triploid frequencies (Table 2). In one population (NL5) the

Table 2. Generation to generation changes in the
triploid frequency of R. pungens populations.
Repeated samples within one generation are pooled.

Population[a]	Generation[b]	Sample size	Triploid frequency
NL4	I, 1985	30	0.63
NL4	II, 1984	35	0.74
NL4	I, 1984	63	0.89
NL4	II, 1983	28	0.86
NL4	I, 1983	324	0.82
NL4	II, 1982	53	0.89
NL4	I, 1982	26	0.62
NL4	I, 1981	23	0.61
NL4	I, 1980	25	0.72
NL5	I, 1985	23	0.04
NL5	II, 1983	34	0.06
NL5	II, 1982	12	0.00
NL5	I, 1981	11	0.09
NL7	I, 1985	30	0.83
NL7	II, 1984	24	0.71
NL7	II, 1983	10	0.90
NL7	I, 1983	9	0.89

[a] Codes listed in Table 1.
[b] I, first generation; II, second generation.

triploid frequency remained below 0.10 in four successive years. In the
other populations (NL4 and NL7) the triploid frequencies were much higher:
0.61 to 0.90. Although changes occurred, there were no indications for a
general increase of the triploid frequencies. The initial increase of the
triploid frequency of population NL4 from 1981 to 1984 was followed by a
decrease to about the same level as in 1981.

Reproductive capacity

    The twofold reproductive capacity of the triploid females is perhaps
the most obvious advantage of being parthenogenetic. This advantage is,
however, only realized if there is no concomitant loss in fecundity and if
other reproductive factors are equal, e.g. if both female types have equal
chances to be inseminated.
    Triploid females produced on average 141.3 ± 84.4 (SD, N=12) adult
offspring and diploid females 133.8 ± 61.5 (N=18) (Mann-Whitney test,
$U_{(12,18)} = 109$, $p > 0.10$). Triploids produced also slightly more offspring
than diploid females in cages with 10 pairs of males and females (den
Bieman, 1987c).

In field populations of R. pungens with high triploid frequencies the insemination percentages of the diploids were much higher than those of the triploids (Table 3). Nearly all diploids appeared to be inseminated in contrast to less than 50% of the triploids. In populations with a low triploid frequency, a higher fraction of the triploids was inseminated (Table 3). Population NL4 was sampled regularly and, interestingly, the highest insemination percentage of the triploids was found in the sample with the lowest triploid frequency.

The differences in mating success between the two female types may be the result of "passive" or "active" discrimination. Ribautodelphax females deposisit their eggs in small groups. Consequently, diploid females are more likely to be near a male (e.g. a brother) when they emerge than are triploid females, also because of the low mobility of planthopper larvae and brachypterous adults. Such a process is "passive" in that mate discrimination is not required. Because of the low densities of most R. pungens populations a study of micro-distributional differences between diploids and triploids was impossible. However, passive discrimination might also be caused by phenological differences. Laboratory and field observations showed that the first adults emerging were generally males (den Bieman, 1987c) and shorter developmental periods of the diploid females than those of the triploids would result in higher insemination chances of the former. A sample of larvae (3th to 5th instars) was taken of the NL4 population on 3.IV.1984. In an outdoor insectarium these larvae were reared to adults and each day the newly emerged adults were collected and the females karyotyped. The day of emergence of the first female was taken as reference point. Males appeared to have the shortest developmental time: 2.4 ± 3.6 days (SD, N=10), diploid females: 8.7 ± 7.5 days (N=11) and triploids 13.9 ± 7.9 (N=61). The differences between the diploid and triploid females were not significant ((Mann-Whitney test corrected for ties: $U_{(11,61)} = 378.5$, $t_s = 0.675$, p > 0.50). Therefore, the differences in mating success can not be explained by phenological differences.

Active mate discrimination was tested in the laboratory. Males inseminated more diploids in 28 of the 40 cages, showed no preference in 9 cages and preferred triploids over diploids in only 3 cages (Sign test, T = 25, p < 0.001). In total, 56% of the diploid females and 22% of the triploid

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Table 3. Insemination frequencies of pseudogamous tripoid and bisexual diploid females in field populations of R. pungens.

Popu-[a] lation	Sample data	Triploid[b] frequency	Diploid females		Triploid females	
			Sample size	% Insemination	Sample size	% Insemination
NL4	15.V.85	0.67	7	85.7	14	42.9
NL4	9.VIII.84	0.74	5	100.0	21	30.0
NL4	20.VIII.83	0.86	4	100.0	24	33.3
NL4	28.V.83	0.93	4	100.0	56	33.9
NL4	26.IV.83	0.81	25	88.0	112	27.7
NL4	3.VIII.82	0.89	6	100.0	41	26.8
NL7	15.V.85	0.83	4	100.0	25	48.0
FR20	26.IV.84	0.89	4	100.0	34	41.2
YU3	8.VII.83	0.88	4	100.0	26	23.1
NL5	15.V.85	0.04	22	95.5	1	100.0
NL5	20.VIII.83	0.06	32	93.8	2	100.0
NL9	20.VIII.83	0.25	15	100.0	5	60.0
FR21	27.IV.84	0.30	14	92.9	6	100.0
GR2	21.VII.83	0.33	10	100.0	5	100.0

[a] Codes listed in Table 1.
[b] Based on karyotypic observations.

females were inseminated. Males showed no preference for either macropterous or brachypterous females, of the diploids 56 and 56% and of the triploids 23 and 21% were inseminated, respectively. In this experiment, males inseminated 0.77 ± 0.34 females/day (Mean $\pm$ SD, N = 40).

Many mixed populations of R. pungens could be reared in the laboratory for ten or more generations. Thus, triploids did not outcompete the sexual diploids in mass rearings. From several cages with triploid frequencies of 0.33-0.52 of population NL4 (3th generation) females were collected, karyotyped and checked for insemination. A significantly higher fraction of the diploids (86%, N = 170) appeared to be inseminated compared to only 49% (N = 165) of the triploids ($x^2_{(1)}$ = 51.86, p < 0.001). This difference must have been caused by active mate discrimination because differences in micro-distribution between the two female types can not play an important role in these small cages with several hunderds of males and females.

Mate discrimination behaviour.

In the mate selection experiment, fourteen "trios" consisting of a male, a diploid and a triploid female were observed for maximally one hour

Table 4. Changes in the triploid frequency
within the first generation of 1983 of
population NL4 of <u>R. pungens</u>. L: larvae; A:
adults.

Sample date	Sample size	Triploid frequency
29.X.82[a]	53 (L)	0.68
29.III.83	73 (L)	0.86
26.IV.83	138 (A)	0.81
28.V.83	60 (A)	0.93

[a] Overwintering larvae.

each. Within this period, six of the diploid females and only one triploid
female were inseminated. This limited experiment revealed an interesting
difference in acoustic communication between the two female types. In
planthoppers, it is usually the male that starts singing and the female
that answers; next the male starts searching, while the female waits.
During this search the acoustic communication is continued. The first 30
exchanges of signals of each trio were recorded. In 87% of the cases the
male signal was answered first by the diploid female followed by the call
of the triploid female. Sometimes the male emitted a signal directly after
the call of the diploid female, leaving no opportunity for the triploid
female to respond. Whether this difference in response-delay period is
really important to mate discrimination remains uncertain. The single
inseminated triploid resulted from a trio in which the diploid female
always called before the triploid female.

<center>Larval mortality</center>

Differences in larval mortality were not observed between the two
female types in laboratory rearings. However, in field populations the
first generation of adults develops from overwintering larvae and
differential mortality might occur between diploids and triploids. One
sample of larvae from the same locality was taken before and one after the
winter, and subsequently reared to adulthood in the laboratory and
karyotyped (Table 4). Triploids appeared to have a significantly lower
larval winter mortality ($x^2_{(1)}$ = 6.061, $0.05 > p > 0.02$). The increasing
triploid frequency towards the end of the first generation (Table 4) is

probably due to differences in longevity between mated and unmated females.
Nearly 100% of the diploid females was inseminated in field populations
(Table 3). Laboratory observations showed that mated females, both diploids
and triploids, lived much shorter than unmated females, probably due to
oviposition wastage. For this reason most of the diploid females will have
died already long before the end of the generation.

DISCUSSION

Drosopoulos (1976, 1977) discovered pseudogamy for the first time in
planthoppers. He and Booij & Guldemond (1984) studied the coexistence of
pseudogamous triploid females and the diploid species _Muellerianella_
fairmairei (Perris). They found a stable triploid frequency in several
populations, but a stabilization mechanism was not discovered. Field
observations, however, suggested that sexual females might have better
insemination chances (Booij & Guldemond, 1984). In mixed laboratory
populations, the pseudogamous triploid females rapidly outcompeted the
sexual _M. fairmairei_ (Drosopoulos, 1977) in contrast to the results for
mixed laboratory populations of _R. pungens_.

In laboratory experiments, no significant differences were found
between the reproductive capacity of the sexual diploid females of _R._
pungens and that of the pseudogamous triploid females living associated
with this species. Consequently, since triploid females have twice the
growth potential of the bisexual species, the triploids would competitively
exclude the diploids, if the two compete for a common limiting resource.
But this would bring about the demise of the triploids, because they would
be deprived then of their sperm source. The common limiting resource of the
two female types might be the availability of sperm, for the insemination
capacity of _R. pungens_ males is low, also compared to that of other
planthopper species (den Bieman, 1987c).

Field observations showed that triploid frequencies remained
relatively stable, suggesting that the twofold reproductive advantage of
the triploids is not realized. _R. pungens_ males actively discriminate
against triploid females in choice experiments, but still some of the
triploids are inseminated. This behavioral mechanism regulating the sperm
availability to the triploids results in low, 20-50%, insemination
frequencies of triploids in mixed field populations with high triploid

frequencies, while nearly 100% of the diploid females are inseminated. In this respect, R. pungens resembles comparable complexes in fishes and salamanders where mate discrimination is an important mechanism to stabilize mixed populations (McKay, 1971; Moore, 1984, 1979; Moore & McKay, 1971, Uzzell, 1964, 1969; Uzzell & Goldblatt, 1967).

To enable stable coexistence, this behavioral mechanism has to be frequency dependent (Stensteth et al., 1985). Data on the insemination frequencies of the triploids of population NL4 suggest that a higher percentage of the triploids was inseminated with decreasing triploid frequencies. In field populations with a rather low triploid frequency (< 40%) nearly all triploid females are inseminated and mate discrimination seems ineffective. Field populations with such low triploid frequencies are stable, but the stabilizing mechanism is unknown. One possibility might be that, because of active or passive mate discrimination, the diploid females are inseminated before the triploids, consequently leaving a shorter reproduction period for these triploids.

In planthoppers, acoustic communication might play an important role in mate selection (Claridge, 1985). Den Bieman (1986, 1987a) described the acoustic signals of two Ribautodelphax species with mixed populations and of eight species without pseudogamous females. Compared to these eight species, the acoustic signals of the triploid and diploid females of R. pungens showed a remarkable variability within and between populations. Differences observed in female acoustic signals, e.g. more spontaneous calls, shorter response-delay periods, longer signals etc., might be important for mate selection by males, but their actual importance has still to be demonstrated experimentally.

Perfection of the male's discrimination against pseudogamous triploid females would be advantageous to the males. Mating with a pseudogamous triploid is a waste of sperm and time for a male because his genetic material is not passed to the offspring of the triploid. The association of pseudogamous females and a bisexual species might be a transitory stage in an evolutionary sequence leading either to the extinction of the pseudogamous females or to the independence of the triploids of the sexual species (Wilbur, 1971). Extinction would follow the perfection of mate discrimination. Truely parthenogenetic Ribautodelphax females have not yet been found, but recently the first truely parthenogenetic triploid planthopper population was discovered within the genus Delphacodes (den

Bieman & de Vrijer, 1987).

Triploid frequencies of mixed R. pungens populations vary enormously (0.05-0.90). No geographic variation pattern was discovered and nearby populations showed strong and consistent differences. The factors responsible for these dramatic differences in the success of pseudogamous triploid females relative to sexual diploid females are unknown. Similar differences were found among mixed populations of the planthopper M. fairmairei (Booij & Guldemond, 1984), Poeciliopsis fishes (Vrijenhoek, 1978), Ambystoma salamanders (Wilbur, 1971 Uzzell, 1964) and of the bark beetle Ips acuminatus (Bakke, 1968). Booij & Guldemond (1984) found that the triploid frequency of mixed populations of M. fairmairei tended to increase with altitude and latitude and was related to several climatic variables. The model of Stenseth et al. (1985) suggests that interpopulation differences in mate selection or location might influence the triploid frequencies. For none of these factors indications were found in R. pungens. However, the slightly different larval winter mortality of triploids and diploids suggests that ecologic differences might exist between the two female types and these differences could influence the triploid frequency.

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TAXONOMIC EVALUATION OF THE <u>RIBAUTODELPHAX</u> <u>COLLINUS</u> COMPLEX (HOMOPTERA, DELPHACIDAE).

by

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ABSTRACT

This paper is the last of a series of papers on the biosystematics of the <u>Ribautodelphax</u> <u>collinus</u> complex. Male genital structures are reanalysed and are combined with the earlier assessed differences in acoustics, host-plant relationships, allozymes and hybridization capacity. The hirtherto recognized species of this complex represent true biological species: <u>R. fanari</u> Asche, Drosopoulos & Hoch, <u>R. collinus</u> (Boheman), <u>R. imitans</u> (Ribaut), <u>R. pungens</u> (Ribaut) and <u>R. angulosus</u> (Ribaut). Lectotypes have been designated for the last three species. Four new species were described likewise on multidisciplinary criteria: <u>R. imitantoides</u>, <u>R. vinealis</u>, <u>R. kalonerensis</u> and <u>R. ventouxianus</u>. Three other new species had to be described on the basis of morphology only: <u>R. noguerae</u>, <u>R. nevadensis</u> and <u>R. libanonensis</u>. A key to the males of the European species of the <u>R. collinus</u> complex is given. Comparison with two outgroup species shows that the <u>R. collinus</u> complex is more related to <u>R. pallens</u> (Stål) than to <u>R. albostriatus</u> (Fieber). The taxonomic status of the pseudogamous triploid females associated with <u>R. pungens</u> and <u>R. imitantoides</u> is discussed.

INTRODUCTION

The planthopper genus <u>Ribautodelphax</u> (Delphacidae) was erected by Wagner (1963) to include <u>R. pallens</u> (Stål, 1854), <u>R. albostriatus</u> (Fieber, 1866) and a group of species close to <u>R. collinus</u> (Boheman, 1847): <u>R. imitans</u> (Ribaut, 1953), <u>R. angulosus</u> (Ribaut, 1953) and <u>R. pungens</u> (Ribaut, 1953). Some species have been described in the last 20 years mainly from the Soviet Union. The genus now comprises 19 species. Besides those mentioned above three species occur in Europe: <u>R. ochreatus</u> (Vilbaste,

1965), R. falakron Asche, Drosopoulos & Hoch, 1986 and a further species of the R. collinus complex, R. fanari Asche, Drosopoulos & Hoch, 1986.

The genus Ribautodelphax was named in honour of the late French homopterologist Henry Ribaut. He was the first (1953) to study the male genital structures of these species in detail, which resulted in the discovery of three new species. He already noticed that some structures of the male genitalia might be variable, e.g. the number of teeth on the aedeagus. However, some of the structures considered by Ribaut as species specific proved also to be variable, e.g. the orientation of the appendages of the anal tube. This variability and the slight morphological differences between most species of the R. collinus complex raised many problems of identification and doubts about the taxonomic status of the known species (e.g. LeQuesne, 1960). Field research in recent years resulted in the discovery of a number of populations of unknown taxonomic status indicated in previous papers (den Bieman, 1986, 1987a - d) as "Taxa 1 to 4".

Solving these taxonomic problems required extensive analysis of biological differentiation within the R. collinus complex. To allow an evaluation of the interspecific differences, the intraspecific variability had to be studied too. So populations from throughout Europe were analysed. Studies on hybridization capacity and on differentiation in acoustic communication, host-plant relations and allozyme patterns provided compelling evidence that the known species and new Taxa of this complex are distinct but very closely related species (den Bieman, 1986; 1987a-d; den Bieman & Eggers-Schumacher, 1987). In addition to these multidisciplinary studies this paper deals with a reevaluation of the male genitalia. Ribautodelphax females are morphologically rather uniform and have not been studied in detail.

This multidisciplinary approach to the R. collinus complex allowed the solution of many taxonomic problems. A formal taxonomic treatment is given in this paper. Especially the differentiation between the most related species is discussed. In addition to the experimentally studied taxa, several new species only known from museum collections are included. The already described species are redrawn because of the confusion about some of them and to allow delimination of the new species.

A second line in the study of the R. collinus complex was initiated after the discovery of pseudogamous triploid Ribautodelphax females. These females reproduce parthenogenetically but need to be inseminated for

reproduction. However the genetic material of the male is not passed on to the all-female offspring of the triploid pseudogamous females. These females occur in populations of R. pungens and of a new species provisionally called "Taxon 4" (den Bieman, 1987c,e).

MATERIAL AND METHODS

Because of the taxonomic confusion about most Ribautodelphax species, all records had to be verified. A list of the material that was studied is included. For each locality, the corresponding province is given. Records already included in Asche, 1982a,b; Asche & Hoch, 1982; Asche & Remane, 1982; Drosopoulos, 1982 and Drosopoulos et al., 1985 are omitted.

Ribaut's type material of R. pungens, R. imitans and R. angulosus (MNHN) was reinvestigated. He indicated no holotypes and the specimens that he drew (Ribaut, 1953) are not in a condition to be used as lectotypes. Therefore, I selected other specimens from his type series as lectotypes.

The following abbreviations are used:
*   First recorded from that country.
AUW   Agricultural University Wageningen, collection of the Laboratory of Entomology, Netherlands.
B   brachypterous wing form.
BMNH   British Museum of Natural History, London, England.
CB   collection of C.F.M. den Bieman, Bennekom, Netherlands.
HNHM   Hungarian National History Museum, Budapest, Hungary.
KBIN   Koninklijk Belgisch Instituut voor Natuurwetenschappen, Brussels, Belgium.
L1-L5 Instars.
M   macropterous wing form.
MNHN   Muséum national d'Histoire Naturelle, Paris, France.
NCSU   North Carolina State University insect collection, Raleigh, United States.
RMNH   Rijksmuseum van Natuurlijke Historie, Leyden, Netherlands.
RR   Collection of R. Remane, Marburg, W. Germany.
ZMA   Instituut voor Taxonomische Zoölogie, Zoölogisch Museum, Amsterdam, Netherlands.
ZMH   Zoological Museum Helsinki, Finland.

For all figures, the abdomina of the specimens have been macerated (concentration of KOH 100 g/l). The genitalia were transferred into glycerol and gelatin and drawings were made with a camera lucida.

Ribautodelphax collinus complex

The species of the R. collinus complex are characterized by structures of the male genitalia. The parameres have an arched finger-shaped distal part and are laterally widened into a flat lobe (Fig. 1D-E). The aedeagus is sharply arched, has a subapical phallotrema at the right side and teeth at both sides (Fig. 1H-J). None of the stronger developed teeth on the left of the aedeagus is very pronounced. In lateral aspect, the pygofer has a small incision in the upper part (Fig. 1B) and ventrally it has a concave incision (Fig. 1C). The central diaphragm bears two tooth-like processes (Fig. 1A, C). The pointed appendages of the anal tube are well developed and cross each other or are parallel (Fig. 1F). Note. For convenience, the anal tube is included in the male genital structures.

Besides the species discussed in this paper, the R. collinus complex comprises also R. affinis Logvinenko, 1965 from the USSR: Georgia; Ukraine; R. exquisitus Anufriev, 1970 from the USSR: C. Siberia and Mongolia; and R. hyrcanus Dlabola, 1981 from N. Iran. The taxonomic status of these species has to be checked.

Ribautodelphax collinus (Boheman, 1847).

Delphax collina Boheman, Handl. Svenska Vet. Akad. 1847:51
Delphax concinna Fiebér, 1866, Verh. Zool. Bot. Ges. Wien 16:525; Pl VIII, Fig. 28 (Primary homonym).
Liburnia biarnica J. Sahlberg, 1871, Not. Fennica (n.s.) 9 (12): 429.
Delphacodes annae Kirkaldy, 1906, Canadian Ent. 38:156; nom. nov. for D. concinna Fieber.
Description. The holotype of R. collinus has been drawn by Ribaut (1953) and a good description has been given by Ossiannilsson (1978). The main differences from the other Ribautodelphax species in male genital structures are listed. The parameres are long and reach almost to the lateral incision of the pygofer (Fig. 1A). Parameres straight without a mediad edge (Fig. 1D,E). The ventral incision of the pygofer is flanked by

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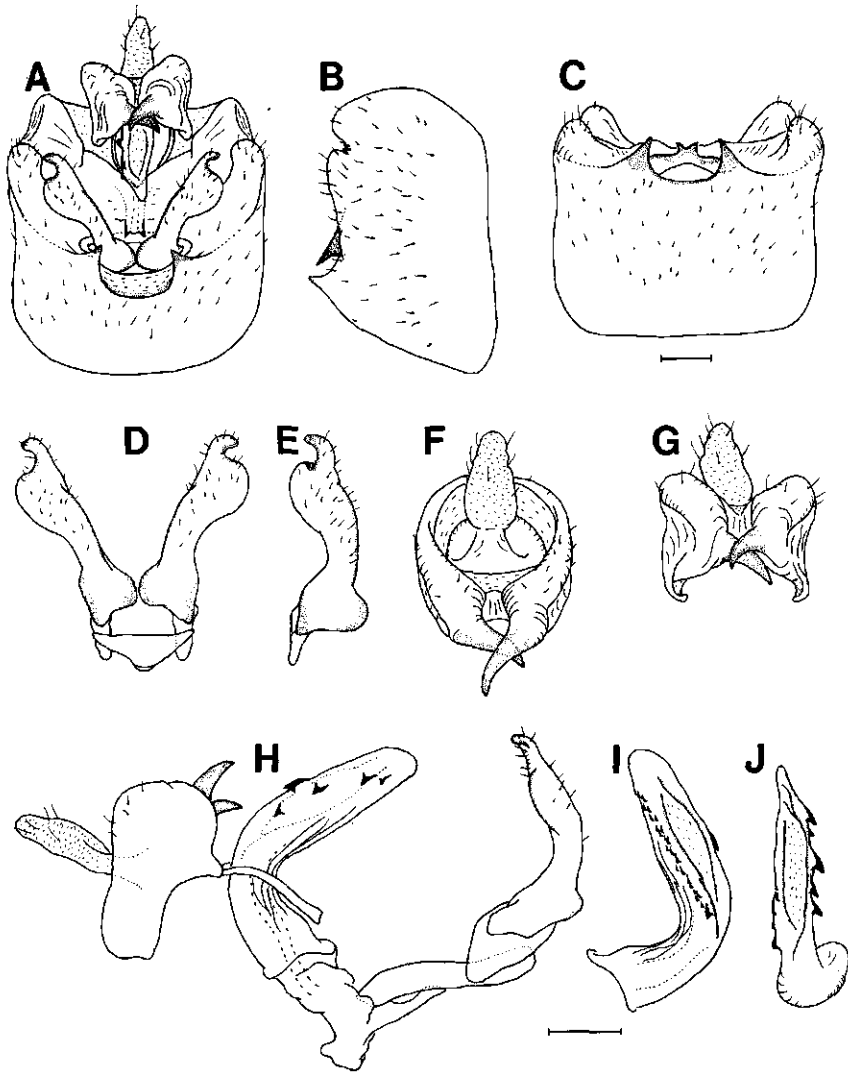
Fig. 1. A–J, R. collinus (Boheman): Netherlands, Limburg, Plasmolen, 25.VIII 1981, leg. C. den Bieman (CB). A ♂ genitalia, ventrocaudal view. B. Pygofer, right lateral view. C. Pygofer, ventral view. D. Parameres, ventrocaudal view. E. Left paramere, maximal view. F. Anal tube, caudal view. G. Anal tube, ventral view. H. ♂ genitalia without pygofer, left lateral view. I. Aedeagus from right side. J. Aedeagus, caudal view. Bars: 0.1 mm.

long and pointed processes (Fig. 1C). The central diaphragm has two well developed ventrad directed tooth-like appendages (Fig. 1A-C). Aedeagus as figured in 1H-J. The appendages of the anal tube are crossed with the left appendage below the right one (Fig. 1 F-G). The mirror-image form is found in small numbers. Length: ♂ B 2.1-2.4 mm; ♂ M 3.6-3.9 mm; ♀ B 2.4-2.7 mm; ♀ M 3.9-4.1 mm.

Remarks. R. collinus is distinguished from the other species of the R. collinus complex by the acoustic signals of the males and females, by its host plant and by the morphology of the male genitalia (den Bieman, 1986, 1987b). R. collinus hybridizes under laboratory conditions with all species tested of the R. collinus complex, but the number of hybrids is low (den Bieman, 1987c).

Three synonyms are known for R. collinus. One (D. annae) is only a new name for D. concinna Fieber because this name was preoccupied. Most of Fieber's types are deposited in the MNHN. The material of D. concinna in the collections of Noualhier and Perrier concerned R. collinus. The type of L. biarnica Sahlberg was studied (ZMH) and belongs to R. collinus.

Biology. R. collinus is monophagous on Agrostis capillaris (L) (A. tenuis Sibth.) in dry biotopes. It is bivoltine in W. and C. Europe, hibernates as L2-L4, and reaches to altitude about 1500 m.

Distribution. Material of the following localities was studied. NETHERLANDS. Limburg, Plasmolen, many samples 1977/1984 (AUW, CB); this population was used for most experimental studies; several specimens were parasitized by Strepsiptera and by the Dryinid Dicondylus bicolor Hal. (det. M. Olmi). Limburg, Melik-Herkenbosch, 21.VIII.1955, 1 ♂ B, leg. R.H. Cobben (AUW). Limburg: Roermond, VIII.1953, 1 ♂ M, leg. R.H. Cobben (AUW). Limburg, Tienray, 28.IV.1987, 19 ♂♂ B 7 ♀♀ B, leg. C. Booij (CB). Limburg, Beegden, 29.V.1982, 1 ♂ B, leg. C. den Bieman (CB). Noord-Brabant, Waalre, 25.VII.1941, 1 ♂ B, leg. Blöte (RMNH). Noord-Brabant, Valkenswaard, 16.VIII.1940, 1 ♂ B, leg. Blöte (RMNH). Noord-Brabant, Berlicum, 25.VII.1928, 1 ♂ B, leg. Geijskes (RMNH). Gelderland, Wageningen, many samples 1955/1958, leg. R.H. Cobben (AUW). Gelderland, Otterlo, 20.V.1956, 2 ♂♂ B, leg. W. Gravestein (ZMA). Gelderland, Hatert, 13.VIII.1979, 1 ♂ M. Utrecht, Veenendaal, 8.V.1982, 2 ♂♂ B. Utrecht, Maarn, 10.VIII.1983, 5 ♂♂ B 7 ♀♀ B. All leg. C. den Bieman (CB). Friesland, Terschelling, 10.IX.1979, 31 ♂♂ B 20 ♀♀ B 1 ♀ M, leg. C. Booij & C. den Bieman (AUW). SWEDEN. Lund, 22.VIII.1977, 28 ♂♂ B 16 ♀♀ B, leg. C. Booij (AUW). FINLAND. Regio aboensis, Kukäla, 30.VI.1979, 3 ♂♂ B 1 ♀ B, leg. Albrecht (ZMH). Regio aboensis, Lojo, VII.1931, 1 ♂ B, leg. Lindberg (ZMH). Karelia borealis, Kontiolahti, 15/25.VIII.1942, 1 ♂ B 1 ♀ B, leg. Lindberg (ZMH). W. GERMANY. Schleswig Holstein, Ratzeburger See, 15.VII.1934, 1 ♂ B 2 ♀♀, leg. E. Wagner (ZMA). Schleswig Holstein, Amrum, 24/31.VII.1954, 1 ♂ B, leg. R. Remane (Zoological Museum Hamburg). Schleswig Holstein, Husum, 2 ♂♂ B 2 ♂♂ M 1 ♀ M, 13.V.1934, leg. W. Wagner (ZMA; NCSU). Schleswig Holstein, Bamberg, 21.VII.1949, 1 ♂ M, leg. T. Schneid (NCSU). Hamburg, 8.VIII.1934,

1 ♂ B 1 ♀ B. Hamburg, Boberg, 6.V.1934, 2 ♂♂ B; 8.VIII.1934, 2 ♀♀ B, 1 ♀ M. Hamburg, Ramelsloh, 14.VIII.1947, 1 ♂ B; 12.VIII.1947, 1 ♂ B. All leg. W. Wagner (NCSU). Hamburg, Horst a.d. See, VIII.1944, 6 ♂♂ B 1 ♂ M 1 ♀ B, leg. E. Wagner (ZMA, NCSU, HNHM). Nieder Sachsen, Göhrde, 23.VII.1931, 2 ♂♂ B 2 ♀♀ B (RMNH, NCSU). Hessen, Frankfurt a. M., 2.VIII.1939, 1 ♂ B, leg. W. Wagner (NCSU). Hessen, Wiesbaden (Rabengrund), 19.VII.1937, 2 ♂♂ M 1 ♀ M (NCSU). Rheinland-Pfalz, Oberwesel, 7.VII.1959, 1 ♂ B, leg. C. de Jong (RMNH). Bayern, Streitberg, VIII.1942, 1 ♂ B, leg. Lanzka (NCSU). Bayern, Fürth, 16.VII.1932, 1 ♂ B, leg. R. Schmidt (MNHN). BELGIUM. Liege, Plombieres, 27.V.1983, 1 ♂ B (CB). Liège, Esneux, 28.III.1930, 2 ♂♂ B 1 ♀ B, leg. G. Vreurick (KBIN). Liège, Landenne, 8.V.1967, 7 ♂♂ B 2 ♀♀ B, leg. R. Detry (KBIN). Limburg, Beringen, 25.VII.1982, 1 ♂ M 1 ♂ B, leg. C. den Bieman (CB). FRANCE. Pyrenées Atlantiques, Escot, 15.VIII.1985, 3 ♂♂ B, leg. R.H. Cobben (AUW). Haute Pyrenées, Gèdre, 8.IX.1917, 1 ♂ M, leg. H. Ribaut (MNHN). Haute-Garonne, Saint-Béat, IV.1929, 1 ♂ B 2 ♂♂ M; 16.VIII.1938, 1 ♂ B, 1 ♀ M, leg. H. Ribaut (MNHN). Dordogne, 20 km SE of Périgueux, 17.VIII.1985, 13 ♂♂ B 7 ♀♀ B 1 ♀ M. Moselle, Lutzelbourg, 8.VII.1959, 1 ♂ B. All leg. R.H. Cobben (AUW). SPAIN*. Huesca, Campo N. of Barbastro, 8.VII.1963, 2 ♂♂ M, leg. R. Remane (RR). PORTUGAL*. Castelo Branco, Penhas da Saùde, 4/6.VI.1959, 1 ♂ B, leg. Lindberg (ZMH) (= C. pallens in Lindberg (1960). YUGOSLAVIA. Srbija, Trstenik, 11.VII.1980, 5 ♂♂ B 7 ♀♀ B, leg. C. den Bieman (CB). See also Asche (1982a). BULGARIA. Sofiya, Mt. Vitos, 5.VIII.1939, 1 ♂ B, leg. Lindberg (ZMH). Kyustendil, Rilski Manastir, 1440-1460 M, 13.VII.1983, 7 ♂♂ B 7 ♀♀ B, leg. C. den Bieman (CB). See also Asche (1982a). GREECE. Trikkala, Meg. Panagia (Pindos Mts), 22.VII.1982, 6 ♂♂ B 8 ♀♀ B, leg. C. den Bieman & S. Drosopoulos (CB). See also Asche & Hoch (1982) and Drosopoulos et al. (1985). R. collinus is further reported from Hungary (Asche, 1982b) and Austria, Czechoslovakia, E. Germany, Italy, Poland and the USSR (Nast, 1972), especially the reports from the USSR should be checked.

R. pungens (Ribaut), R. vinealis n.sp, R. noguerae n.sp.

R. pungens, R. vinealis and R. noguerae are morphologically closely related. The first two have been experimentally studied (den Bieman, 1986, 1987a-d; den Bieman & Eggers-Schumacher, 1987). R. pungens and R. vinealis live on different host plants and could not be reared on each other's host plants. The two are well differentiated in acoustic signals of the males. Differences in female acoustic signals are less pronounced because of the strong variation between individuals in the signals of R. pungens. The two species differ one from another in allozyme patterns. Crossing between the two was unsuccessful. Morphologically R. pungens and R. vinealis are clearly distinguished by the position of the mediad edge of the paramers. These differences warrant a species status for the two.

A common characteristic of R. pungens and R. vinealis is the variability in the orientation of the appendages of the anal tube. The third species, R. noguerae, differs from the first two because it lacks

this variability. Moreover the orientation of the anal-tube appendages is slightly different, and the appendages are stouter developed. The host plant of R. noguerae differs from those of the other Ribautodelphax species.

Ribautodelphax pungens (Ribaut, 1953).

C. pungens Ribaut, 1953; Bull. Soc. Hist. nat. Toulouse 88, 247-248, fig. 15-16.

Description. R. pungens has been described by Ribaut (1953) and Ossiannilsson (1978) and differs in a number of structures of the male genitalia of the remaining Ribautodelphax species. The ventral incision of the pygofer has no angular corners (Fig. 2A, C). The parameres have a well developed mediad edge (Fig. 1A, D, E). The filiformous appendages of the anal tube have a strongly variable orientation (Fig. 2I-P). Ribaut (1953), LeQuesne (1960) and Ossiannilsson (1978) did not report this variation. In some specimens, the appendages are parallel, resulting in confusion with R. imitans or R. imitantoides. The aedeagus (Fig. 2 F-H) has a variable number of teeth. Two small mediad directed processes are placed on the central diaphragm (Fig. 2A-C). Length: ♂ B 2.2-2.5 mm; ♂ M 3.2-3.7 mm; ♀ B 2.6-3.0 mm; ♀ M 3.7-4.3 mm.

Remarks. A brachypterous male of Ribaut's type series is designated as lectotype: France, Haute-Garonne, Saint Béat, no date (MNHN). The acoustic signals of the males, the host plants and the morphology of the male genitalia distinguish R. pungens from the other Ribautodelphax species. R. pungens hybridizes in the laboratory with several species but the number of offspring is low.

Biology. R. pungens lives on Brachypodium species. In W. and C. Europe on B. pinnatum (L.) Beauv., in the Mediterranean region also on B. phoenicoides (L.) Roemer & Schultes and in Greece also on B. sylvaticum (Hudson) Beauv. (den Bieman, 1987a). It is bivoltine in W. Europe and may have more generations in S. Europe. R. pungens was sampled from altitude 70 to 1500 m. It hibernates as L2-L5 in W. Europe. R. pungens inhabits dry biotopes and occurs in W. Europe mainly on calcareous soils probably because of the ecology of its host plant. Triploid pseudogamous females occur in most populations of R. pungens (den Bieman, 1987e) and are morphologically indistinguishable from the diploid bisexual females.
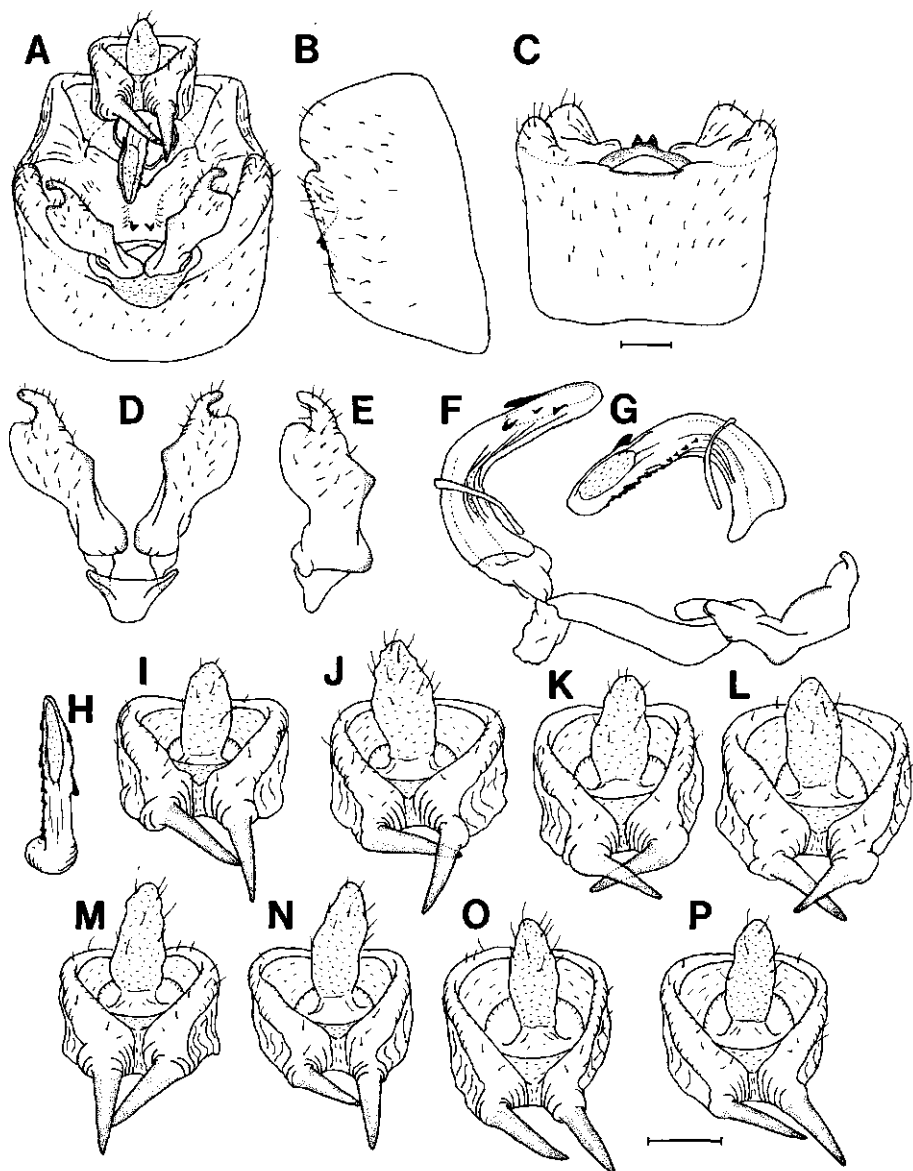
Fig. 2. A-P, R. pungens (Ribaut). A-I; France, Haute-Garonne, Saint-Béat, lectotype, leg. H. Ribaut (MNHN); J-L: Netherlands, Limburg, Bemelen, 30.VIII.1979: M; France, Alpes de Haute Provence, Montfuron, 7.IV.1983; N: W. Germany, Baden-Württemberg, Tübingen, 26.VIII.1982; O-P: Belgium, Namur, Nismes, 21.VII.1982. All leg. C. den Bieman (CB). A. ♂ genitalia, ventrocaudal view. B. Pygofer, right lateral view. C. Pygofer, ventral view. D. Parameres, ventrocaudal view. E. Left paramere, maximal view. F. ♂ genitalia without pygofer and anal tube, left lateral view. G. Aedeagus from right side. H. Aedeagus, caudal view. I-P. Anal tube, caudal view. Bars: 0.1 mm.

Several populations are heavily parasitized by Pipunculidae, Strepsiptera and Dryinidae (Dicondylus bicolor Hal. and Donisthorpina pallida Ceb., det. M. Olmi).

Distribution. Material of the following localities was studied.
NETHERLANDS. Limburg, Bemelen, many samples 1951 to 1985, this population was termed population A in den Bieman (1987a) and used for many experiments (AUW, CB, RMNH, ZMA). Limburg, Kunrade, (Kunderberg), samples from 1980 to 1985 (AUW, CB). Limburg, Wijlre (Wrakelberg), samples from 1967 to 1985 (AUW, CB, RMNH, ZMA). Limburg, Wahlwiller, samples from 1981 to 1984 (CB). Limburg, Maastricht (Pieterberg), samples from 1931 to 1984 (AUW, CB, RMNH, ZMA). Limburg, Schin op Geul, 23.V.1953, 1 ♂ B 8 ♀♀ B, leg. W. Gravestein (ZMA). Limburg, Termoors, 25.V.1986, 1 ♂ B, leg. C. den Bieman (CB). BELGIUM*. Namur, Jemelle, 29.VII.1981, 14 ♂♂ B 20 ♀♀ B 2 L5, leg. R.H. Cobben (AUW); 17.VII.1982, 35 ♂♂ B, 80 ♀♀ B, 3 L5. Namur, Dinant, 20.VII.1982, 4 ♂♂ B 22 ♀♀ B 2 L5. Namur, Yvoir-Spontin, 20.VII.1982, 1 ♂ B 3 ♀♀ B. Namur, Nismes, 21.VII.1982, 13 ♂♂ B 57 ♀♀ 1 ♀ M. Namur, Han sur Lesse, 19.VII.1982, 1 ♂ B 24 ♀♀ B 2 L5. Namur, Mariembourg, 21.VII.1982, 2 ♂♂ B 7 ♀♀ B 1 ♀ M. Namur, Rochefort (Abbaye St. Remy), 17 ♂♂ B 44 ♀♀ B 12 L5. All leg. C. den Bieman (AUW, CB). Luxembourg, Torgny, 28.VII.1981, 5 ♂♂ B 11 ♀♀ B 2 L5, leg. R.H. Cobben (AUW); 22.VII.1982, 17 ♂♂ B 51 ♀♀ B 2 ♀♀ M 3 L5, leg. C. den Bieman (AUW). ENGLAND. Gloucester, Withington, IV, 1 ♂ B, VI, 1 ♂ B, leg. J. Edwards (Hope Entomologycal Collection, Oxford). Dorset: Worth, 28.V.1938, 3 ♂♂ B, (BMNH). W. GERMANY. Nordrhein-Westfalen, Rolandswerth (Rodderberg), 10.VIII.1982, 3 ♂♂ B 14 ♀♀ B, leg. C. den Bieman (CB). Hessen, Frankfurt, a.M. (Bergen), 22.VII.1939, 2 ♂♂ B. Hessen, Frankfurt a. M. (Enkheim), 22.VII.1939, 2 ♂♂ B 1 ♀ B. Hessen, Wiesbaden (Mainzer Beckens, Rabengrund), 27.VII.1935, 1 ♂ B 1 ♀ M. All leg. W. Wagner (NCSU). Hessen, Amöneberg, 27.VIII.1979, 42 ♂♂ BB 13 ♀♀ B, leg. R. Remane, R.H. Cobben, C. den Bieman & C. Booij; 12.VIII.1982, 25 ♂♂ B, 31 ♀♀ B, leg. C. den Bieman (AUW). Baden-Württemberg, Kaiserstühl, 23.VII.1938, 1 o B, leg. W. Wagner (NCSU). Baden-Württemberg, Tübingen (Spitzberg), V.1953, 2 ♂♂ B; VI.1953, 2 ♂♂ B; VII.1954, 1 ♂ B, leg. W. Schwoerbel (coll. W. Schwoerbel, Biberach, W. Germany); 15/16.VIII.1982, 36 ♂♂ B 37 ♀♀ B. Baden-Württemberg, Hayingen, 17.VII.1982, 4 ♂♂ B 24 ♀♀ B. Baden-Württemberg, Hundersingen, 17.VIII.1982, 4 ♂♂ B 12 ♀♀ B. Rheinland-Pfalz, Ebernburg (Lemberg), 13.VIII.1982, 17 ♂♂ B 17 ♀♀ B. Rheinland-Pfalz, Bad Kreuznach (Rotenfels near Bad Munster am Stein), 20.VIII.1982, 2 ♂♂ B. All leg. C. den Bieman (AUW, CB). Bayern, Königsbrunn (Königsbrunner heide), 16.VII.1939, 1 ♂ B, leg. W. Wagner (NCSU). Bayern, Retzbach, 9.VI.1964, 1 ♂ B, leg. Lindberg (ZMH). Bayern, Bamberg, 14.VIII.1932, 1 ♂ B (MNHN). E. GERMANY. Erfurt, Eichsfeld (Börnhagen), 26.VII.1928, 1 ♂ B (Zoological Museum, Hamburg). Gera, Ronneburg (Brahmental), 14.VII.1946, 1 ♂ B 1 ♀ M, leg. M. Nicolaus (ZMA). SWITSERLAND. Wallis, Zeneggen, 1370 m, 19.VII.1953, 2 ♂♂ B, leg. Lindberg (ZMH). FRANCE. Meuse, Apremont, 240-260 M, 28.IV.1984, 2 ♀ B. Jura, Pont d' Hery, 400-420 m, 27.IV.1984, 4 ♂♂ B 24 ♀♀ B. Jura, Salins-les-Bains, 540-560 m, 27.IV.1984, 4 ♂♂ B 21 ♀♀ B. Jura, Chemille, 440-460 m, 26.IV.1984, 1 ♀ B 1 L5. Ain, Treffort, 280-300 m, 26.IV.1984, 3 ♂♂ B 42 ♀♀ B. All leg. C. den Bieman (AUW). Ardèche, Coulens, 29.IV.1983, 3 ♂♂ 10 ♀♀ B, leg. R.H. Cobben (AUW). Ardèche, Jaujac, 16.IV.1984, 2 ♂♂ B. Ardèche, Asperjoc, 320-340 m, 16.1V.1984, 9 ♂♂ B 22 ♀♀ B 5 L5. Gard, Lasalle, 18.IV.1984, 1 ♂ B 2 ♀♀ B. Vaucluse, Ansouis, 300-320 m, 8.IV.1982, 2 ♂♂ B. Alpes de Haute Provence, Montfuron, 600-620 m, 9.IV.1982, 1 ♂ B 1 ♀ B; 7.IV.1983, 25 ♂♂ B 8 ♀♀ B; termed population C. den

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Bieman (1987a). Bouches du Rhône, La Ciotat, 280–300 m, 9.IV.1982, 3 ♂♂ B 1 ♀ B. All leg. C. den Bieman (CB). Puy-de-Dôme, Royat, 26.VII.1922, 1 ♂ B, leg. H. Ribaut (MNHN). Puy-de-Dôme, Besse en Chandesse, 19.VIII.1955, 1 ♂ M 1 ♀ B 1 ♀ M (RMNH). Haute-Garonne, Saint Béat, 5 ♂♂ B 2 ♀♀ B (type series Ribaut!, MNHN, NCSU). Haute-Garonne, Eup, 32.VIII.1925, 2 ♂♂ B. Tarn, Tonnac, 5.VI.1932, 1 ♂ B 1 ♀ B. Haute Loire, Le Puy, 2.V.1933, 1 ♂ B. All leg. H. Ribaut (MNHN). Haute Loire, St. Privat d'Allier, 880–900 m, 2.VII.1985, 1 o B 1 ♀ B. Lozère, Le Massegras, 840–860 m, 17.VII.1985, 1 ♂ B 2 ♀♀ B. Aude, Quillan 340–360 m, 4.VII.1985, 1 ♂ B. All leg. C. den Bieman (CB). SPAIN* Gerona, Olot, 10.VII.1986, 5 ♂♂ B 18 ♀♀ B 3 ♀♀ M. Teruel, Alcala de la Selva (Sierra de Gúdar), 19.VII.1986, 1480–1500 m, 3 ♂♂ B 9 ♀♀ B. All leg. C. den Bieman (CB). ITALY. Ascoli Piceno, Montefortino, 7.IX.1977, 2 ♂♂ B, leg. R. Remane (RR). YUGOSLAVIA. Slovenija, Postojna, 4.VII.1983, 9 ♂♂ B 10 ♀♀ B 22 L5. Slovenija, Unec (10 km east of Postojna), 4.VII.1983, 1 ♂ B. Croatia, Zdihovo, 5.VII.1983, 3 ♂♂ B 17 ♀♀ B. Bosna-Hercegovina, Bosanska Petrovac, 7.VII.1983, 1 ♂ B. Bosni-Hercegovina, Jajce, 8.VII.1983, 38 ♀♀ B 3 ♀♀ M 19 L5. All leg. C. den Bieman (AUW, CB). See also Asche (1982a). GREECE. Fokis, Pentapolis (Skaloula), 16.IV.1980, 4 ♂♂ B 11 ♀♀ B, leg. R.H. Cobben & S. Drosopoulos (AUW). Fokis, Elaion, 13/16.IV.1980, 4 ♂♂ B, leg. R.H. Cobben & S. Drosopoulos (AUW); 23.VII.1983, 26 ♂♂ B 20 ♀♀ B 4 ♀♀ M 2 L5, leg. C. den Bieman & S. Drosopoulos (AUW); termed population B in den Bieman (1987a). Florina, Kotas, 21.VII.1983, 18 ♂♂ B 19 ♀♀ B 5 ♀♀ M 1 L5, leg. C. den Bieman & S. Drosopoulos (CB). Kozani, Vourinos Mt (near Siatista), 1300 m, 18.VIII.1983, 11 ♂♂ B 43 ♀♀ B 14 L4–L5, leg. S. Drosopoulos & R.H. Cobben (AUW). Trikkala, Meg. Panagia (Pindos Mts), 22.VII.1983, 2 ♂♂ BB 9 ♀♀ B, leg. S. Drosopoulos & C. den Bieman (AUW). Other localities were listed in Asche & Remane (1982), Drosopoulos (1982) and Drosopoulos et al. (1985). R. pungens is further reported from Austria, Poland and Czechoslovakia (Nast, 1972) and Sweden (Gotland) (Ossiannilsson, 1978). Note. Several of the females listed might concern pseudogamous triploid females.

Ribautodelphax vinealis n.sp. (Fig. 3A–R).

Description. R. vinealis resembles R. pungens in habit and coloration. Differences are found in the structures of the male genitalia. The ventrale incision of the pygofer has no angular corners or processes (Fig. 3A, C). Laterally the pygofer has a small incision in the upper part (Fig. 3B). The parameres have a mediad edge placed near to the base (Fig. 3E, F). The parameres do not reach the lateral incision of the pygofer (Fig. 3A). The aedeagus is sharply arched, has a variable number of teeth and a subapical phallotrema at the right (Fig. 3G–I). The smaller teeth on the right form a ridge. The filiformous and sharply pointed anal-tube appendages have a variable orientation (Fig. 3J–R). The central diaphragm has a pair of small distally directed processes (Fig. 3B–D). Length: ♂ B 2.1–2.4 mm; ♂ M 3.4–3.5 mm; ♀ B 2.4–3.0 mm; ♀ M 3.7–4.0 mm.

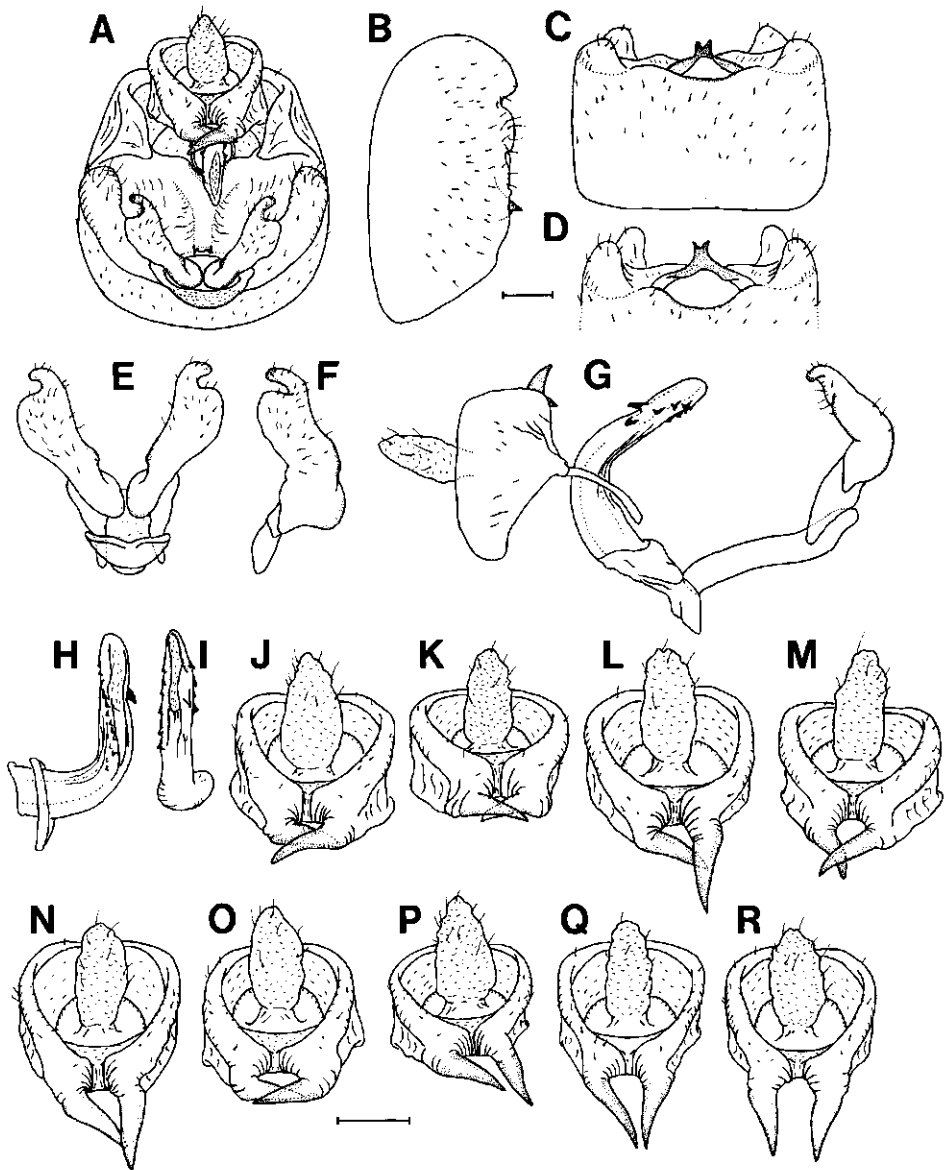Remarks. R. vinealis was termed "Taxon 1" in my previous papers (den

Fig. 3. A-R, R. vinealis n.sp.: Netherlands, Gelderland, Hoge Veluwe.
A-C, E-K, Q: 3.VI.1983, paratypes; D, L, N-P, R: 23.V.1981,
paratypes; M: 20.VI.1979, paratype. All leg. C. den Bieman (CB). A.
♂ genitalia, ventrocaudal view. B. Pygofer, left lateral view. C-D.
Pygofer, ventral view. E. Parameres, ventrocaudal view. F. Left
paramere, maximal view. G. ♂ genitalia without pygofer, left lateral
view. H. Aedeagus from right side. I. Aedeagus, caudal view. J-R.
Anal tube, caudal view. Bars: 0.1 mm.

Bieman 1986, 1987a – d, den Bieman & Eggers-Schumacher). The acoustic signals of the males and females, its host plant and the male genital structures clearly distinguish R. vinealis from the remaining species of the R. collinus complex and warrant its species status despite its potential hybridization capacity with some other Ribautodelphax species under forced conditions. The number of hybrids produced was always low.

Biology. R. vinealis lives on Agrostis vinealis Schreber in dry sandy biotopes. It is bivoltine with hibernation in the 2nd to 3rd instars. A small number of individuals was parasitized by Strepsiptera and Dryinidae.

Distribution. R. vinealis seems to be endemic in the Netherlands. It is geographically separated from R. pungens probably by the ecology of their host plants. R. pungens occurs only in the calcareous region of the Netherlands, the far south of Limburg, while R. vinealis is found in the remaining part. Schiemenz (1969) published on R. pungens from E. Germany. The descriptions of his sample localities suggest that the northern populations might be R. vinealis. Unfortunately this material could not be studied.

Material studied. Holotype: NETHERLANDS. Gelderland, De Hoge Veluwe, 23.V.1981, 1 ♂ B, leg. C. den Bieman (ZMA). Paratypes, same locality, 23.V.1981, 45 ♂♂ B 16 ♀♀ B 63 L4-L5; 20.VI.1979, 1 ♂ B 1 ♀ B 1 ♀ M; 5.IX.1979, 6 ♂♂ B 3 ♀♀ B; 17.IV.1980, sample of larvae; 14.IX.1980, 15 ♂♂ B 6 ♀♀ B; 8.VI.1982, 1 ♂ B 2 ♀♀ B; 3.VI.1983, 22 ♂♂ B 24 ♀♀ B; 30.VIII.1983, 3 ♂♂ B 5 ♀♀ B. All leg. C. den Bieman (CB, AUW). Same locality, 27.VII.1975, 4 ♂♂ B 2 oo M; 12.VIII.1975, 1 ♂ B; 31.VIII.1977, 2 ♂♂ B. All leg. R.H. Cobben (AUW). Many other samples were taken to study the phenology but were not conserved. In the spring of 1984, management practices destroyed this population in the Hoge Veluwe 'nature reserve'. Gelderland, Arnhem, 28.VII.1908, 2 ♂♂ B, leg. Bierman (ZMA); Utrecht, Hollandse Rading, 14.V.1953, 11 ♂♂ B 3 ♂♂ M 7 ♀♀ B 1 ♀ M, leg. W. Gravestein (ZMA). Utrecht, Rhenen (Grebbeberg), 23.V.1953, 1 ♂ M, leg. R.H. Cobben (AUW). Noord-Brabant, Waalre, 2.VII.1942, 1 ♂ B 1 ♀ B, leg. Blöte (ZMA). Noord-Brabant, Heeze, 6.VI.1959, 1 ♂ B 2 ♀♀ M, leg. W. Gravestein (ZMA). Noord-Brabant, Valkenswaard, 24.VII.1938, 1 o B; 1/8.VIII.1943, 1 ♂ M 2 ♀♀ M, leg. Blöte (RMNH). Limburg, Roermond, 11.V.1953, 1 ♂ B 6 ♀♀ B; VIII.1953, 1 ♂ B; 11.IX.1953, 2 ♂♂ B, leg. R.H. Cobben (AUW).

Ribautodelphax noguerae n.sp. (Fig. 4A-J)

Description. R. noguerae is morphologically close to R. pungens. It resembles this species in habit though R. noguerae is somewhat smaller and darker coloured. As in the preceding two species, the ventral incision of the pygofer is not flanked by processes (Fig. 4A-C). Laterally the pygofer has a small incision. The parameres are similar to those of R. pungens.
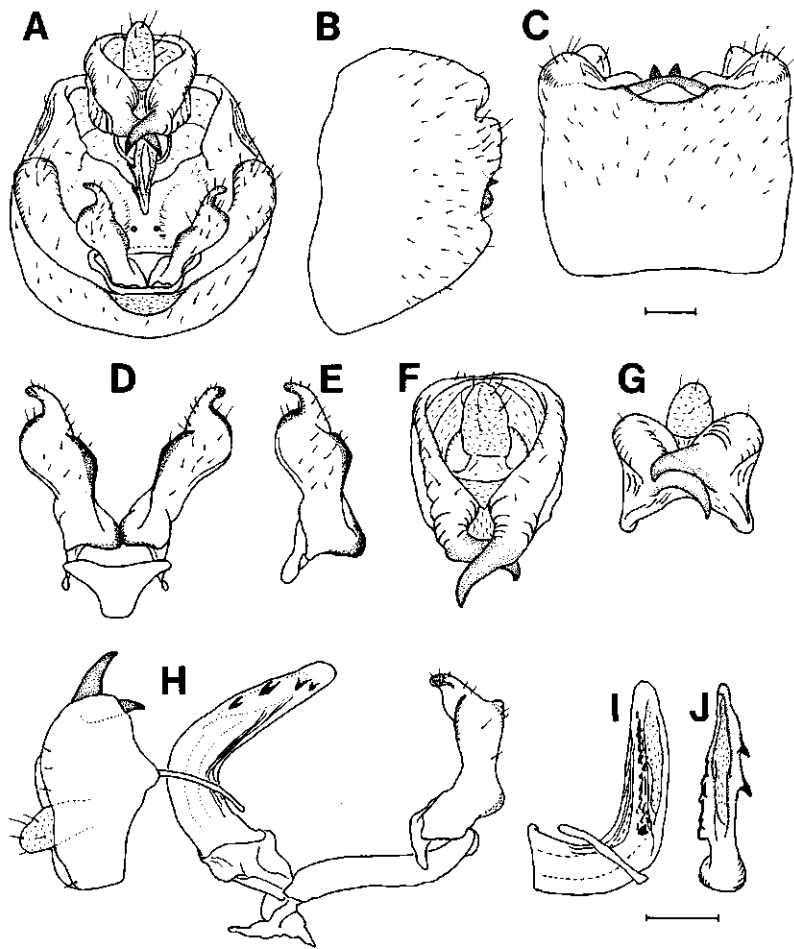
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Fig. 4. A–J, R. noguerae n.sp.: Spain, Teruel, Sierra de Albarracin,
4 km NW Noguera, 15.VII.1986, paratype, leg. C. den Bieman (CB). A. ♂
genitalia, ventrocaudal view. B. Pygofer, left lateral view, C.
Pygofer, ventral view. D. Parameres, ventrocaudal view. E. Left
paramere, maximal view. F. Anal tube, caudal view. G. Anal tube,
ventral view. H. ♂ genitalia without pygofer, left lateral view. I.
Aedeagus from right side. J. Aedeagus, caudal view. Bars: 0.1 mm.

However the mediad edge has a flat top (Fig. 4D-E). The central diaphragm
has two small distad processes (Fig. 4A-C). The anal tube appendages are
stouter than those of the preceding two species and have only one
orientation: the left one is always bent below the right one, the mirror
image is not found (Fig. 4F-G). The left anal tube appendage is bent
inwards; this orientation is never observed in R. pungens and R. vinealis.
The aedeagus is similarly shaped to that of R. pungens and has a variable
number of spines (Fig. 4H-I). Length: ♂ B 2.1-2.2 mm; ♀ B 2.6-2.8 mm; ♀ M
3.5-3.8 mm.

Biology and distribution. R. noguerae is only known from Spain.
Holotype: SPAIN, Teruel, Sierra de Albarracin, 4 km NW of Noguera, 1600-
1620 m, 15.VII.1986, 1 ♂ B, leg. C. den Bieman (ZMA). Paratypes: same
locality and date: 4 ♂♂ B 4 ♀♀ B 1 ♀ M and 16.VII.1986, 26 ♂♂ B 10 ♀♀ B 1 ♀
M, leg. C. den Bieman (CB, AUW). R. noguerae was taken from Carex nigra
(L.) in a wet and heavily grazed mountain meadow. Teruel, Montes
Universalis, near Guadalaviar in the Tago Valley, 6.VIII.1978, 1978, 2 ♂♂
B, leg. R. Remane (RR).

R. imitans (Ribaut), R. imitantoides n.sp.

R. imitans and R. imitantoides are morphologically closely related
species that hybridize readily under forced conditions (den Bieman, 1987c)
and produce fully fertile hybrids. Also their allozyme patterns indicate a
close relationship (den Bieman & Eggers-Schumacher, 1987). Nevertheless,
they are considered to be separate species, because they show clear mating
preferences for their own species in choice tests (den Bieman, 1987c).
Moreover they live on different host plants and R. imitans could not be
reared on the host plant of R. imitantoides. The two differ in acoustic
signals of the males and in male genital structures.

Ribautodelphax imitans (Ribaut, 1953).

C. imitans Ribaut, 1953; Bull. Soc. Hist. nat. Toulouse 88, 246-248,
Fig. 9-11.

Description. R. imitans and R. imitantoides are characterized by the
parallel orientation of the anal-tube appendages (Fig. 5J). These
appendages diverge at the top, a characteristic never observed in the
specimens of R. pungens or R. vinealis with parallel appendages. The
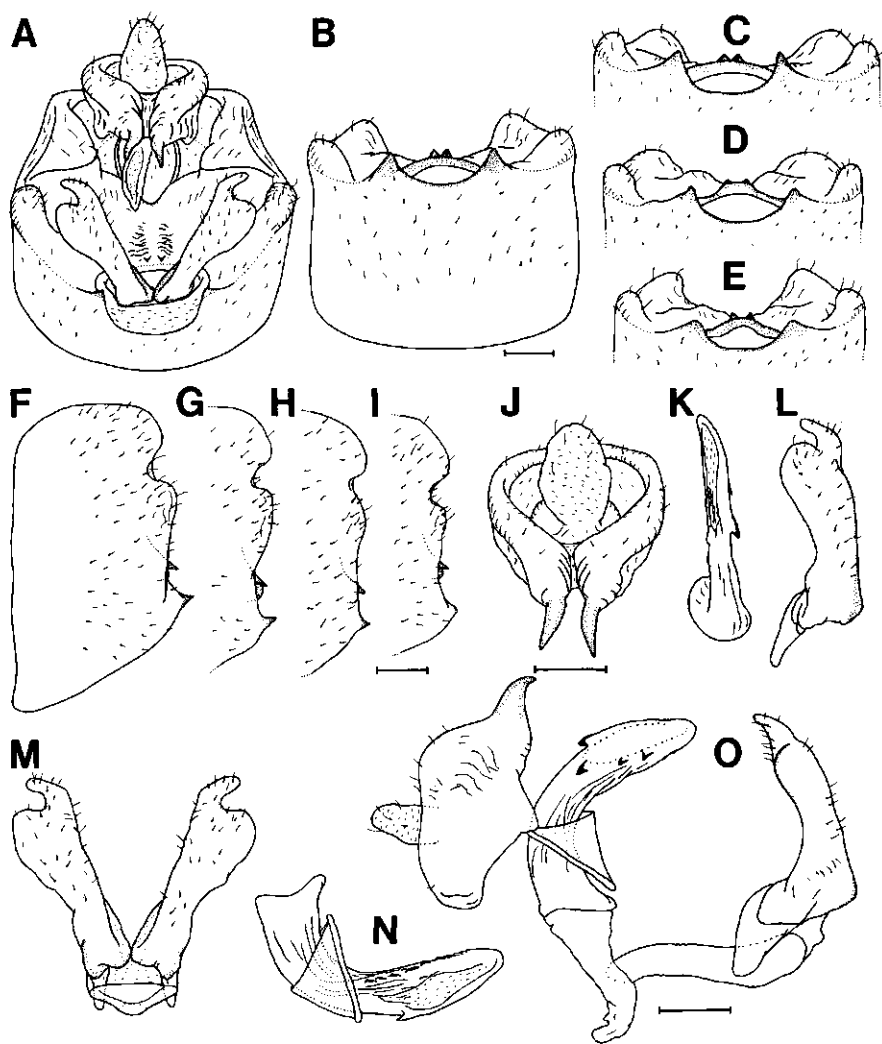ventral incision of the pygofer is flanked by processes (Fig. 5B-E), well

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Fig. 5. A–O, R. imitans (Ribaut). A–B, F, J–O: France, Tarn, Albi,
29.III.1936, lectotype, leg. H. Ribaut (MNHN). C,G: Greece, Fokis,
Elaion, 15.IV.1980, leg. R.H. Cobben (CB). D,H: France, Ardèche,
Coulens, 30.IV.1983, leg. R.H. Cobben (CB). E, I, France, Pyrenées
Orientales, St. Cyprien Plage, 17.VI.1984, leg. R.H. Cobben (CB). A.
♂ genitalia, ventrocaudal view. B–E. Pygofer, ventral view. F–I.
Pygofer, left lateral view. J. Anal tube, caudal view. K. Aedeagus,
caudal view. L. Left paramere, maximal view. M. Parameres,
ventrocaudal view. N. Aedeagus from right side. O. ♂ genitalia
without pygofer, left lateral view. Bars: 0.1 mm.

developed in most individuals (Fig. 5B-D) and less pronounced in others (Fig. 5E). The long parameres have no edge at the inner margin (Fig. 5A, M). The central diaphragm has two small ventrad processes (fig. 5F-I). The arched aedeagus (Fig. 5N-O) is shorter than in most other Ribautodelphax species. Length: ♂ B 2.2-2.6mm; ♂ M 3.5-3.9 mm; ♀ B 2.5-2.9 mm; ♀ M 3.7-4.1 mm.

Remarks. A brachypterous male of Ribaut's type series, France, Tarn, Albi, 29.III.1936, is designated as lectotype. The acoustic signals of the two sexes, its host plant, genital structures and allozyme patterns separate R. imitans from the congeneric species (den Bieman, 1986, 1987a-b, den Bieman & Eggers-Schumacher, 1987). Under forced conditions a small number of hybrids was produced with several Ribautodelphax species apart from R. imitantoides (den Bieman, 1987c).

Biology. R. imitans lives on Festuca arundinacea ssp fenas (Lag.) Arcangeli in dry biotopes. It occurs up to 1600 m. Probably this species has three or more generations in the Mediterranean region.

Distribution. R. imitans occurs mainly in the E. Mediterranean region but its area of distribution extends to S.W. Germany and even to S. England. FRANCE. Tarn, Albi, 29.III.1936, 4 ♂♂ B 1 ♀ B, leg. H. Ribaut (MNHN, Ribaut's type series!). Haut-Garonne, St. Béat, 18.III.1940, 12 ♂♂ B 5 ♀♀ B, leg. H. Ribaut (MNHN). Aude, Puilaurans, 6.X.1932, 2 ♂♂ B 1 ♂ M (MNHN). Ardèche, 3 km E. of St. Fortunat, 19.VII.1985, 1 ♂ B 1 ♀ B, leg. C. den Bieman (CB). Ardèche, Coulens, 29.IV.1982, 6 ♂♂ B 7 ♀♀ B, leg. R.H. Cobben (AUW). Ardèche, Asperjoc, 320-340 m, 16.VII.1984, 1 ♂ B, leg. C. den Bieman (CB). Pyrenées Orientales, St. Cyprien Plage, 21.VI.1981, 2 ♂♂ B 5 ♂♂ M 5 ♀♀ M; 17.VI.1984, 4 ♂♂ B 4 ♂♂ M 7 ♀♀ M; 20.VI.1985, 3 ♂♂ M 1 ♀ B 4 ♀♀ M; 15.VII.1985, 2 ♂♂ B 4 ♀♀ B, leg.R.H. Cobben, Th. Heijerman, P. de Vrijer & C. den Bieman (AUW, CB). Alpes de Haute Provence, Mt. de Lure, 1600 m, 19.VI.1982, 1 ♂ M, leg. M. Wilson (BMNH). ENGLAND. Dorset, Southwell, Portland, 3.VIII.1945, 3 ♂♂ B (Hope Entomological Collection, Oxford). W. GERMANY. Baden-Württemberg, Kaiserstuhl (Aichkarren), 31.VII.1953, 2 ♂♂ B, leg. W. Wagner (NCSU). Baden-Württemberg, Tübingen (Spitzberg), VII.1953, 2 ♂♂ B, leg. W. Schwoerbel (coll. Schwoerbel, Biberach, W. Germany). AUSTRIA. Osterreich, Bad Vöslau, 1 ♂ B (ZMH). ITALY. Marche, Monti Sibillini, infra Castelluccio, about 1350 m, 8.IX.1977, 1 ♂ B, leg. R. Remane (RR). YUGOSLAVIA*. Istra, Mumjan 6 km NE of Baje, 9.VII.1966, 2 ♂♂ B (ZMA). GREECE. Fokis, Elaion, 16.IV.1980, 21 ♂♂ B 13 ♀♀ B, leg. R.H. Cobben & S. Drosopoulos; 23.VII.1983, 32 ♂♂ B 16 ♀♀ B 3 ♀♀ M 2 L5, leg. C. den Bieman & S. Drosopoulos (AUW, CB). Other localities from Greece were listed in Asche & Hoch (1982), Asche & Remane (1982), Drosopoulos (1982) and Drosopoulos et al. (1985). Reports of R. imitans from Czechoslovakia, Poland, Romania (Nast, 1972) and Hungaria (Soos, 1976) should be checked. The erroneous report of R. imitans from the Netherlands was based on confusion with R. pungens.

Ribautodelphax imitantoides n.sp. (Fig. 6A-O).

Description. R. imitantoides is morphologically close to R. imitans and resembles this species in coloration and in proportions. Differences of this from other species of the R. collinus complex are found in the male genitalia. The ventral incision of the pygofer is not flanked by processes (Fig. 6B-E), though some individuals have ventral incisions with slightly angular corners. Laterally, the pygofer has a small incision in the upper part (Fig. 6F-I). The appendages of the anal tube (Fig. 6J) and the parameres (Fig. 6L-M) are similar to those of R. imitans. The central diaphragm has two small tooth-like processes, some dorsad, others caudad. (Fig. 6F-I). These processes are placed on a well developed elevation of the central diaphragm and R. imitantoides differs in this respect from R. imitans. As in R. imitans, the aedeagus is shorter than in most other species of the R. collinus complex, has a variable number of teeth and a subapical phallotrema. Length: ♂ B 2.2-2.8 mm; ♂ M 3.4-3.9 mm; ♀ B 2.3-2.8 mm; ♀ M 3.6-4.1 mm.

Remarks. R. imitantoides was termed "Taxon 4" in my previous papers (den Bieman, 1986; 1987a-d; den Bieman & Eggers-Schumacher). Differences in male acoustic signals between the Tuchan population (code: FR10) and the remaining populations suggested that this population might represent a new species (den Bieman, 1986). However, this distinction is not supported by analysis of the allozyme patterns and morphology of the male genitalia (den Bieman & Eggers-Schumacher, 1987; this paper, Fig. 6D). Moreover all populations, including the Tuchan population, were sampled from the same host plant (den Bieman, 1987a). The Tuchan population hybridized freely with several other R. imitantoides populations. These data support the conclusion that the Tuchan population has to be included in R. imitantoides. However the specimens of this population are not considered as paratypes. R. imitantoides differs from the other species of the R. collinus complex in the same respects as R. imitans. However R. imitantoides lives on the same host plant as some of the R. pungens populations (den Bieman, 1987b). The variability of the female acoustic signals of R. imitantoides limits their applicability as a species-specific characteristic (den Bieman, 1987a).

To avoid confusion with R. imitans due to morphologic variability, identifications should preferably make use of more than one male of a
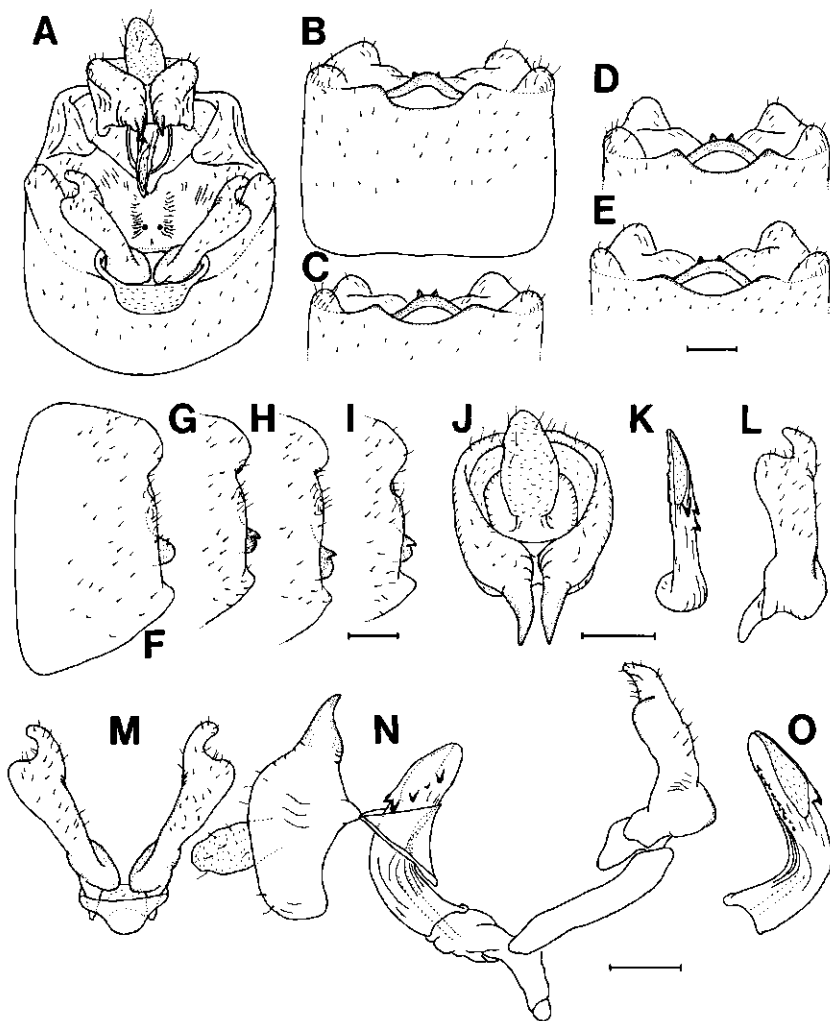
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Fig. 6. A-O, R. imitantoides n.sp. A-C, F-G, J-O: France, Vaucluse, St. Estève, 3.IV.1983, paratypes. D,H: France, Aude, Tuchan, 15.VI.1980. E,I: France, Pyrénées Orientales, Gorges de Galamus,. 21.VI.1982, paratypes. All leg. C. den Bieman (CB). A. ♂ genitalia, ventrocaudal view. B-E. Pygofer, ventral view. F-I. Pygofer, left lateral view. J. Anal tube, caudal view. K. Aedeagus, caudal view. L. Left paramere, maximal view. M. Parameres, ventrocaudal view. N. ♂ genitalia without pygofer, left lateral view. O. Aedeagus from right side. Bars: 0.1 mm.

population.

Biology. R. imitantoides lives on Brachypodium phoenicoides. It is a very common planthopper in the W. Mediterranean region often occurring in deserted vineyards, orchards and meadows. It occurs up to 1000 m, has three or more generations and hibernates as L2-L4. Parasitization by Strepsiptera, Dryinidae and Entomophthora fungi is observed. Pseudogamous triploid females occur in several populations of R. imitantoides, which are morphologically indistinguishable from the diploid bisexual females (den Bieman, 1987d).

Distribution. R. imitantoides occurs in the W. Mediterranean region and is, consequently, partly geographically separated from R. imitans. Holotype: FRANCE, Vaucluse, St. Estève, 520-540 M, 3.IV.1983, 1 ♂ B, leg. C. den Bieman (ZMA). Paratypes. Same locality and date, 88 ♂♂ B 54 ♀♀ B 10 L3-L5; 5.IV.1982, 22 ♂♂ B 13 ♀♀ B 4 L5; 24.IV.1984, 15 ♂♂ B 13 ♀♀ B 33 L3-4, leg. C. den Bieman (CB, AUW), this population was termed population A in den Bieman (1987b). Vaucluse, Crillon-le-Brave, 300-320 m, 4/7.IV.1982, 24 ♂♂ B 20 ♀♀ B; 3.IV.1983, 10 ♂♂ B 10 ♀♀ B; 25.IV.1984, 6 ♂♂ B 4 ♀♀ B. Vaucluse, Bedoin, 280-300 m, 3.IV.1982, 6 ♂♂ B 12 ♀♀ B. Vaucluse, Flassan, 320-340 m, 1 ♂ B. Vaucluse, Javon, 640-660m, 8.IV.1983, 2 ♂♂ B 4 ♀♀ B. Vaucluse, Pertuis, 22.IV.1984, 2 ♂♂ B. Vaucluse, Col de Murs, 627 m, 23.IV.1984, 1 ♂ B 11 ♀♀ B. Vaucluse, Lourmarin 5 km N of Cadenet, 23.IV.1984, 1 ♂ B. Bouches du Rhône, St. Remy de Provence, 200 m, 4.IV.1983,6 ♂♂ B 13 ♀♀ B. Bouches du Rhône, les Baumettes 3 km E. of Mouries, 4.IV.1983, 8 ♂♂ B 23 ♀♀ B; 19.IV.1984, 18 ♀♀ B, termed population B in den Bieman (1987b). Bouches du Rhône, Gargaron (Camargue), 0-20 m, 5.IV.1982, 2 ♂♂ B. Bouches du Rhône, Alleins, 7.IV.1983, 1 ♂ B. Var, Rians, 340-360 m, 1 ♂ B 4 ♀♀ B. Aude, Tuchan, 15.VI.1980, 4 ♂♂ B 5 ♀♀ B; 12.VIII.1981, 5 ♂♂ B 11 ♀♀ B 3 L3. Aude, 8 km N of Quillan, 4.VII.1985, 4 ♂♂ B 1 ♂ M 8 ♀♀ B. Aude, 12 km N of Carcasonne, 260-280 m, 3.VII.1985, 1 ♂ B 1 ♀ B. Pyrenées Orientales, Gorges de Galamus, 16.VI.1980, 5 ♂♂ B 16 ♀♀ B 4 ♀♀ M. All leg. C. den Bieman (CB, AUW). SPAIN. Lerida, 12 km SW of Organa, 5.VII.1985, 3 ♂♂ B 16 ♀♀ B. Lerida, 5 km W of Tremp, 560-580 m, 6.VII.1985 1 ♂ B 2 ♀♀ B. Huesca, 9 km W of Huesca, 660-680 m, 1 ♂ M 2 ♀♀ B. Huesca, Arén, 6.VII.1985, 1 ♂ B. Zaragoza, Nuevalos, 900-920 m, 9.VII.1985, 3 ♂♂ B 1 ♂ M 3 ♀♀ B. All leg. C. den Bieman (CB). Valencia, Sagunto, 17.V.1982, 7 ♂♂ B 1 ♂ M 4 ♀♀ B 7 ♀♀ M, leg. R.H. Cobben (AUW). Alicante, Agres, 14.VI.1979, 5 ♂♂ B 4 ♀♀ B, leg. M.R. Wilson (BMNH). Information on more than 20 populations from the provinces: Alicante, Cuenca, Gerona, Jaen, Lerida, Murcia, Soria, Teruel and Valencia, will be published by R. Remane (RR). PORTUGAL. Estre, Sintra, 23/26.VII.1957, 1 ♂ B 2 ♀♀ B, leg. R.H. Cobben (ZMH). MOROCCO. Ifrane, 2.VII.1935, 1 ♂ B, leg. J.M. Mimeur (MNHN); 28.V.1981, 3 ♂♂ B 1 ♀ B, leg. R. Remane (RR). Haute Atlas, N of Asni, 22.V.1981, 1 ♂ B, leg. R. Remane (RR). Daïet Haoua, 1500 m, 17.VII.1935, 2 ♂♂ B, leg. J.M. Mimeur (MNHN). Note. Several of the females listed might be pseudogamous triploid females.

Ribautodelphax fanari Asche, Drosopoulos & Hoch, 1986.

R. fanari Asche, Drosopoulos & Hoch, 1986; Marburger Ent. Publ. 2(3), 198-199, fig. 6-10.

Description. R. fanari has been recently described by Asche et al. (1986) and is only drawn in this paper to allow comparison with the other species of the R. collinus complex. The main differences in male genitalia are listed. The ventral incision of the pygofer is flanked by long processes, which are apically rounded (Fig. 7A-C). The parameres are short and slender, and have a well developed mediad edge. Differences between the material from the type locality in Greece and from France have not been found. Length: ♂ B 2.4-2.8 mm; ♂ M 3.7-3.9 mm; ♀ B 2.9-3.2 mm; ♀ 4.0-4.2 mm.

Remarks. The acoustic signals of both sexes, the host plants and the male genitalia clearly distinguish this species from the remaining species of the R. collinus complex (den Bieman, 1986, 1987b). The taxonomic relationship between this species and R. exquisitus Anufriev needs to be studied (Asche et al., 1986).

Biology. R. fanari lives on Elymus hispidus (Opiz) Melderis in Greece and on E. pycnanthus (Godron) Melderis in France. It inhabits salt marshes near to the Mediterranean sea-shore. The number of generations is unknown.

Material studied. GREECE. Xanthi, W. of Porto Lagos (type locality), 16.VII.1983, 7 ♂♂ B 1 o M 5 ♀♀ B 19 L3-L5, leg. C. den Bieman (CB); 23.VIII.1983, 1 ♂ B, leg. R.H. Cobben & S. Drosopoulos (AUW). Xanthi, E. of Porto Lagos, 15/16.VII.1983, 10 ♂♂ B 5 ♀♀ B 30 L2-L5, leg. C. den Bieman (AUW,CB). See also Asche et al. (1986). YUGOSLAVIA* Istra, 2 km ESE of Valtura, 14.VII.1966, 2 ♂♂ B 1 ♀ B (ZMA). FRANCE*. Bouches du Rhône, Etang de Vaccarès (Camargue), 5/6.IV.1983, 12 ♂♂ B 7 ♀♀ B; 9.IV.1984, 2 ♂♂ B, leg. C. den Bieman (CB). R. fanari was also reported from Turkey (Asche et al. 1986).

Ribautodelphax angulosus (Ribaut, 1953).

C. angulosa Ribaut, 1953; Bull. Soc. Hist. Nat. Toulouse 88, 247-248, fig. 12-14.

Description. A good description of R. angulosus is given by Ossiannilsson (1978). The main differences with the other Ribautodelphax species are given. The corners of the ventral incision of the male pygofer
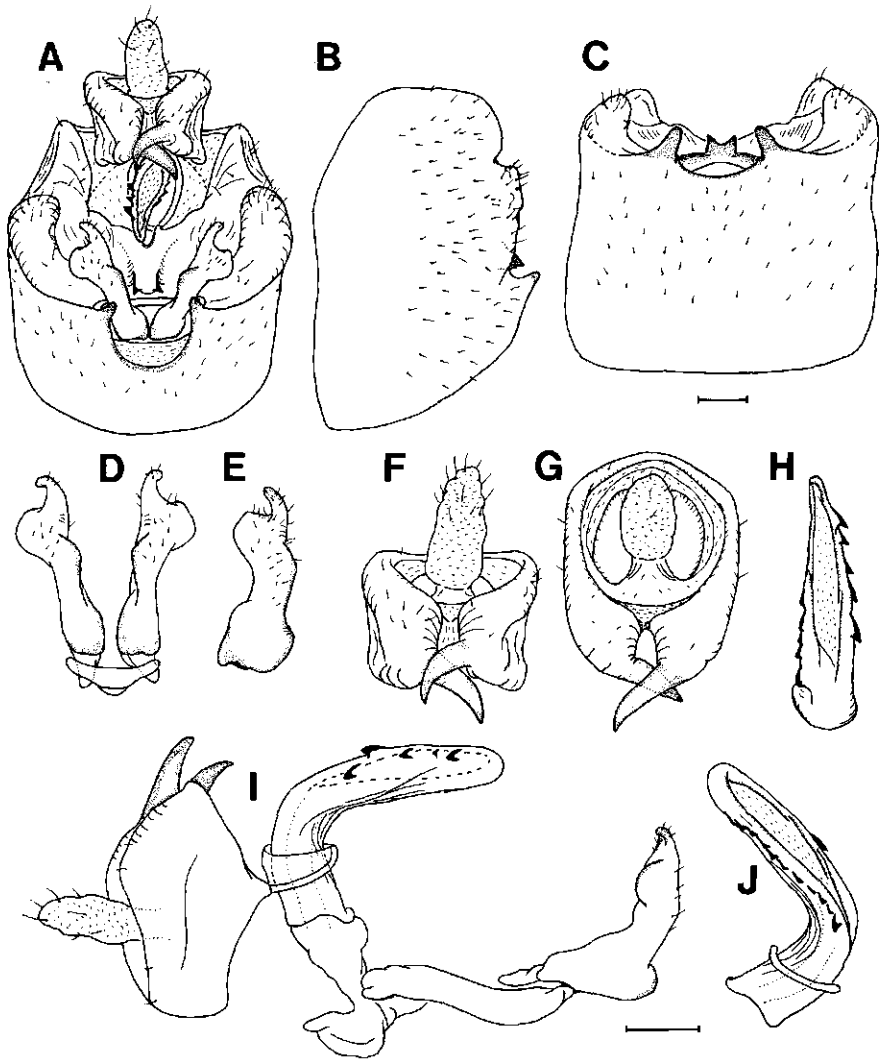
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Fig. 7. A–J, R. fanari Asche, Drosopoulos & Hoch: France, Bouches du Rhône, Etang de Vaccarès, 6.IV.1983, leg. C. den Bieman (CB). A. ♂ genitalia, ventrocaudal view. B. Pygofer, left lateral view. C. Pygofer ventral, view. D. Parameres, ventrocaudal view. E. Left paramere, maximal view. F. Anal tube, ventral view. G. Anal tube, caudal view. H. Aedeagus, caudal view. I. ♂ genitalia without pygofer, left lateral view. J. Aedeagus from right side. Bars: 0.1 mm.
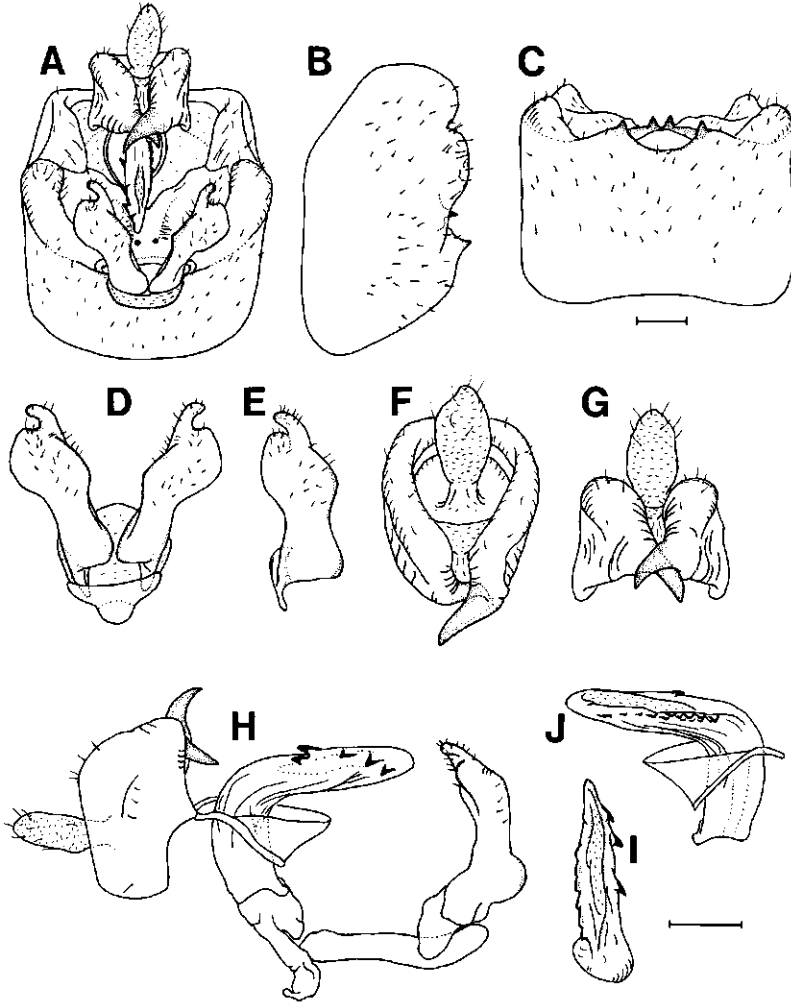
Fig. 8. A-J, R. angulosus (Ribaut): France, Haute-Garonne, Saint-
Béat, IV.1929, lectotype, leg. H. Ribaut (MNHN). A. ♂ genitalia,
ventrocaudal view. B. Pygofer, left lateral view. C. Pygofer, ventral
view. D. Parameres, ventrocaudal view. E. Left paramere, maximal
view. F. Anal tube, caudal view. G. Anal tube, ventral view. H. ♂
genitalia without pygofer, left caudal view. I. Aedeagus, caudal
view. J. Aedeagus from right side. Bars: 0.1 mm.

are distinctly angular but do not protrude much (Fig. 8A, C). Parameres stout with a mediad directed edge (Fig. 8D-E). The stout appendages of the anal tube cross with the left one beneath the right one (Fig. 8F-G). The mirror image is observed in a few specimens (LeQuesne, 1960). The arched aedeagus has a variable number of teeth (Fig. 8H-J). Length: ♂ B 2.0-2.3 mm; ♂ M 3.0 mm; ♀ B 2.5-2.8 mm; ♀ M 3.4-3.8 mm.

Remarks. R. angulosus is often confused with other Ribautodelphax species. The acoustic signals of both sexes, the host plant and the male genitalia are distinctly species-specific (den Bieman, 1986; 1987b). Only some combinations produced a few hybrids. A brachypterous male of Ribaut's type series is designated as lectotype: France, Haute-Garonne, Saint Béat, IV.1929 (MNHN).

Biology. R. angulosus seems a rare planthopper, though its host plant Anthoxanthum odoratum L. is a common grass in W. Europe. It is bivoltine in W. Europe and occurs in dry biotopes. It hibernates as 2nd to 4th instars.

Material studied. FRANCE. Haute-Garonne, Saint Béat, no date, 6 ♂♂ B 1 ♂ M 4 ♀♀ B 5 ♀♀ M; 12.VII.1921, 1 ♂ B; 16.VII.1921, 1 ♂ B 1 ♂ M 1 ♀ B; IV.1929, 4 ♂♂ B 3 ♀♀ B; 30.VII.1930, 1 ♂ B; 6.VII.1930, 1 ♂ B; 25.VII.1939, 1 ♂ B, 1 ♀ B; 20.III.1940, 3 ♂♂ B 1 ♀ B; 2.IX.1940, 1 ♂ B. All leg. H. Ribaut (MNHN, NCSU). Haute-Garonne, Arlos, 8.VIII.1930, 2 ♂♂ M 1 ♀ M (MNHN). Aude, Puilaurens, 13.VII.1934, 1 ♂ B 1 ♂ M 1 ♀ M (MNHN). Ariège, Ariège, 1400 m, 27.VII.1978, 1400m, 2 ♂♂ B, leg. R. Remane (RR). THE NETHERLANDS. Utrecht, Leersum, many samples 1972/1984 (AUW, CB), this population was used for most experimental studies. Noord-Holland, Laren, 22.VIII.1954, 1 ♂ B 4 ♀♀ B, leg. W. Gravestein (ZMA). Noord-Holland, Nieuwkoop,15.VIII.1951, ♂,leg. Lindberg (ZMH). Gelderland, Wageningen, many samples 1953/1959, leg. R.H. Cobben (AUW). Limburg, Asselt-Boukoul, 12.VIII.1953, 1 ♂ M, leg. R.H. Cobben (AUW). SWEDEN. Ostgotland, Rystad, 15/16.VI.1933, 1 ♂ B, leg. Lindberg (ZMH). FINLAND. Alandia, Aland, 2 ♂♂ B. Regio aboensis, Lojo, 3.VII.1920, 1 ♂ B; 25.VII.1931, 1 ♂ B. All leg. Lindberg (ZMH). W. GERMANY. Hessen, Frankfurt a.M. (Mönchbruch), 29.VII.1939, 2 ♂♂ B, leg. W. Wagner (ZMH). Rheinland-Pfalz, Lemberg a.d. Lahe, 1.VIII.1939, 3 ♂♂ B 1 ♀ B, leg. W. Wagner (ZMH, NCSU). Bayern, Oberstdorf, VIII.1936, 1 ♂ B, leg. W. Wagner (NCSU).SWITZERLAND. Vaud, Lausanne, VIII.1926, 1 ♂ M, leg. P. Kupka (NCSU). AUSTRIA. Steiermark, Admont, VIII.1941, 1 ♂ B 3 ♀♀ B 1 ♀ M, leg. W. Wagner (NCSU). Steiermark, Barndorf, 28.VII.1930, 1 ♂ M, leg. Singer (MNHN). GREECE*. Drama, Rodopi Mt., around Megali Panagia, 1400-1600 m, 19.VII.1983, 4 ♂♂ B 10 ♀♀ B 1 L5, leg. C. den Bieman & S. Drosopoulos (CB). R. angulosus is further reported from E. Germany, England, Mongolia, Poland, Romania and USSR (Nast, 1972), these records should be carefully checked. All the material of R. angulosus from the eastern part of the USSR that I studied concerned other Ribautodelphax species.

R. ventouxianus n.sp., R. kalonerensis n.sp.

R. ventouxianus and R. kalonerensis are morphologically hardly
distinguishable. Nevertheless, they are considered to be separate species
because of the following biological differences. Their host plants and the
acoustic signals of males and females are different (den Bieman, 1986,
1987b). The two hybridize in the laboratory but their number of hybrids is
low. However their most conspicuous difference concerns differentiation in
hibernation. R. kalonerensis could be reared continuously in the laboratory
at 20 °C and long-day conditions (18 h light). R. ventouxianus, however,
shows an obligatory diapause and could not be reared continuously under
similar condtions. The diapause could not be broken by various prolonged
cold or heat treatments. Finally, the two are geographically separate.

Ribautodelphax ventouxianus n.sp. (Fig. 9A-J).

Description. The deep concave incision of the ventrocaudal margin of
the male pygofer has pronounced sharp corners (Fig. 9A-C). The lateral
incision of the pygofer is well developed (Fig. 9B). The long parameres
have a clear mediad edge (Fig. gD-E). This edge is more basally situated
than, for instance, in R. angulosus or R. pungens. The filiformous anal
tube appendages are long and crossed, always with the left beneath the
right (Fig. 9F-G). In most specimens, the tips of the appendages just
overlap but six males showed slightly more overlap. The central diaphragm
has two small ventrad tooth-like processes (Fig. 9A-C). The aedeagus is
arched as in the other species of the R. collinus complex, has a supapical
phallotrema and a variable number of spines. R. ventouxianus is one of the
biggest Ribautodelphax species: ♂ B 2.8-3.5 mm; ♂ M 3.2-3.8 mm; ♀ B 4.3-4.7
mm; ♀ M 4.4-4.9 mm.
Remarks. This species was termed "Taxon 3" in my previous papers. The
differences in host plants and in male and female acoustic signals from the
remaining members of the R. collinus complex warrant species status for R.
ventouxianus (den Bieman, 1986; 1987b).
Biology. R. ventouxianus is a mountain species occurring at 1360 -1600
m in dry shaded biotopes. It lives on Festuca rubra rubra L. It is probably
univoltine and the 2nd and 3rd instars hibernate. Several individuals were
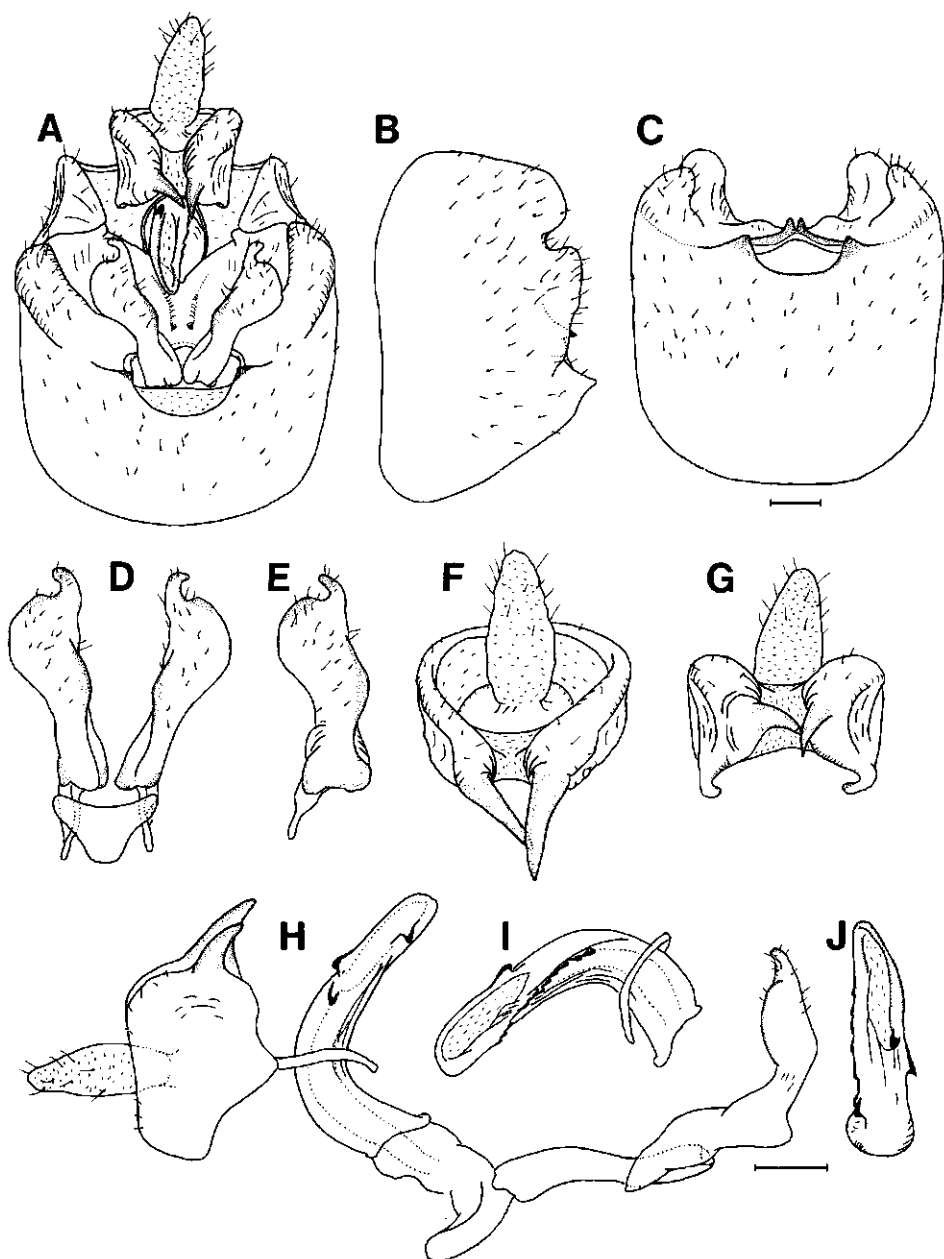parasitized by Pipunculidae.

Fig. 9. A-J, R. ventouxianus n.sp.: France, Vancluse, Mont Ventoux,
24.VII.1986, paratype, leg. C. den Bieman (CB). A. ♂ male genitalia,
ventrocaudal view. B. Pygofer, left lateral view. C. Pygofer, ventral
view. D. Parameres, ventrocaudal view. E. Left paramere, maximal
view. F. Anal tube, caudal view. G. Anal tube, ventral view. H. ♂
genitalia without pygofer, left lateral view. I. Aedeagus from right
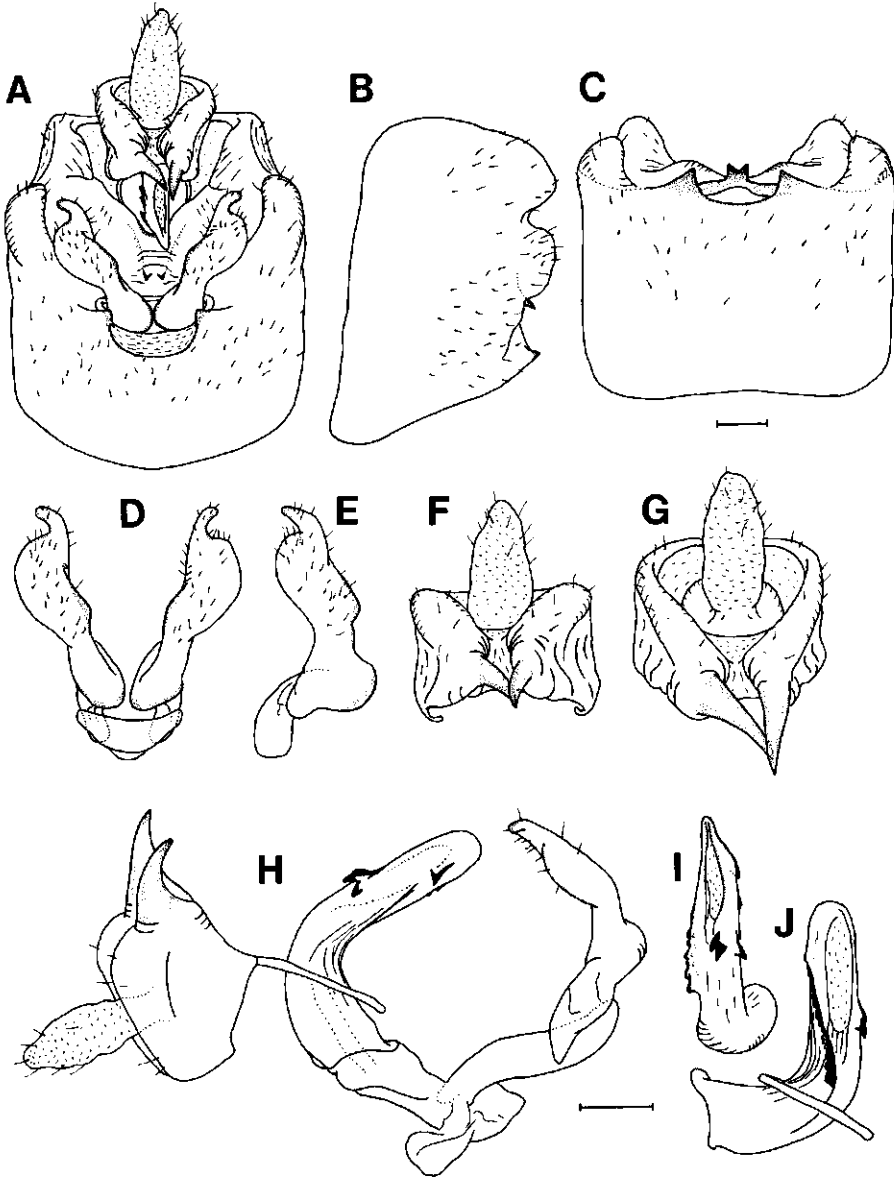side. J. Aedeagus, caudal view. Bars: 0.1 mm.

Fig. 10. A-J, R. kalonerensis n.sp.: Greece, Florina, Kalo Nero Mt.,
30.VII/2.VIII.1982, paratype, leg. S. Drosopoulos (CB). A. ♂
genitalia, ventrocaudal view. B. Pygofer, left lateral view. C.
Pygofer, ventral view. D. Parameres, ventrocaudal view. E. Left
paramere, maximal view. F. Anal tube, ventral view. G. Anal tube,
caudal view. H. ♂ genitalia without pygofer, left lateral view. I.
Aedeagus, caudal view. J. Aedeagus from right side. Bars: 0.1 mm.

Distribution. R. ventouxianus appears to be restricted to SE. France. Holotype: FRANCE, Vaucluse, Mont Ventoux, 1360-1380 m, 24.VII.1986, 1 ♂ B, leg. C. den Bieman (BMNH). Paratypes: same locality and date, 27 ♂♂ B 4 ♂♂ M 22 ♀♀ B 6 ♀♀ M, leg. C. den Bieman (CB, AUW, ZMA). Same locality, 27.VI.1979, 7 ♂♂ B 6♂♂ M 7 ♀♀ B 2 ♀♀ M; 26.VII.1979, 1 ♂ b 3 ♂♂ M 1 ♀ B 2 ♀♀ M, all leg. M.R. Wilson (BMNH); samples of larvae were taken at 5.IV.1982, 9.IV.1983 and 24.V.1984. The larvae were reared to adulthood in the laboratory. Alpes de Haute Provence, Mt. de Lure, 1600 m, 19.VI.1982, 1 ♂ M 1 ♀ M, leg. M. Wilson (CB).

Ribautodelphax kalonerensis n.sp. (Fig. 10A-J).

Description. R. kalonerensis is largely like the preceding species in proportions but most individuals are lighter coloured. The deep concave ventral incision of the male pygofer has sharp corners (Fig. 10A-C), which are slightly more pronounced than of R. ventouxianus. Laterally, the incision of the pygofer is well developed. The parameres are similarly shaped to those of R. ventouxianus, but the mediad edge is less pronounced (Fig. 10D-E). As in R. ventouxianus, the tips of the filiformous anal tube appendages are crossed with the left always beneath the right (Fig. 10F-G). The tooth-like processes of the central diaphragm are more developed than for R. ventouxianus (Fig. 10A-C). The arched aedeagus (Fig. 10H-J) has a subapical phallotrema and a variable number of teeth. The differences in the number of caudal teeth between R. kalonerensis (Fig. 10I) and R. ventouxanius (Fig. 10J) concern variation between individuals. Length: ♂ B 2.7-2.9 mm; ♂ M 3.9 mm; ♀ B 3.0-3.4 mm.

Remarks. This species was termed "Taxon 2" in previous papers (den Bieman, 1986, 1987a-e) and R. spec. cf. collinus in Drosopoulos et al. (1985). R. kalonerensis differs from the remaining species of the R. collinus complex in host plants and acoustic signals. A few hybrids were produced with only three of the Ribautodelphax species tested.

Biology. R. kalonerensis was collected from Arrhenatherum elatius (L.) Beauv. in a mountain meadow at 1300-1500 m. One female was parasitized by Stresiptera. The number of generations and the hibernating instars are unknown.

Distribution. R. kalonerensis has as yet only been collected in NW. Greece. Holotype: GREECE, Florina, Kalo Nero Mt., Southern Slope, supra Agia Triada, 1300-1600m, 30.VII.1982, ♂ B, leg. S. Drosopoulos (coll. Drosopoulos, Athens). Paratypes: same locality, 30.VII-2.VIII.1982, 12 ♂♂ B (1 ♂ parasitized by Strepsiptera) 12 ♀♀ B, leg. S. Drosopoulos & M. Asche (coll. Asche, Marburg, CB); 21.VII.1983, 4 ♂♂ B 4 ♀♀, leg. S. Drosopoulos & C. den Bieman (AUW, CB).

R. libanonensis n.sp., R. nevadensis n.sp.

The morphologically related species R. libanonensis and R. nevadensis are only known from museum collections. Their male genitalia are sufficiently different mutually and from the other Ribautodelphax species to describe them as new species.

Ribautodelphax libanonensis n.sp. (Fig. 11 A-J).

Description. The deep concave incision of the ventral margin of the pygofer is flanked by two long and pointed processes as in R. collinus (Fig. 11 A-C). Laterally, the pygofer has a small incision in the upper part (Fig. 10 B). The long slender parameres have a rounded mediad edge and, as in R. kalonerensis and R. ventouxianus, this edge is situated more basally than in e.g. R. pungens (Fig. 11 D-E). The long anal tube appendages are crossed with the left always beneath the right (Fig. 11 F-G). As in the other species of the R. collinus complex, the arched aedeagus has a subapical phallotrema, a number of larger teeth on the left and rows of smaller teeth on the right (Fig. 11 H-J). The tooth-like appendages of the central diaphragm are well separated (Fig. 11 A,C). All brachypterous specimens have long forewings nearly reaching the tip of the abdomen. Such long brachypterous forewings are very uncommon in the other Ribautodelphax species. Lenght: ♂ B 2.0-2.6 mm, ♀ B 2.6 mm; ♂ M 3.6 mm; ♀ M 3.8 mm.

Biology. Unknown.

Distribution. Holotype: LIBANON, Mazraat Kfar, Zebiane (el Qanator), 24.VII.1983, 1 ♂ B, leg. H. Abdul-Nour (ZMA). Paratypes: same date, locality and collector 3 ♂♂ B, 2 ♂♂ M, 2 ♀♀ B, 2 ♀♀ M (CB, AUW).

Ribautodelphax nevadensis n.sp. (Fig. 12 A-J).

Description. The deep concave incision of the ventrocaudal margin of the pygofer of this species, of the preceding one and of R. collinus is flanked by long pointed processes (Fig. 12 A,C). The pygofer margin has on the side a small incision in the upper part (Fig. 12 B). The long and slender parameres have a rounded mediad edge (Fig. 12 D-E). R. nevadensis and R. libanonensis have similarly shaped and oriented anal tube appendages (Fig. 12 F-G). A conspicuous difference from R. libanonensis is found in the tooth-like processes of the central diaphragm. In the other species of
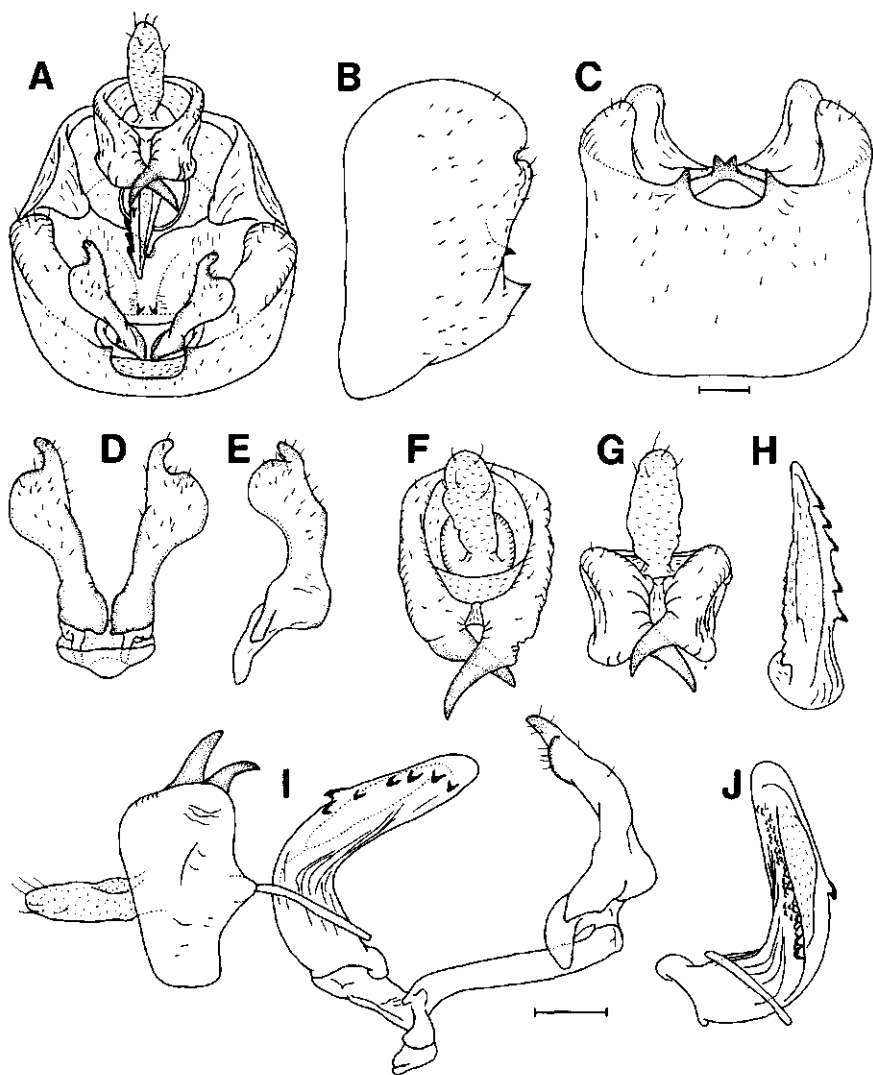
Fig. 11. A–J, R. libanonensis n.sp.: Libanon, Mazraat Kfar, Zebiane (el Qanater), 24.VII.1982, paratype, leg. H. Abdul–Nour (CB). A. ♂ genitalia, ventrocaudal view. B. Pygofer, left lateral view. C. Pygofer, ventral view. D. Parameres, ventrocaudal view. E. Left paramere, maximal view. F. Anal tube, caudal view. G. Anal tube, ventral view. H. Aedeagus, caudal view. I. ♂ genitalia without pygofer, left lateral view. J. Aedeagus from right side. Bars: 0.1 mm.
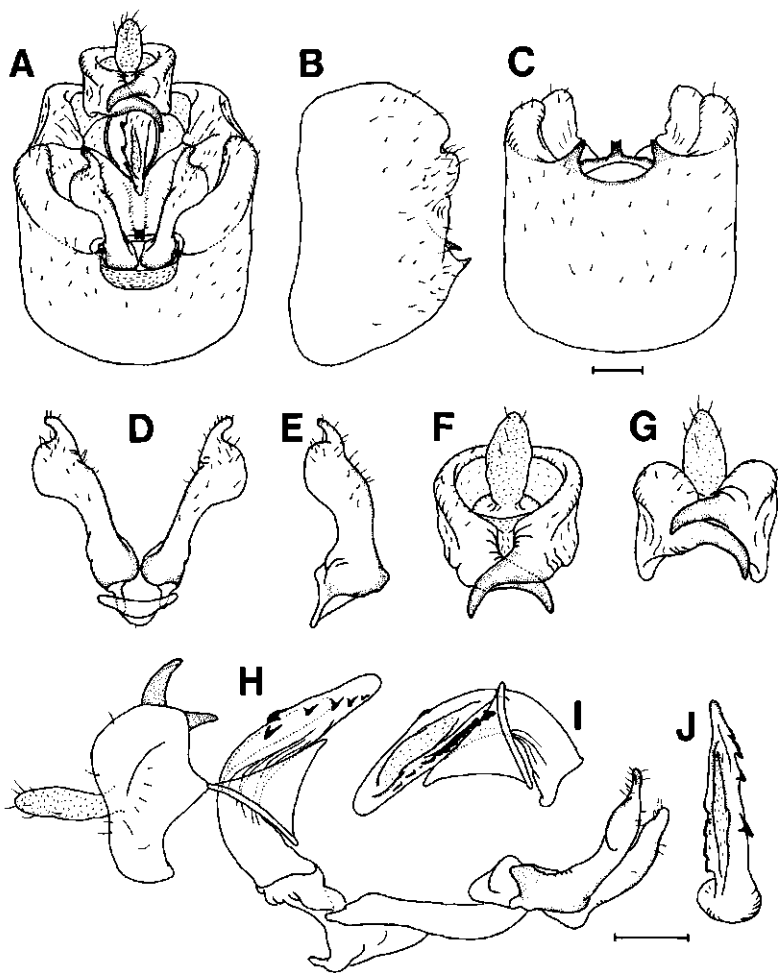
Fig. 12. A–J, R. nevandensis n.sp.: Spain, Granada, Sierra Nevada, Laguna de las Yeguas, 25.VI.1972, paratype, leg. R. Remane (CB). A. ♂ genitalia, ventrocaudal view. B. Pygofer, left lateral view. C. Pygofer, ventral view. D. Parameres, ventrocaudal view. E. Left paramere, maximal view. F. Anal tube, caudal view, G. Anal tube, ventral view. H. ♂ genitalia without pygofer, left lateral view. I. Aedeagus from right side. J. Aedeagus, caudal view. Bars: 0.1 mm.

the R. collinus complex, these processes are well separated but they are
basally fused in R. nevadensis forming one indented process as, for
instance, in e.g. R. ochreatus (Fig. 12 B,C). The aedeagus is similar to
that of most species of the R. collinus complex (Fig. 12 H-J). Brachypters
have normally developed forewings reaching to the 6th or 7th abdominal
segment. Length: ♂ B 2.0-2.1 mm; ♀ B 2.6-2.7 mm.

Biology. Unknown.

Distribution. Holotype: SPAIN, Granada, Sierra Nevada, Laguna de las
Yeguas, 2900 m, 25.VI.1972, 1 ♂ B, in a wet meadow, leg. R. Remane (R.R.).
Paratypes: same locality and date: 3 ♂♂ B, 2 ♀♀ B, leg. Remane (RR, CB).

KEY TO THE MALES OF EUROPEAN SPECIES OF THE R. COLLINUS COMPLEX

1   Anal tube appendages parallel, diverging at the top (Fig. 5 J, 6 J);
     parameres without an edge at the inner margin (Fig. 5 M, 6 M)........2

-    Anal-tube appendages not parallel and diverging at the top...........3
2   Ventral incision of pygofer with sharp corners (Fig. 5 C-E). Processes
     of central diaphragm not placed on an elevation (Fig. 5 F-I); host
     plant: Festuca arundinacea spp. fenas......................R. imitans

-    Ventral incision of pygofer without well developed angular corners
     (Fig. 6 B-E); processes of central diaphragm placed on an elevation
     (Fig. 6 F-I); host plant: Brachypodium phoenicoides....R. imitantoides

3   Ventral incision of pygofer not flanked by processes or with sharp
     corners (Fig. 2C; 3C-D; 4C)..4

-    Ventral incision of pygofer flanked by processes or with sharp
     corners..6

4   Parameres with a basally placed mediad-directed edge (Fig. 3 E); host
     plant: Agrostis vinealis..................................R. vinealis

-    Parameres as in Figures 2 D and 4 D..................................5

5   Anal tube appendages filiformous with variable orientation (Fig. 2 I-
     P); host plants: Brachypodium spp...........................R. pungens

-    Anal tube appendages stout, the left always bent beneath the right,
     and the left appendage bent inwards (Fig. 4 F); host plant: Carex
     nigra..R. noguerae

6   Parameres without an edge at the inner margin (Fig. 1 D), almost
     reaching to the lateral pygofer incision (Fig. 1 A); ventral incision
     of pygofer flanked by long processes (Fig. 1C); host plant Agrostis
     capillaris..R. collinus
-    Parameres with an edge at the inner margin..........................7

7   Processes of ventral pygofer incision apically rounded (Fig. 7 C); parameres short (Fig. 7 A); host plants: Elymus spp..........R. fanari

-    Processes of ventral pygofer incision apically not rounded...........8

8   Ventral incision of pygofer with sharp corners (Fig. 8 A, 9 A, 10 A).9

-    Ventral incision of pygofer flanked by long processes...............10

9   Length: ♂ B 2.0-2.3 mm; ♂ M 3.0 mm; anal tube appendages crossed as in Fig. 8 F,G; host plant: Anthoxanthum odoratum.............R. angulosus

-    Length: ♂ B 2.7-3.5 mm; ♂ M 3.2-3.9 mm; anal tube appendages crossed as in Fig. 9 F, 10 G..
                R. ventouxianus (France; host plant: Festuca rubra rubra)
                R. kalonerensis (Greece; host plant: Arrhenatherum elatius)

10   Processes of central diaphragm well separated (Fig. 11 A, C).........
     ....R. libanonensis

-    Processes of central diaphragm fused (Fig. 12C)..........R. nevadensis

Relationships between the R. collinus complex and outgroup species.

Two outgroup species, R. pallens and R. albostriatus, have been studied experimentally. Descriptions and drawings of male genitalia have been given by Ossiannilsson (1978). Morphological data suggest that R. pallens is more like the R. collinus complex than R. albostriatus. The last species differs in the following structures of the male genitalia from the species of the R. collinus complex: pygofer in lateral aspect with an incision in the lower part and not in the upper part; pygofer without a ventral incision; central diaphragm without paired processes; parameres without a lateral lobe; aedeagus evenly curved with big teeth; anal tube with short appendages. R. pallens, however, resembles the R. collinus complex in the shape of the pygofer margin, in parameres, in anal tube appendages, and by the presence of paired processes on the central diaphragm. These relationships are supported by acoustic, electrophoretic and hybridization data (den Bieman, 1986, 1987c, den Bieman & Eggers-Schumacher, 1987).

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Pseudogamous triploid <u>Ribautodelphax</u> females.

Parthenogenetic organisms present special taxonomic problems, including the question whether these forms should be provided with formal names. Recently, several solutions for this last problem have been proposed (Cole, 1985; Dubois & Günther, 1982; Walker, 1986). In the past, many authors have dealt with these forms as if they were "biological" species, and have given them formal taxonomic recognition (e.g. Maslin, 1968; Walker, 1986). Others proposed informal systems using letters or figures (Walker, 1986) or applied hyphenated names reflecting their hybrid composition and genomic constitution (Drosopoulos, 1976; Schultz, 1969).

Dubois & Günther (1982) proposed a solution to the treatment of pseudogamous forms. They created new systematic categories and proposed nomenclatural rules. I will not apply their system to the pseudogamous triploid <u>Ribautodelphax</u> females, firstly because their system is not followed in the recent literature; secondly because they assumed that a hybrid origin was one of the main common features of pseudogamous forms. However extensive multidisciplinary investigations on the pseudogamous triploid <u>Ribautodelphax</u> females indicate their autopolyploid origin (den Bieman, 1987a-d; den Bieman & Eggers-Schumacher, 1987). Apart from the difference in ploidy, no differences have been found between the pseudogamous triploid females and their diploid sperm-donor species. The pseudogamous triploid females cannot survive alone in nature because of their sperm dependency. This relationship results in a very restricted evolutionary potential for the pseudogamous forms. Moreover, the sexual species and the pseudogamous forms are not genetically isolated because the pseudogamous triploid females repeatedly originate from the same sexual species. Consequently the two should be considered as one dynamic unit.

To allow discussion and reference to the pseudogamous <u>Ribautodelphax</u> females, I propose an informal system by indicating them with a combination of the name of their ancestral species followed by 3N i.e. <u>R. pungens</u> 3N and <u>R. imitantoides</u> 3N. In most cases the ancestral bisexual species will be the acting sperm donor. However potentially the triploid females may coexist with several other bisexual species (den Bieman, 1987a,c) and therefore similarity in biological characteristics as ecology, acoustic behaviour or allozyme patterns should also be taken into account.

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SAMENVATTING

Dit proefschrift behandelt de biosystematiek van het Ribautodelphax collinus komplex (Homoptera, Delphacidae). De soorten uit dit komplex vertonen slechts geringe morfologische verschillen, terwijl enkele diagnostische kenmerken ook nog variabel zijn. Deze geringe verschillen waren een bron van veel identifikatieproblemen en leidden tot twijfel over de status van de beschreven soorten. Daarnaast zijn in de loop van dit onderzoek enkele populaties ontdekt, waarvan de taxonomische status onduidelijk was. Om deze taxonomische verwarring op te kunnen lossen zijn de soorten* uit dit komplex vergeleken wat betreft waardplantrelaties, geluidsproduktie, chromosoomaantallen, kruisbaarheid, eiwitten en genitaalbouw.

De ontdekking van triploide ♀♀, die zich pseudogaam parthenogenetisch voortplanten vormde het begin van een tweede onderzoeksrichting. Deze triploide ♀♀ komen voor in populaties van R. pungens en Taxon 4. De ontstaanswijze van de triploide ♀♀ is uitgebreid onderzocht en de ekologische relaties tussen de pseudogame triploide ♀♀ en de bisexuele soorten, die fungeren als spermaleverancier, zijn geanalyseerd.

De familie van de Delphacidae bestaat uit kleine (2-8 mm) cikaden, die gekenmerkt worden door een beweeglijk spoor aan de acherpoten, waaraan ze hun Nederlandse naam "spoorcikaden" te danken hebben. In Europa komen ongeveer 250 soorten voor. Spoorcikaden zijn sap zuigende insekten, die in Europa hoofdzakelijk leven op grassen, zeggen en biezen. De spoorcikaden gebruiken hun waardplanten niet alleen als voedselbron, maar ook als paringsplaats en als ovipositiesubstraat. De mannetjes en vrouwtjes kommuniceren met behulp van geluidssignalen, die zich primair via de grasstengels voortplanten.

In Europa omvat het genus Ribautodelphax naast de soorten uit het R. collinus komplex nog een viertal soorten. Ter vergelijking met het

* Gemakshalve wordt in deze samenvatting van "soorten" gesproken, hoewel het vaststellen van hun taxonomische status het doel van deze studie vormde. Met "Taxon 1 tot 4" worden de recent ontdekte populaties aangeduid.

R. collinus komplex zijn hiervan twee soorten, R. pallens en R. albostriatus, nader bestudeerd.

De waardplantrelaties van 11 Ribautodelphax soorten worden in hoofdstuk 1 beschreven. Elke soort is in het veld gebonden aan één of enkele verwante grassoorten, de zgn. "veldwaardplant(en)". Slechts twee Ribautodelphax soorten komen op dezelfde grassoort voor. De waardplanten zijn kenmerkend voor de Ribautodelphax soorten en enkele nieuwe soorten werden ontdekt dankzij verschillen in waardplanten. Via keuze- en kweekproeven zijn de waardplantrelaties nader geanalyseerd. Alle soorten, zowel ♂♂ als ♀♀, vertonen een sterke voorkeur voor de veldwaardplant, maar in enkele gevallen zijn verwante waardplanten ook aantrekkelijk. De beste kweekresultaten werden behaald op de veldwaardplant. Toch kunnen een aantal soorten gekweekt worden op de waardplanten van andere Ribautodelphax soorten. Opvallend is, dat vrijwel alle soorten zich kunnen voortplanten op het gras Festuca arundinacea ssp. fenas. Pseudogame triploide ♀♀ en de bisexuele soorten waarmee zij samen voorkomen, vertonen geen verschillen in waardplantrelaties.

De geluidssignalen van de 11 Ribautodelphax soorten zijn geregistreerd (hoofdstuk 2). De ♂♂ produceren komplexe signalen, waarin vier sekties onderscheiden kunnen worden, waarvan er twee geschikt zijn voor kwantitatieve analyse. De ♂♂ produceren een voor elke soort karakteristiek signaal. De signalen van de ♀♀ hebben een eenvoudige struktuur, maar desondanks produceren alle soorten, m.u.v. R. pallens en R. fanari, specifieke signalen. In hoeverre de verschillen in zangpatronen van belang zijn voor de reproduktieve isolatie moet nader onderzocht worden.

Van enkele Ribautodelphax soorten zijn meerdere populaties geanalyseerd. Hoewel sommige populaties aanzienlijke intraspecifieke verschillen laten zien, zijn er geen geografische trends in variabiliteit gevonden. De signalen van de ♂♂ van één populatie van Taxon 4 zijn dermate afwijkend, dat de populatie mogelijk tot een nieuwe soort behoort.

Verrassend is de grote variabiliteit in de geluidssignalen van de ♀♀ van de twee Ribautodelphax soorten met gemengde populaties van bisexuele diploiden en pseudogame triploide ♀♀. Grote verschillen komen voor tussen en binnen populaties, maar de triploide en diploide ♀♀ uit dezelfde populaties vertonen weinig verschillen. De gevonden variatiepatronen pleiten voor een autopolyploide ontstaanswijze van de triploide ♀♀, waarbij

de triploide ♀♀ ontstaan zijn uit de lokale populatie van de diploide soort.

Onder laboratorium kondities kan bijna de helft van de 8 getoetste soorten uit het R. collinus komplex suksesvol onderling gekruist worden (Hoofdstuk 3). Het merendeel van de kombinaties resulteert in een gering aantal hybriden door de lage inseminatiegraad van de heterospecifieke ♀♀. Vrijwel alle hybriden, ♂♂ én ♀♀, zijn fertiel, maar het aantal nakomelingen van een deel van de terugkruisingen is gering, omdat er opnieuw slechts weinig ♀♀, geïnsemineerd worden. R. imitans en Taxon 4 kruisen gemakkelijk, maar vertonen toch een duidelijke paringsvoorkeur voor conspecifieke partners. Uit de experimenten blijkt, dat barrières die interspecifieke paring voorkomen, belangrijk zijn voor het in stand houden van de reproduktieve isolatie. Postmating barrières zoals verlengde ontwikkelingsduur van de hybriden, steriele ♂♂ en afwijkende chromosoombeelden in de eikern, zijn slechts incidenteel waargenomen. Geen van de experimenten heeft aanwijzingen opgeleverd, dat triploide ♀♀ via kruising en terugkruising ontstaan zouden zijn.

De Ribautodelphax soorten bezitten 15 paar chromosomen en hebben een XX-XO sex-determinatie systeem (Hoofdstuk 4). Enkele ♀♀ vertoonden afwijkende chromosoomaantallen met 31 en 32 chromosomen. Twee ♀♀, één van R. collinus en één van Taxon 1, bevatten naast eieren met 32 chromosomen ook een ei met 64 chromosomen; één ♀ van R. pungens bevatte alleen eieren met 60 chromosomen. Deze vondsten zijn van belang omdat ze een mogelijke ontstaansweg van de triploide ♀♀ aangeven. Als de tetraploide eieren nl. een reductiedeling ondergaan en daarna bevrucht worden door haploid sperma zou dit kunnen resulteren in de synthese van nieuwe triploide ♀♀.

Tussen de triploide ♀♀ is een aanzienlijke mate van variatie in chromosoomaantallen gevonden. Terwijl het basisgetal 3N=45 is, kwamen er in populaties van R. pungens triploide ♀♀ voor met 40 tot 46 chromosomen. Slechts enkele triploide ♀♀ uit populaties van Taxon 4 zijn onderzocht en deze ♀♀ bezaten 41, 44 of 45 chromosomen. Enkele ♀♀ uit twee populaties van R. pungens vertoonden een opvallende karyologische variatie: eieren met 45 univalenten én met een variabel aantal univalenten, bivalenten en trivalenten kwamen in één ♀ voor. In kweken van ♀♀ met dit type variatie verminderde het percentage ♀♀ met deze storingen snel en resteerden vnl. ♀♀ met 45 univalenten. Deze gegevens en ook enkele kweekwaarnemingen

ondersteunen een autopolyploide oorsprong van de triploide ♀♀.

De electroforetische eiwitpatronen van de diploide <u>Ribautoldelphax</u> soorten en de pseudogame triploide ♀♀ zijn vnl. geanalyseerd om de ontstaanswijze van de triploide ♀♀ op te helderen (Hoofdstuk 5).

De resultaten van dit beperkte onderzoek, waarin slechts 10 loci zijn geanalyseerd, suggereren dat de soorten uit het <u>R. collinus</u> komplex nauwer verwant zijn met elkaar, dan met de twee soorten die niet tot dit komplex behoren: <u>R. pallens</u> en <u>R. albostriatus</u>.

Binnen het nakomelingschap van individuele triploide ♀♀ is geen variatie gevonden in eiwitpatronen hetgeen pleit voor een parthenogenetische voortplantingswijze van de triploide ♀♀.

Indien triploide ♀♀ van hybride, allopolyploide oorsprong zijn, moeten zij kenmerken van de twee oudersoorten vertonen, aannemende dat alle drie genomen van de triploide ♀♀ tot expressie komen. Autopolyploiden bezitten, afgezien van mutationele veranderingen, alleen kenmerken van hun ene oudersoort. De expressie van de drie genomen van de triploide ♀♀ is voor één enzym (Pgm) aangetoond en is overigens ook bij vele andere triploide dieren gekonstateerd. Een autopolyploide oorsprong van de triploide <u>Ribautodelphax</u> ♀♀ wordt ondersteund door de grote overeenkomsten in eiwitpatronen tussen triploiden ♀♀ en de diploide bisexuelen die tot dezelfde populatie behoren. De triploide ♀♀ vertonen duidelijke verschillen met de overige <u>Ribautodelphax</u> soorten en ook de triploide ♀♀ uit populaties van <u>R. pungens</u> en Taxon 4 onderscheiden zich.

In het algemeen wordt aangenomen, dat polyploide parthenogenetische dieren een hybride oorsprong hebben. De onderzoeken bij <u>Ribautodelphax</u> wijzen er echter op dat de triploide ♀♀ autopolyploiden zijn, die polyfyletisch ontstaan zijn dwz. de triploide ♀♀ zijn meerdere malen ontstaan uit de lokale bisexuele soort, waarmee zij samen voorkomen. Deze hypothese vormt een logische verklaring voor de gevonden variatiepatronen in de eiwitten en in de geluidssignalen van de ♀♀. Verder verklaart deze hypothese waarom de bisexuele diploide soorten en de daarmee geassocieerde triploide ♀♀ volledig overeenkomen in waardplantrelaties en paringsvoorkeur. Een autopolyploide oorsprong stemt ook overeen met enkele karyologische vondsten, diverse waarnemingen in laboratorium kweken en de mislukking om via kruisingsproeven triploiden te synthetiseren.

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Pseudogame triploide ♀♀ zijn afhankelijk van de diploide bisexuele soort, waarmee zij samen voorkomen, omdat de triploide ♀♀ geïnsemineerd moeten worden om tot voortplanting te komen. Anderzijds zijn de triploide ♀♀ potentieel in staat de diploide soort te verdringen door hun tweemaal zo grote relatieve toename kapaciteit, aangezien de triploide ♀♀ alleen vrouwelijke nakomelingen produceren. Zonder een stabiliserend mechanisme zou het percentage triploide ♀♀ in mengpopulaties een konstante toename vertonen. Tenslotte verdwijnt de diploide soort, als de laatste ♂♂ bij toeval alleen paren met triploide ♀♀, waarna ook de triploide ♀♀ uitsterven bij gebrek aan ♂♂.

De ekologische relaties tussen de pseudogame triploide ♀♀ en de diploide bisexuelen van R. pungens worden in hoofdstuk 6 beschreven. Het percentage triploide ♀♀ in mengpopulaties varieert aanzienlijk, zelfs tussen dichtbij gelegen populaties. Drie populaties zijn gedurende 4-5 jaar bemonsterd en het percentage triploide ♀♀ bleef relatief konstant zowel in een populatie met een laag als in de twee populaties met een hoog percentage triploide ♀♀. In een aantal populaties met een hoog percentage triploide ♀♀ was minder dan 50% van deze ♀♀ geïnsemineerd tegenover echter bijna 100% van de diploide ♀♀. Deze verschillen komen ook voor in gemengde laboratoriumkweken. R. pungens ♂♂ vertonen een duidelijke paringsvoorkeur voor de diploide bisexuele ♀♀ in keuzeproeven. De grotere relatieve toename kapaciteit van de triploide ♀♀ wordt dus gekompenseerd door een lage inseminatiekans. Echter in veldpopulaties met een laag percentage triploide ♀♀ worden vrijwel alle diploide én triploide ♀♀ geïnsemineerd; voor deze populaties is geen stabiliserend mechanisme gevonden.

De gegevens uit de voorafgaande hoofdstukken worden in het laatste hoofdstuk geëvalueerd als basis voor een oplossing van de taxonomische onduidelijkheden binnen het R. collinus komplex. Daarnaast wordt de verspreiding van de soorten en de differentiatie in de genitaalstrukturen van de ♂♂ beschreven. De vijf reeds beschreven en de vier recent ontdekte Taxa verschillen in waardplantrelaties, geluidsignalen en genitaalstrukturen, terwijl kruisingsproeven isolatiebarrieres aantonen. Deze gegevens rechtvaardigen een soortstatus voor de beschreven soorten en de recent ontdekte Taxa. In totaal worden zeven nieuwe soorten beschreven, waarvan er drie alleen uit museumkollekties bekend zijn.

CURRICULUM VITAE

Cornelis (Kees) Franciscus Maria den Bieman werd op 10 februari 1953 te
Wamel geboren. Het HBS-B diploma werd in 1971 behaald aan het Pax Christi
College te Druten. Aansluitend begon hij zijn studie Biologie (specialisa-
tie populatie/ecosystemen) aan de Landbouwuniversiteit te Wageningen met
als hoofdvakken Entomologie en Dierfysiologie en als bijvak Diertaxonomie
doktoraal onderzoek werd in Nigeria verricht. In januari 1978 heeft hij
zijn studie beëindigd en is vervolgens tot 1982 aan de Landbouwuniversteit
te Wageningen verbonden geweest. In deze periode werd onderwijs verzorgd in
de diergeografie en biosystematiek en leeronderzoek begeleid in de
geïntegreerde bestrijding van plagen in boomgaarden. Het promotieonderzoek
werd in deze periode gestart en werd van 1982 tot 1985 voortgezet met
subsidie van BION-ZWO. Momenteel volgt hij de kursus 'Nieuw Elan Banken-
projekt'.