# Biosystematics of the *Muellerianella* complex (Homoptera, Delphacidae), in Western Europe

**Patterns of variation, interrelations and speciation**

C. J. H. Booij

# Biosystematics of the *Muellerianella* complex (Homoptera, Delphacidae), in Western Europe

## Patterns of variation, interrelations and speciation

**Proefschrift**

ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. ir. C. C. Oosterlee,
hoogleraar in de veeteeltwetenschap,
in het openbaar te verdedigen
op vrijdag 4 juni 1982
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen
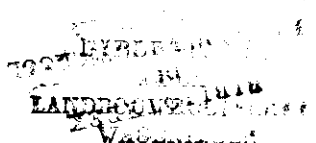
Omslag: Frederik von Planta
Muellerianella ♀ en ♂

# STELLINGEN

1. Naast het gebruik van genitaalkenmerken bij de diagnostiek van cicaden, verdienen andere morfologiese en niet-morfologiese kenmerken meer aandacht.

   Dit proefschrift.

2. Doordat het biologiese soortsbegrip niet van toepassing is op parthenogenetiese vormen, is de classificatie van deze organismen vaak willekeurig. Er dienen daarom betere criteria ontwikkeld te worden voor het afgrenzen van parthenogenetiese taxa.

   Maslin, T. P. (1968). Systematic Zoology 17: 219-231.

3. Het herkennen van gynogenetiese vormen binnen populaties van verwante sexuele diersoorten wordt bemoeilijkt door de geringe morfologiese verschillen tussen sexuele en gynogenetiese vrouwtjes en door het ontbreken van eenvoudige technieken om meiose in eicellen te bestuderen. Gynogenetiese vormen zijn daarom vermoedelijk minder zeldzaam dan wordt verondersteld.

4. Het percentage gynogenetiese vrouwtjes in mengpopulaties van sexuele en gynogenetiese cicaden kan niet zonder meer van de sex-ratio worden afgeleid. Het is daarom beter een meer directe schattingsmethode te gebruiken.

   Drosopoules, S. (1977). Mededelingen Landbouwhogeschool Wageningen 77: 1-133. Dit proefschrift.

5. Het weinig selectieve gedrag dat mannetjes van veel diersoorten vertonen bij de partnerkeuze, kan het voortbestaan van populaties bedreigen wanneer gynogenetiese vormen in het spel zijn.

6. De cladistiese methode van Hennig kan veelal niet doeltreffend worden toegepast bij een fylogenetiese reconstructie van complexen van nauwverwante soorten.

   Arnold, E. N. (1981). Zeitschrift für Zoologische Systematik und Evolutionsforschung 19: 1-35.

7. Een botanies gezien goed beheer van vegetaties is niet automaties gunstig voor de insektenfauna. Het verdient daarom aanbeveling meer onderzoek te doen naar de effecten van verschillende beheersmaatregelen op de insektenfauna.

   Morris, M. G. (1981). Journal Applied Ecology 18: 107-123.

8. Het „sib-competition model" van Maynard-Smith (1976, 1978) zou meer inzicht kunnen geven in de korte termijn voordelen van sexuele reproduktie, indien het aantal „environmental patches" wordt gevarieerd en meer rekening gehouden wordt met „random extinctie" van bepaalde genotypen.

   Maynard-Smith, J. (1976). Journal of Theoretical Biology 63: 245-258. Maynard-Smith, J. (1978). The Evolution of Sex. Cambridge University Press.

9. Nestkastpopulaties van holenbroeders zijn in verschillende opzichten niet representatief voor natuurlijke populaties van holenbroeders.

   Van Balen, J. H., C. J. H. Booij, J. A. van Franeker and E. R. Osieck (1982). Ardea 70: 1-24. Nilsson, S. G. (1975). Vår Fågelvärld 34: 207-211.

10. „De wetenschap", „de vrouw" en „onkruid" bestaan noch vergaan.

    Feyerabend, P. K. (1977). In strijd met de methode. Aanzet tot een anarchistiese kennistheorie. Boom Meppel. 't Hart, M. (1982). De vrouw bestaat niet. De arbeiderspers A'dam. Zonderwijk, P. (1979). De bonte berm. Zomer en Keunig Ede.

11. In navolging van de snip op het briefje van $f$ 100,—, lijkt de kwartel de aangewezen vogelsoort om op een nieuw briefje van $f$ 25,— te worden afgebeeld.

Proefschrift van C. J. H. Booij.
Biosystematics of the Muellerianella complex (Homoptera, Delphacidae), in Western Europe.
Wageningen, 4 juni 1982.

Aan mijn ouders
Aan Corrie

# Voorwoord

Het verschijnen van dit proefschrift, als afronding van enkele jaren onderzoek, schenkt mij veel voldoening. De vele mensen die op enigerlei wijze hebben bijgedragen aan het onderzoek en de totstandkoming van het proefschrift, wil ik hier daarom bedanken voor alle hulp en steun. Een aantal mensen wil ik daarbij met name noemen.

Veel dank ben ik verschuldigd aan mijn promotor Prof.Dr. R.H. Cobben die mij de vrijheid heeft gelaten het onderzoek naar eigen inzicht op te zetten en uit te voeren. Zijn voortdurende interesse en begeleiding hebben veel bijgedragen aan het uiteindelijke resultaat.

Ook mijn co-promotor Prof.Dr. J. Sybenga moet ik bedanken voor zijn belangstelling voor mijn onderzoek, voor zijn adviezen bij het cytogeneties werk en voor het doornemen van een aantal manuscripten.

Met mijn voorganger in dit onderzoek, Dr. Sakis Drosopoulos heb ik vele levendige discussies gehad over de Muellerianella's. Zijn hulp en informatie zijn van groot belang geweest bij het opzetten en uitvoeren van het onderzoek. Hiervoor mijn dank.

Mijn collega Peter de Vrijer dank ik voor de techniese hulp en voor de vele waardevolle suggesties en gesprekken tijdens het onderzoek en het schrijfwerk.

Ook Kees den Bieman heeft met veel enthousiasme het nodige bijgedragen aan het onderzoek. Met veel plezier denk ik terug aan de verzameltrips en de vele discussies. Mijn dank hiervoor.

Jan-Adriaan Guldemond moet ik bedanken voor de grote inzet en kreativiteit waarmee hij een doctoraalonderzoek bij mij deed. Zijn werk heeft veel bijgedragen aan het uiteindelijk onderzoeksresultaat. Terecht is hij dan ook medeauteur van twee artikelen.

Ook Els Miedema werkte met veel enthousiasme aan de Muellerianella's. Mijn dank voor de plezierige samenwerking en de vele uren die je aan het opnemen en verwerken van cicadengeluiden besteedde.

Vanuit het buitenland heb ik veel medewerking gehad in de vorm van informatie en al dan niet levend materiaal. Alle betrokkenen die in de acknowledgements van de verschillende artikelen genoemd worden, ben ik zeer

# Inhoud - Contents

# Inleiding

Dit proefschrift is het tweede dat aan het cicaden-geslacht
*Muellerianella* is gewijd. Vanwaar de belangstelling voor deze dieren?

Het geslacht *Muellerianella* wordt gevormd door enkele zeer nauw verwante
soorten, zogenaamde sibling species. Vanuit biosystematies oogpunt zijn zulke
soorten interessant omdat ze veelal recent ontstaan zijn uit een gemeenschap-
pelijke vooroudersoort. Door alle mogelijke verschillen tussen dergelijke
soorten te bestuderen kan een beeld gevormd worden van hun onderlinge ver-
wantschap, hun mogelijke wordingsgeschiedenis en van de mechanismen die hun
onafhankelijk voortbestaan verzekeren (isolatiemechanismen).

Drosopoulos (1977) liet voor de twee Nederlandse soorten *M. fairmairei*
en *M. brevipennis* zien dat er verschillen zijn wat betreft hun oecologie en
paringsgedrag, die van belang zijn voor de reproduktieve isolatie. Hoewel in
het laboratorium hybriden werden verkregen, werden deze in het veld niet ge-
vonden.

Het meest opmerkelijke dat door Drosopoulos werd ontdekt, en dat het
geslacht *Muellerianella* bijzonder aantrekkelijk maakte voor voortgezet onder-
zoek, is het bestaan van triploide parthenogenetiese vormen die morfologies
niet te onderscheiden zijn van *M. fairmairei* vrouwtjes, waarmee ze gewoonlijk
samen voorkomen. Deze triploide vormen bleken zich voort te planten door
gynogenese (pseudogamie). Bij deze zeldzame vorm van ongeslachtelijke voort-
planting is wel copulatie nodig, maar het sperma dient slechts om de eiont-
wikkeling op gang te brengen. Deze triploide gynogenetiese *Muellerianella's*
zijn te beschouwen als reproduktie-parasieten, aangezien ze sperma onttrekken
aan de verwante soorten *M. fairmairei* en *M. brevipennis*. Het idee van
Drosopoulos was dat deze vormen door hybridizatie van de twee sexuele soorten
ontstaan zijn en alleen zouden voorkomen in geografiese gebieden waar beide
sexuele soorten ook voorkomen.

Om een beter inzicht te krijgen in het soortsvormingsproces van de
*Muellerianella* soorten en in het ontstaan en de verspreiding van de triploide
gynogenetiese vormen, werd dit onderzoek op grotere geografiese schaal voort-
gezet. Bovendien werd het onderzoek uitgebreid doordat een derde soort,

1

*M. extrusa*, werd onderscheiden.

Om aan de taxonomiese verwarring die rond de *Muellerianella* soorten bestond een einde te maken, werden de soorten opnieuw morfologies en taxonomies gedefinieerd en veel oude en nieuwe verspreidingsgegevens werden bewerkt (artikel 1).

Omdat de mate van isolatie tussen de soorten geografies kan varieren, werden populaties uit verschillende delen van Europa vergeleken wat betreft hun oecologie (artikel 2), hun gedrag (artikel 3), en hun onderlinge kruisbaarheid (artikel 4)

Om een beeld te krijgen van de geografiese verspreiding van de triploiden en hun binding met bepaalde habitats en voedselplanten, werden zoveel mogelijk populaties met triploiden opgespoord en bemonsterd (artikel 6). Doordat deze triploiden slechts door het maken van chromosoompreparaten van *M. fairmairei* vrouwtjes te onderscheiden zijn, werd bovendien een opmerkelijke variatie in chromosoomaantallen bij deze vormen ontdekt (artikel 5).

Op grond van alle verzamelde gegevens werd tenslotte gepoogd een model te construeren voor de evolutie van de *Muellerianella* soorten (artikel 7).

2

# BIOSYSTEMATICS OF THE *MUELLERIANELLA* COMPLEX (HOMOPTERA, DELPHACIDAE), TAXONOMY, MORPHOLOGY AND DISTRIBUTION

by

## C. J. H. BOOIJ

*(Department of Entomology, Agricultural University, Wageningen, The Netherlands)*

## SUMMARY

The closely related species and forms of the plant-hopper genus *Muellerianella* have been studied. In Western Europe this complex consists of three bisexual species and a number of triploid forms which reproduce by gynogenesis. *M. extrusa* Scott 1871 appears to be a distinct species which lives on *Molinia caerulea*. It is here taken out of synonymy from *M. fairmairei* Perris 1857, which lives on *Holcus lanatus*. The third species, *M. brevipennis* Boheman 1847, lives on *Deschampsia cespitosa*. Intra- and interspecific morphological differences in males and females are described in detail, including geographic variation. Triploid forms, which are usually associated with *M. fairmairei* cannot be distinguished from bisexual females of this species. Distribution maps are given for each bisexual species and for the triploid forms which are based on an evaluation of literature data, museum collections and on 150 new samples taken all over Europe.

## INTRODUCTION

Earlier investigations on two sibling species of the genus *Muellerianella* (Auchenorrhyncha, Delphacidae) have shown, that this genus is highly interesting for biosystematic and evolutionary studies. DROSOPOULOS (1976, 1977) discovered that the excess of females, which is often found in populations of *M. fairmairei* is caused by triploid females which coexist with *M. fairmairei* and reproduce by gynogenesis (pseudogamy). This mode of reproduction, which is very rare in insects (WHITE 1978), is asexual, but the eggs can only develop after the female has been inseminated by a male of a related bisexual species. From crossing experiments DROSOPOULOS (1978) concluded that these triploid gynogenetic forms arose by hybridization between *M. fairmairei* and *M. brevipennis*. The ecological differences between these two bisexual species have been extensively studied by DROSOPOULOS (1977). However, most of his work has been based on populations from only two localities in the Netherlands, and many questions were left open.

The striking similarity between the species of this genus has led to great confusion in the past. In the autumn of 1978 we found that *M. extrusa*, previously being regarded identical with *M. fairmairei* (living on *Holcus lanatus*), represents a distinct species which lives on *Molinia caerulea*. Moreover, no reliable differences were known between females

3

of the *Muellerianella* species. The complexity of the genus is further increased by extensive variation between triploid females which resemble *M. fairmairei*.

For the understanding of the evolution of this complex of bisexual and asexual forms much information is needed. Since the process of speciation and differentiation is very complicated, all relevant aspects should be studied.

Ideally, one should have information on past and present distribution, detailed morphological descriptions including geographic variation, ecological data on hostplants, habitat and phenology, information on pre- and postmating isolation mechanisms, results of hybridization experiments, detailed descriptions of karyotypes, and data on biochemical (allelic) differentiation (WHITE, 1978).

Although it is hardly possible to obtain all this information even for a small number of species, we have tried to apply this multidisciplinary approach in our studies on the genus *Muellerianella*, without going into much detail or being too superficial.

The work of DROSOPOULOS (1976, 1977, 1978) formed a good starting-point to extend the work to a wider geographic scale and to go more deeply into some special problems. The results of the investigations of last years will be presented in a series of papers of which this is the first. In the present paper, a taxonomic revision of the genus is given, morphological differences between the species are described and distributional data are evaluated. Later papers will give information on ecology, acoustic behaviour, hybridization, cytogenetics and on the ecological differentiation and coexistence between bisexual and asexual forms.

## NOMENCLATURE

Present knowledge of the *Muellerianella* complex necessitates some corrections in the nomenclature of this genus. Because the sibling species *M. extrusa* and *M. fairmairei* have not been recognized in the past, several unjustified synonyms have been made.

The genus *Muellerianella* was erected by WAGNER (1963). Two species were recognized: *M. fairmairei* Perris 1857 and *M. brevipennis* Boheman 1847. Recently a third species has been described from the Caucasus, named *M. relicta* Logvinenko 1976.

Previously, the name *M. fairmairei* has been used for the species living on *Holcus*, the species which lives on *Molinia caerulea*, and for triploid females which cannot be distinguished from the *Holcus*-species. The original description of *M. fairmairei* Perris 1857 was based on one female which was taken from La Teste (near Bordeaux), France. I

4

examined this female which is deposited in the zoological museum of the École National Supérieur d'Agronomie in Montpellier. It strongly resembles the bisexual species which lives on *Holcus* but it might also be a triploid female. Because it is impossible to discriminate between diploid and triploid forms in museum-material, the taxonomic identity of the holotype cannot be determined with certainty.

Since the nomenclature for parthenogenetic organisms is problematic and the biological species concept cannot be applied to parthenogenetic forms, it seems best to preserve the name *M. fairmairei* Perris 1857 for the bisexual species which lives on *Holcus lanatus*. In order to prevent confusion in the future we have deposited two labeled males of the species from *Holcus* with the holotype female in the Perris collection.

NAST (1971) listed three other names as synonyms for *M. fairmairei*: *Delphax neglecta* Flor 1861, *Liburnia extrusa* Scott 1871 and *Delphax fairmairei signicollis* Rey 1894.

The description of *Delphax neglecta* is based on a series of specimens from the Eastern Baltic. No holotype was designated. Unfortunately the type material contains a mixture of males and females of *M. brevipennis* and a number of females which resemble "*M. fairmairei*" (Vilbaste pers. comm.). Therefore *D. neglecta* was listed as a synonym for both *M. brevipennis* and *M. fairmairei* by NAST (1971), which is not justified however. Since the bisexual species from *Holcus* probably does not occur in the Eastern Baltic, the "*M. fairmairei*" females in the type series may belong to the species from *Molinia* or they might be triploid gynogenetic females. Since in the original publication of FLOR (1861) both males and females of *M. brevipennis* are described and the unclear females in the series cannot be easily identified, I prefer to regard the name *M. neglecta* as a synonym for *M. brevipennis* Boheman 1847. On my request Dr. Vilbaste designated one *M. brevipennis* male in the type series as lectotype for *D. neglecta*. The type series is in the Museum of Tartu (Estonia).

The second name which has been published as synonym for *M. fairmairei* is *Liburnia extrusa* Scott 1871. Males and females of *Liburnia extrusa* were described from England. The type material is stored at the British Museum (Natural History), London. Examination of these series revealed that the holotype and paratypes belong to the species from *Molinia*. Therefore the name *Liburnia extrusa* is not a synonym of *M. fairmairei* and the name for the species from *Molinia* should be *Muellerianella extrusa* Scott 1871.

The present nomenclature for the bisexual species of the genus *Muellerianella* should be as follows:

1. *Muellerianella brevipennis* Boheman 1847, originally described as *Del-*

*phax brevipennis* Boheman 1847. Other previously used invalid names for this species are: *Fulgora flavescens* Fabricius 1794 (primary homonym), *Delphax bivittata* Boheman 1850, *Delphax hyalinipennis* Stål 1854, and Delphax neglecta Flor 1861.

2. *Muellerianella fairmairei* Perris 1857, originally described as: *Delphax fairmairei* Perris 1857.

3. *Muellerianella extrusa* Scott 1871, originally described as *Liburnia extrusa* Scott 1871.

4. *Muellerianella relicta* Logvinenko 1976.

With regard to the triploid gynogenetic forms a lot of difficulties arise in naming these taxa. Since the biological species concept is based on the presence or absence of interbreeding between populations it cannot be applied appropriately to asexual reproducing organisms (Maslin 1968, Mayr 1969, White 1978). Following Schultz (1969) the common triploid forms which coöccur with *M. fairmairei*, were called *M.2-fairmairei-brevipennis* by Drosopoulos (1977). In this name the possible hybrid origin of the triploid forms is incorporated. Triploid forms were obtained after hybridization and backcrossing of *M. fairmairei* and *M. brevipennis* (Drosopoulos 1979). Despite of extensive crossing experiments the synthesis of triploid females could not be repeated however (Booij in prep.). If the hybrid character of the triploid forms can be confirmed by biochemical methods the terminology proposed by Drosopoulos (1977, 1979) seems to be appropriate. On the other hand there is much variation between triploid *Muellerianella* clones (this paper and Booij in prep.). Some triploid forms might have evolved from hybridization between *M. brevipennis* and *M. extrusa* or even by autopolyploidy. As long as the genetic structure of the different triploid forms is not fully understood, I prefer a very neutral terminology. In this paper the term "*triploid form*" or "*triploid females*" will be used for all triploid gynogenetic females which resemble *M. fairmairei* morphologically.

TABLE I
Simplified overview of the *Muellerianella* complex as it is known at present.

| | *M. brevipennis* | *M. extrusa* | *M. fairmairei* | *Triploid forms* |
|---|---|---|---|---|
| hostplant | *Deschampsia cespitosa* | *Molinia caerulea* | *Holcus lanatus* | *Holcus lanatus* |
| wintereggs in | *Deschampsia cespitosa* | *Molinia caerulea* | *Juncus effusus* | *Juncus effusus* |
| generations/year | 1–2 | 1 | 1–4 | 1–2 |
| distribution | temperate and northern Europe | temperate and northern Europe | atlantic-mediterranean | atlantic |
| reproduction | bisexual | bisexual | bisexual | gynogenetic |

The basic biological features of *M. brevipennis*, *M. fairmairei*, *M. extrusa*, and the triploid forms are given in Table 1. In the rest of this paper names of these species and forms will be abbreviated as *Mb*, *Mf*, *Me* and *M3* respectively.

## MORPHOLOGY

In several genera of Delphacidae like *Javesella*, *Muellerianella*, and *Ribautodelphax*, species can only be distinguished by examination of the male genitalia. Although even small differences between species tend to be very constant and characteristic, one should bear in mind that even genital characters can reveal considerable variation due to geographical or environmental factors. This has been shown for several leafhopper-species (LeQuesne and Woodroffe 1976, Müller 1956). Identification of females of *Muellerianella* is very difficult. Differences in the form of the gonocoxae and genital scales, as used by Ossianninilsson (1978) for *Javesella* and *Ribautodelphax*, were found not to be suitable. The gonocoxae are too variable and genital scales are reduced.

The high similarity between *Me* and *Mf*, the difficulties in identifying females, and the association of *M3* and *Mf* in the field which are morphologically extremely alike, necessitated a detailed morphological analysis. The availability of material from different parts of Europe made it possible to include geographic variation in the analysis. *Me* has been studied in less detail because this species was discovered after most of the morphological studies and collecting had been done.

### *Methods*

For morphological investigations males and females were collected by hand from their hostplants in the field. In some cases fieldcollected third to fifth instar larvae were reared to adults under optimal conditions in the laboratory. For females, mainly freshly killed material was used because triploid females and diploid (bisexual) females can only be discriminated by cytological methods. These methods, developed by Drosopoulos (1977) and improved afterwards, will be described in detail in a future paper. Most of the males were measured after mounting. Apart from overall length and the clarity of pigmentation patterns, the characters used are hardly affected by longer preservation. Measurements and observations were made with a binocular microscope (Wild M5) at 50 × magnification and a stereo microscope (Leitz-Dialux 20 EB) at 100 × and 400 × magnification. The measuring-fault of size characters was maximal 0.01 mm for head-width, spur-length, and length of the ovipositor-saw, and maximal 0.02 mm for the other size characters.

Genital characters have been studied after maceration at 100° C in 20% KOH for about 5 minutes or at 60° C in 50% lactic acid for several hours.
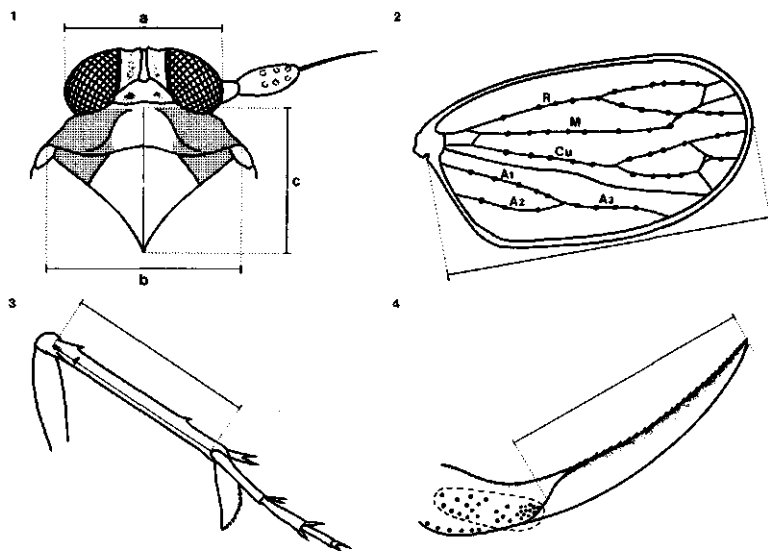


Figs. 1–4. Some quantitative characters used for morphometrics in *Muellerianella*. 1, head-width (a), thorax-width (b), and thorax-length (c). 2, winglength and number of hairspots on veins. 3, length of hind-tibia. 4, sawblade of the ovipositor: length, number of sensillae at base, number of teeth.

## *Morphology of females*

Because no reliable differences between females were known, the morphology of females was studied in great detail. From a variety of 45 characters which were examined in some test-series of *Mb* and *Mf*, 28 were found to be suitable for final analysis. These characters are partly quantitative, like size-characters and meristic characters (Figs 1–4), and partly qualitative like colour and pigmentation characters (Figs 5–20).

After examination of many females it appeared that *Mb* females can be easily distinguished from *Me*, *Mf* and *M3* females by several qualitative characters which are listed in Table 2. Most *Mb* females are darker than females of the other species and the pigmentation patterns are less clearly demarcated and not contrasting with the ground-colour. The pigmentation on the genae differs most characteristically (Figs 5–10), but also the pigmentation on the frons and the abdomen can be used to distinguish *Mb*. In macropterous females, which are always intensively

8

TABLE 2

Qualitative morphological characters which differentiate females of *M. brevipennis* from females of *M. extrusa*, *M. fairmairei* and *triploid forms*.

|  | *M. brevipennis* | *M. extrusa/M. fairmairei/triploids* |
|---|---|---|
| genae pigmentation | vague, not extending to base of antennae | contrasting, extending to base of antennae |
| dark streak between eyes and keels | always absent | always present |
| frons pigmentation | vague, mottled, without a distinct pattern | weakly developed or with a distinct pattern |
| eye colour | black-brown | with greenish reflection |
| ocellus spot | small, circular | often extended, rectangular |
| abdomen pigmentation | with broad, dark lateral bands. | lighter with pattern of stripes and spots. |
| inner wing margins | semi-transparant | white |

pigmented, the pigmentation on the head is most pronounced. Furthermore females of *Mb* are on average smaller than females of *Mf* or *M3*, but larger than females of *Me* (Table 3). However, in most size characters there is considerable overlap, and only head-width and length of the hind-tibia can be used to discriminate females of *Muellerianella* (Fig. 21). From this figure it can also be seen that *Me* females can be distinguished from *Mf* females by the length of their hindtibia. Since large females of *Me* can be easily confused with small females of *Mf* or *M3* one should be cautious to use this character. Differences in colour or pigmentation patterns could not be found between *Me*, *Mf* and *M3*.

The score of 28 characters for many individuals of *Mf* and *M3* in samples from Ireland and the Netherlands did not yield any differences which could be used to distinguish these females. Although the mean value of several characters differed significantly between *M3* and *Mf* females, the ranges are strongly overlapping (Table 4). Moreover some differences which were found in Ireland, appeared to be absent or reversed in the Dutch sample. From table 3 and 4 it can be seen that some characters might indicate whether a female is more likely to be a *M3* or a *Mf* female. These are: head-width, thorax-width, number of hairspots on the wings, number of sensilla on base of the ovipositorsaw and number of teeth on the ovipositorsaw. Two of these characters have been assessed in a great number of animals from different sites in the Netherlands. It appeared that in all samples the mean winglength of *M3* females is significantly shorter than that of *Mf* females (*Mf*: 1.56 $\pm$ 0.11 mm (n = 187) and *M3*: 1.50 $\pm$ 0.11 mm (n = 356), t = 6.04, P < 0.001) and that the mean number of hairspots was higher in *Mf* than in *M3* females (*Mf*: 35.5 $\pm$ 6.0 (n = 178) and *M3*: 26.8 $\pm$ 4.7 (n =

9

TABLE 3

Size differences between females of the *Muellerianella* species and forms. Mean values and standard deviations for different size characters are given in mm. Material used: *Me* Denmark, Germany, Ireland, Great Britain and the Netherlands; *Mf* Spain, Yugoslavia, France, Ireland and the Netherlands; *Mb* Sweden, Finland, Denmark, Poland, Yugoslavia, France, Ireland and the Netherlands; *M3* the Netherlands, Ireland, France and Yugoslavia.

| | *M. extrusa* | | | *M. fairmairei* | | | *Triploid forms* | | | *M. brevipennis* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\bar{x}$ | $s_x$ | n | $\bar{x}$ | $s_x$ | n | $\bar{x}$ | $s_x$ | n | $\bar{x}$ | $s_x$ | n |
| headwidth | 0.72 | ± 0.03 | (60) | 0.76 | ± 0.03 | (85) | 0.79 | ± 0.02 | (53) | 0.81 | ± 0.03 | (131) |
| thorax-width | 0.78 | ± 0.03 | (60) | 0.84 | ± 0.04 | (85) | 0.89 | ± 0.03 | (53) | 0.88 | ± 0.04 | (131) |
| hind-tibia length | 0.88 | ± 0.04 | (60) | 1.08 | ± 0.05 | (85) | 1.09 | ± 0.07 | (53) | 0.91 | ± 0.05 | (131) |
| wing-length (brachypterous) | 1.34 | ± 0.10 | (60) | 1.59 | ± 0.13 | (85) | 1.55 | ± 0.11 | (53) | 1.43 | ± 0.10 | (131) |
| overall-length brachypters | | | | | | | | | | | | |
| freshly killed | 3.00 | ± 0.14 | (54) | 3.54 | ± 0.20 | (96) | 3.52 | ± 0.21 | (66) | 3.28 | ± 0.16 | (72) |
| mounted | 2.62 | ± 0.21 | (55) | 3.12 | ± 0.17 | (45) | | | | 2.75 | ± 0.17 | (79) |
| overall-length macropters | | | | | | | | | | | | |
| fresh or mounted | 3.98 | ± 0.20 | (20) | 4.53 | ± 0.20 | (36) | | | | 4.08 | ± 0.27 | (12) |
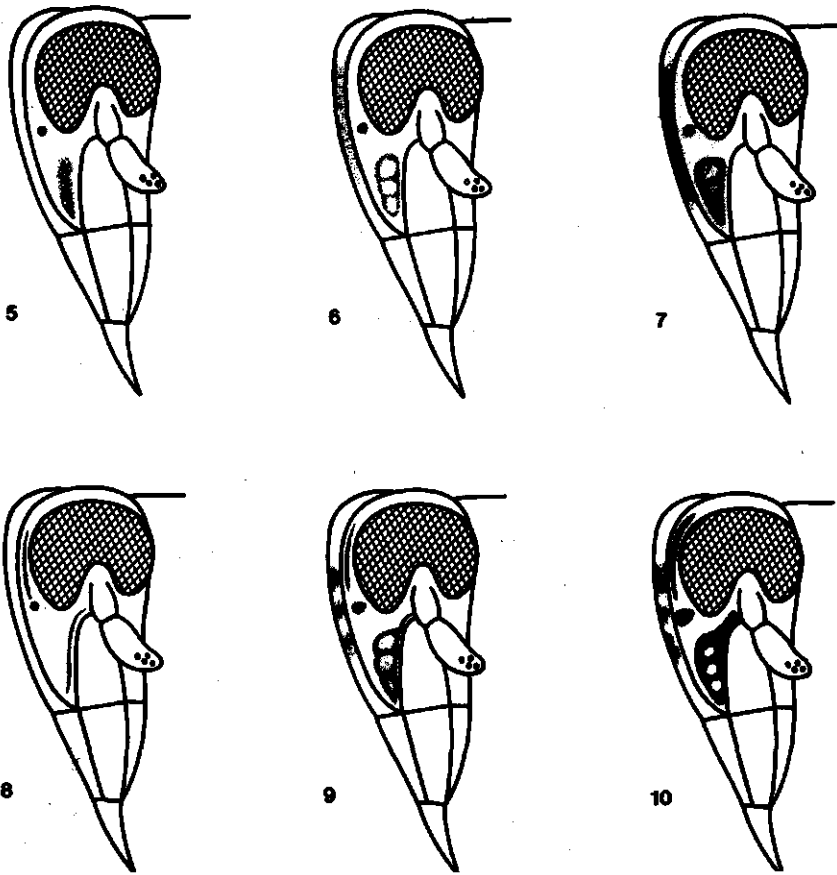
Figs. 5–10. Pigmentation patterns on the genae of *Muellerianella* species. Figs. 5–7 *M. brevipennis*. 5, light specimen. 6, average specimen. 7, dark specimen. Figs. 8–10 *M. extrusa*, *M. fairmairei* and *triploid forms*. 8, light specimen. 9, average specimen. 10, dark specimen.

347), t = 16.9, P < 0.001). However, the variation within and between samples is so great that the diagnostic value of these characters is too low to be useful.

Some differences found, can be interpreted as evidence for the hypothesis that *M3* arose by hybridization between *Mf* and *Mb*. Thorax width, head-width, wing-length, number of hairspots on the wings, number of sensillae and teeth on ovipositorsaw are on average intermediate between *Mf* and *Mb* (table 3). However, the length of hind tibia and of the ovipositor tend to be longer in *M3* than in *Mf* whereas they are shorter in *Mb*.

Apart from the most common triploid forms which live on *Holcus*
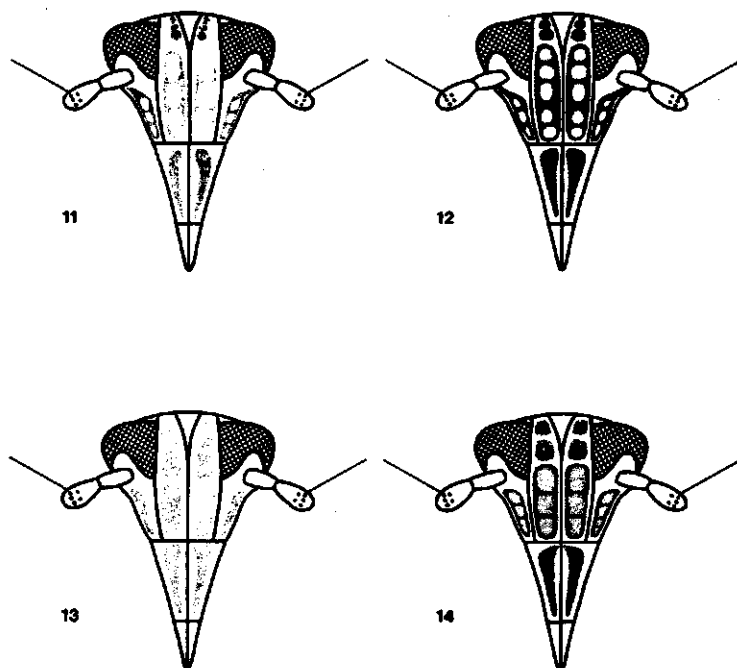
Figs. 11–14. Pigmentation patterns on the frons of *Muellerianella* species. Figs. 11–12, *M. brevipennis.* 11, average specimen. 12, dark specimen. Figs. 13–14, *M. fairmairei, M. extrusa* and *triploid forms.* 13, average specimen. 14, dark specimen.

some clones were found on other hostplants (*M3* 071, 072, 073). These clones were found to differ in several size characters from the common clones. The clones from *Bromus ramosus* and *Deschampsia flexuosa* had a smaller headwidth and shorter legs, and can be easily confused with *Me*-females.

|  | mean head-width | mean hindtibia-length | |
| --- | --- | --- | --- |
| *M3 Holcus lanatus* | 0.79 | 1.08 | (n = 85) |
| *M3 Bromus ramosus* | 0.75 | 0.90 | (n = 10) |
| *M3 Deschampsia flexuosa* | 0.76 | 0.91 | (n = 10) |

For 24 characters measured in *Mf* and *M3* (including those presented in Table 4), coëfficients of variation have been calculated in order to compare the degree of variation between *M3* and *Mf*. Of the 24 coëfficients calculated, 8 were greater in *Mf*, 10 were greater in *M3* and 6 were about the same in *M3* and *Mf*. From this we can conclude that the
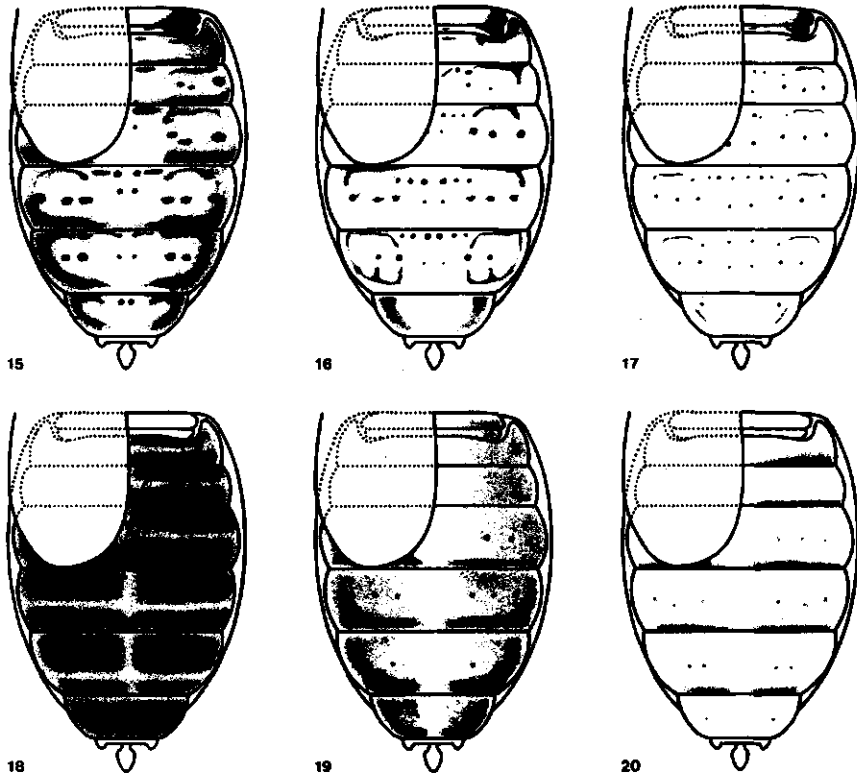
Figs. 15—20. Pigmentation patterns on abdomen of females of *Muellerianella* species. Figs. 15–17 *M. extrusa*, *M. fairmairei* and *triploid forms*. 15, dark specimen. 16, average specimen. 17, light specimen. Figs. 18–20 *M. brevipennis*. 18, dark specimen. 19, average specimen. 20, light specimen.
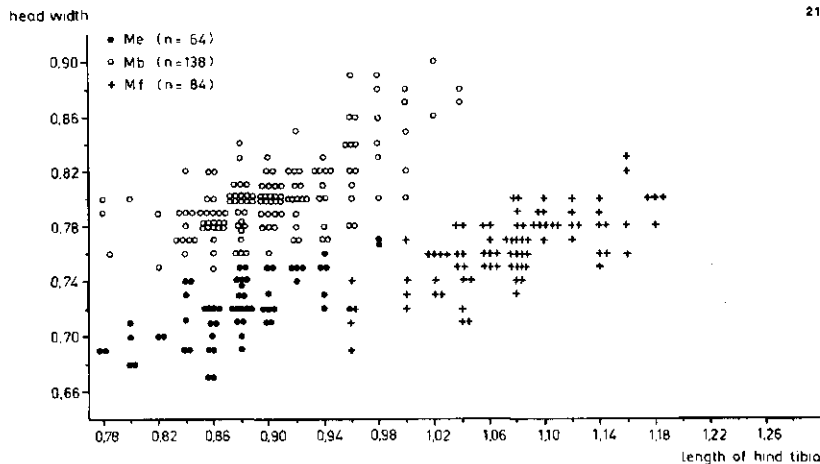


Fig. 21. Differences in some size characters between females of *M. extrusa* (*Me*), *M. brevipennis* (*Mb*), and *M. fairmairei* (*Mf*). Based on material from different parts of Europe.

13

TABLE 4

Morphological differences between females of *M. fairmairei* and *triploid* females in samples from Glengarriff (Ireland) and Leersum (The Netherlands). Means and standard deviations are given. An asterix indicates that the difference between *Mf* and *M3* is significant at least at the 0.05% level.

| | Glengarriff | | Leersum | |
| | M. fairmairei (n = 25) | Triploids (n = 13) | M. fairmairei (n = 24) | Triploids (n = 60) |
|---|---|---|---|---|
| overall length | 3.66 ± 0.14 | 3.74 ± 0.18 | 3.53 ± 0.18 | 3.41 ± 0.16* |
| thorax length | 0.77 ± 0.04 | 0.81 ± 0.04* | 0.75 ± 0.05 | 0.75 ± 0.04 |
| thorax width· | 0.86 ± 0.04 | 0.89 ± 0.03* | 0.87 ± 0.03 | 0.89 ± 0.03* |
| head width | 0.78 ± 0.02 | 0.81 ± 0.02* | 0.77 ± 0.02 | 0.77 ± 0.02 |
| length fore tibia | 0.75 ± 0.03 | 0.77 ± 0.04 | 0.74 ± 0.04 | 0.71 ± 0.04* |
| length hind tibia | 1.10 ± 0.04 | 1.16 ± 0.05* | 1.07 ± 0.04 | 1.05 ± 0.05* |
| wing length | 1.55 ± 0.09 | 1.64 ± 0.10* | 1.59 ± 0.09 | 1.50 ± 0.08* |
| ovipositor length | 0.85 ± 0.03 | 0.86 ± 0.05 | 0.84 ± 0.02 | 0.86 ± 0.04 |
| no. wing hairspots | 61.9 ± 8.3 | 59.5 ± 6.7 | 59.4 ± 9.7 | 47.0 ± 7.6* |
| no. ovip. sensillae | 25.1 ± 3.1 | 22.5 ± 2.9* | 26.1 ± 2.4 | 18.4 ± 1.6* |
| no. ovip. teeth | 29.3 ± 2.3 | 25.5 ± 1.8* | 28.8 ± 3.3 | 24.2 ± 2.1* |
| no. spur teeth | 13.4 ± 2.7 | 14.5 ± 2.3 | 15.0 ± 1.7 | 14.5 ± 1.7 |

morphological variation is of the same magnitude in *Mf* as in *M3*. Thus there is no evidence for more morphological homogeneity in *M3* despite of the clonal structure of *M3* populations.

## Morphology of males

In spite of characteristic differences in genital structures between males of the *Muellerianella* species, there has been much confusion in the past, expecially because *Me* and *Mf* have not been distinguished.

At present the morphological differences between the species are quite clear. Males of *Mb* are easily recognized by the longer median spine on the aedeagus which is bent to the right as drawn in Figure 24. The form of the aedeagus of *M. relicta* (Fig. 25), which has been described by LOGVINENKO (1976), suggests that this species is closely related to *Mb*. In contrast to *Mb* and *M. relicta*, the median spine of the aedeagus of *Mf* and *Me* is bent to the left side (Figs 22, 23). Although the length is rather variable, it is generally shorter than in *Mb*. The males of *Me* and *Mf* can be reliable distinguished by the position of the right spine of the aedeagus, which is directed downward and more to the side in *Mf* (Fig. 23). In *Me* this spine is directed forward and more upward (Fig. 22). The right spine is more variable and smaller in *Mf* than in *Me*.

As shown in Figures 26–28, the lateral concavity of the genital cap-
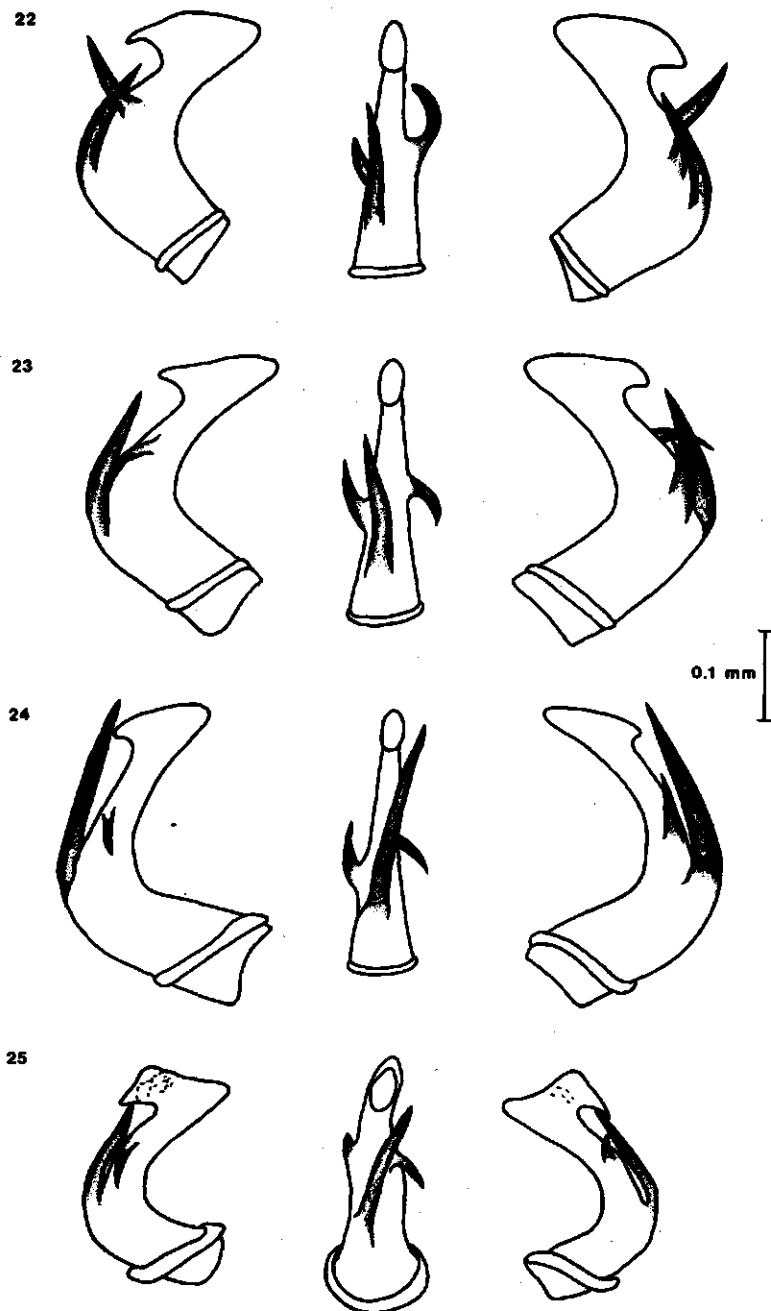
Figs. 22–25. Various views of the aedeagus of the Muellerianella species. 22, *M. extrusa.* 23, *M. fairmairei.* 24, *M. brevipennis.* 25, *M. relicta* (redrawn after Logvinenko, 1976).
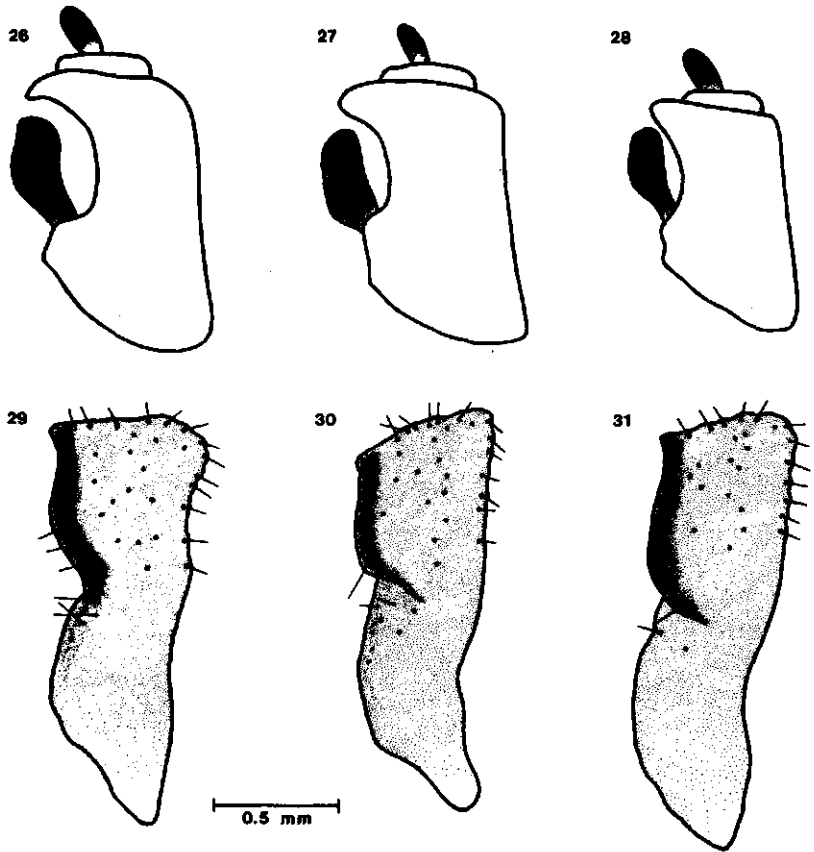
Figs. 26–31. Genital segments and parameres of the *Muellerianella* species. Figs. 26–28 Genital segments. 26, *M. brevipennis*. 27, *M. extrusa*. 28, *M. fairmairei*. Figs. 29–31 Parameres. 29, *M. brevipennis*. 30, *M. extrusa*. 31, *M. fairmairei*.

sule formed by the caudal margin is deeper in *Mb* than in the other species. In *Me* the lateral concavity tends to be somewhat deeper and more angular than in *Mf* (Figs. 27, 28), but this difference is not fully reliable due to variation. The dorso-caudal projection of the genital capsule is sharp and pointed downward in *Mb*, while it is blunt and not pointed downward in *Me* and *Mf* (figs. 26–28).

In Figures 29–31 the most common form of the parameres is given for each species. However, since there is considerable variation within and between populations (see below), and the observed form depends on the angle at which it is viewed, these forms are not reliable for identification.

Males of *Mb*, *Me* and *Mf* can be further characterized by a number of size characters of which especially headwidth and length of the hind

TABLE 5

Size differences between males of the *Muellerianella* species. For all characters mean values and standard deviations are given in mm. Material used: *Me* Finland, Denmark, Great Britain, W. Germany, Ireland and the Netherlands; *Mf* Spain, Yugoslavia, Andorra, France, the Netherlands, Ireland and Denmark; *Mb* Poland, Ireland, Yugoslavia, France, Denmark, Sweden, Finland, Belgium.

| | *M. extrusa* | | | *M. fairmairei* | | | *M. brevipennis* | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\bar{x}$ | $s_x$ | $n$ | $\bar{x}$ | $s_x$ | $n$ | $\bar{x}$ | $s_x$ | $n$ |
| head-with | 0.65 | ± 0.03 | (57) | 0.68 | ± 0.03 | (120) | 0.74 | ± 0.03 | (107) |
| thorax-width | 0.69 | ± 0.03 | (57) | 0.72 | ± 0.03 | (120) | 0.79 | ± 0.04 | (107) |
| hind-tibia length | 0.78 | ± 0.05 | (57) | 0.96 | ± 0.05 | (120) | 0.83 | ± 0.05 | (107) |
| wing-length (brachypterous) | 1.24 | ± 0.19 | (57) | 1.45 | ± 0.13 | (120) | 1.55 | ± 0.14 | (107) |
| overall-length brachypters | | | | | | | | | |
| freshly killed | 2.59 | ± 0.10 | (51) | 2.65 | ± 0.13 | (42) | 2.90 | ± 0.09 | (43) |
| mounted | 2.26 | ± 0.12 | (47) | 2.35 | ± 0.11 | (107) | 2.51 | ± 0.11 | (107) |
| overall-length macropters | | | | | | | | | |
| fresh or mounted | 3.73 | ± 0.16 | (11) | 3.73 | ± 0.10 | (7) | 4.05 | ± 0.17 | (12) |

tibia are useful for identification (Table 5 and Fig. 32). Pigmentation patterns which are useful characters to distinguish females show about the same differences in males. Especially the pigmentation of the genae (Figs. 5–10) can be used to separate *Mb* from *Me* and *Mf*. Males are
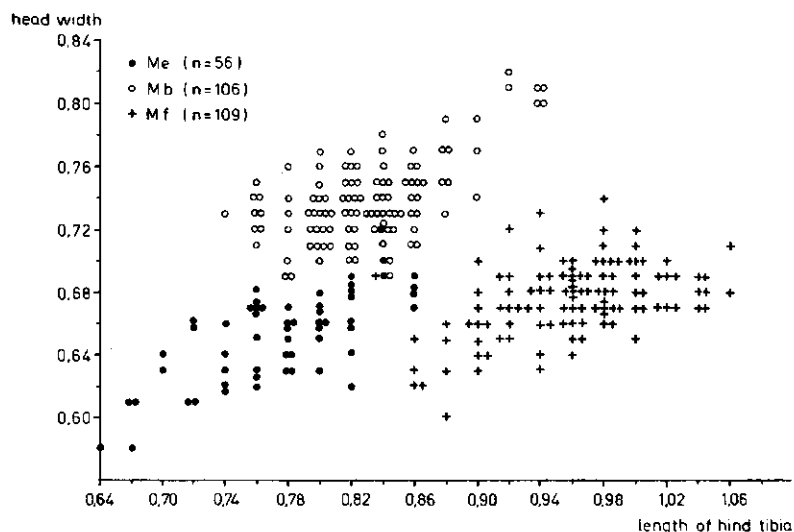


Fig. 32. Differences in some size characters between males of *M. extrusa* (Me), *M. brevipennis* (Mb) and *M. fairmairei* (Mf).

generally more intensively pigmented than females and the ground-colours are more orange-brown in stead of yellowish, especially in *Me* and *Mf* which are often deeply orange and black pigmented.

### Geographic variation in morphology

In order to study the geographic variation, material from different parts of Europe was measured and morphologically compared. For this purpose seven size characters were selected: thorax-width, thorax-length, head-width, length of fore- and hind-tibia, wing-length and overall-length. From each sample both males and females were measured. Moreover, variation in male genitalia was studied.

From the analysis of size characters, no interesting geographic trends
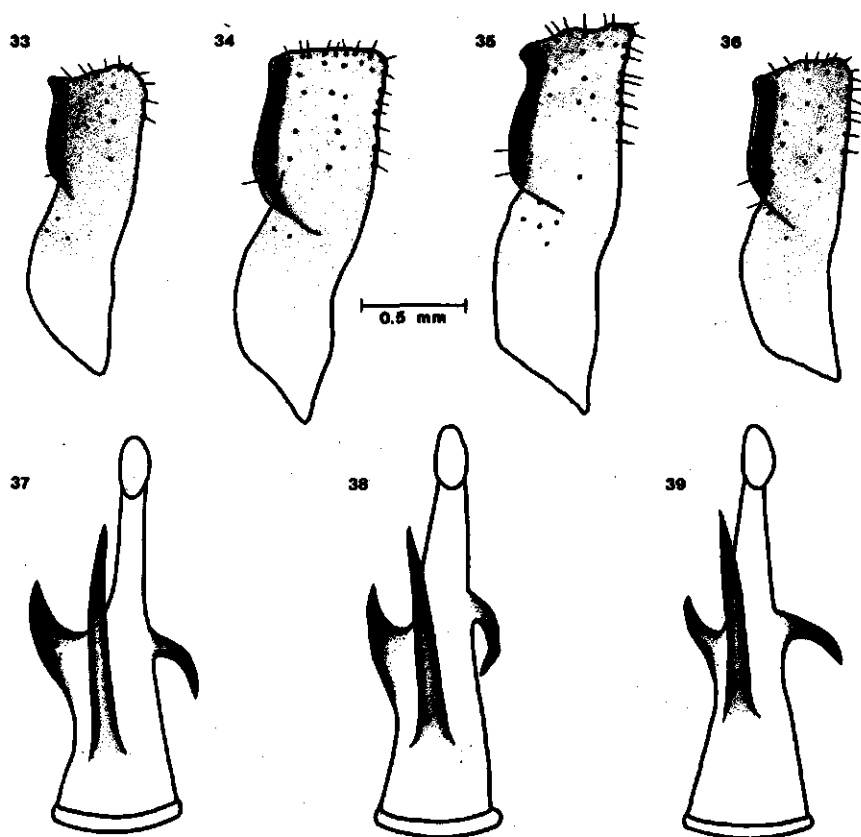


Figs. 33–39. Intraspecific variation in genital structures of *M. fairmairei*. Figs. 33–36 Parameres of specimen from S. Spain, Greece, S. Sweden, and S. France, respectively. Figs. 37–39 Aedeagus of specimen from S. Spain, Greece and S. Sweden, respectively.

18

or patterns were found. In both males and females mean values for characters varied between populations and although the differences were often significant, they appeared to be very irregular and not correlated with macroclimatic conditions. Even on a smaller scale, for example between Dutch populations of *Mf*, we found similar differences. Therefore it seems likely that the size characters are strongly influenced by local conditions (e.g. hostplant quality and microclimate). Therefore one might doubt upon the biosystematic value of geographic analysis of such characters in insects.

One of the populations of *Me* clearly deviates from the other samples of *Me*. This sample was taken from the Castor Hanglands (Cambridgeshire, Great Britain) by MORRIS. Although the measurements were taken from alcohol material, which might slightly affect some characters, the specimens are strikingly bigger than specimens of the other *Me* populations. The samples from Castor Hanglands will be further discussed in a later paper on ecology (BOOY in prep.).

Male genitalia were studied in *Mb* and *Mf* from many different populations all over Europe (Scandinavia, Ireland, The Netherlands, W. Germany, France, Spain, Portugal, Yugoslavia and Greece). Geographic variation was found in parameres and the shape of the aedeagus. The form of the parameres in *Me* and *Mb* seems to be rather constant, but there is appreciable geographic variation in the parameres of *Mf* (Figs 33–36). However, there is much variation within populations as well.

Also the shape of the aedeagus is surprisingly constant in *Me* and *Mb*, but quite a lot of variation was found within and between populations of *Mf* (Figs 37–39). In populations from Spain, Portugal and the Azores the aedeagus of most of the specimens reveal a strongly developed left spine, which is nearly always less developed in populations from other parts of Europe. The size of the median spine and the position of the right spine show considerable variation as well, but no geographic trend could be detected.

It is interesting to note that the variation found in the position and size of the spines on the aedeagus of *Mf* is of about the same magnitude as the differences between the species *Mf* and *Me*. This means that although small difference in genital structures might be characteristic for species, one has to be careful to describe species only on the basis of such small differences as long as more biological information is lacking.

## DISTRIBUTION

Distributional data for *M. brevipennis* and *M. fairmairei* have been listed by NAST (1972) and subsequently discussed by DROSOPOULOS (1977). In

the last few years our knowledge of the distribution of *Muellerianella* species and forms has greatly increased because many new data have been collected all over Europe. Moreover, since *Mf* and *Me* have previously been confused, older records for "*M. fairmairei*" had to be reanalysed. Several museums and specialists were requested to send material and information. In this way part of the available material could be traced and older records could be checked. Because females of *Mf* and *M3* cannot be distinguished in museum material, *Mf*—like females were considered to indicate the presence of *Mf* at the locality. Reliable records for *M3* can only be obtained by cytological examination. In a few cases however, highly skewed sex-ratio's in favour of females were regarded as evidence for the presence of *M3*.

A list of all presently known records of the *Muellerianella* species and forms can be obtained on request at the authors address. This list includes all critically evaluated data from literature and museum-collections and detailed information is given on over 150 samples taken by the author and his colleagues all over Europe from 1977–1980.

Although more information is desirable, especially from Eastern Europe, the available data are sufficiently accurate to produce distribution maps, and to suggest factors which limit the distribution of the species. Distribution maps for the *Muellerianella* species and forms, and their hostplants are given in Figures 40–46. Since the *Muellerianella* species are more or less specific feeders, their occurrence is closely correlated with the distribution of their hostplants.

In the past *Mf* was considered to be more widespread and abundant than *Mb* (DROSOPOULOS 1977). This wrong impression is caused by the confusion of *Mf* and *Me* and because *Mf* is caught more easily and in higher numbers than *Mb* and *Me*. The latter two species live deep in the vegetation and are rarely caught by sweepnet. When more intensive sampling methods are used, for example a suction apparatus, it appears that all three species can be influent or dominant at suitable localities in the autumn (*Mf:* DROSOPOULOS 1977; *Me:* SCHIEMENZ 1971; *Mb:* TORMÄLÄ and RAATIKAINEN 1976).

The distribution of *M. fairmairei* is typical atlantic-mediterranean (Fig. 40). Although *Holcus lanatus* extends farther N and NE, *Mf* seems to be absent from places with cold winters and short seasons, like S. Finland, Poland, the eastern Baltic, and even in submontaneous areas of Central Europe. As far as could be checked allmost all older records for "*M. fairmairei*" from Sweden, Finland and eastern Europe (KONTKANEN 1947, 1950, SCHIEMENZ 1971, 1975, 1976, VILBASTE 1974, HIEBSCH et al 1978, OSSIANNILSSON 1978) refer to *M. extrusa*. Other records from Ireland (MORRIS 1974) and W. Germany (REMANE 1958) refer to both species.
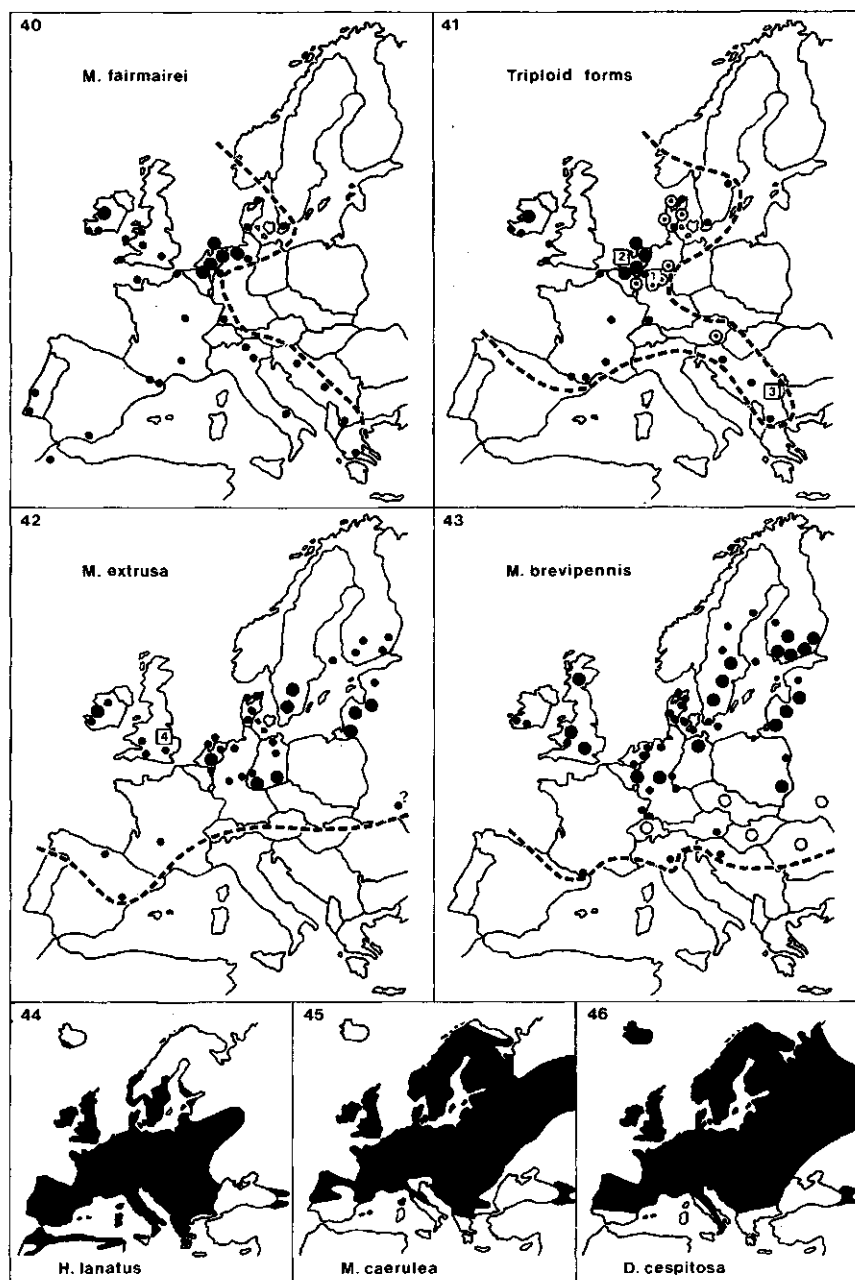
Figs. 40–46. Distribution of the species and forms of the *Muellerianella* complex and their hostplants. 40, *M. fairmairei*. 41, *Triploid forms*. 42, *M. extrusa*. 43, *M. brevipennis*. Small solid circles indicate records from 1–3 sites, large solid circles records from 3–10 sites, large open circles not confirmed records for countries and open circles with points indicate records of triploid forms coexisting with *M. brevipennis*. Specialized hostplant-clone of triploid forms on *Deschampsia flexuosa, Calamagrostis canescens* and *Bromus ramosus* are indicated by ☐, ☑ and ☒ respectively. ☒ indicates a population of *M. extrusa* on *Arrhenatherum elatius*. Figs. 44–46 Distribution of the hostplants. 44, *Holcus lanatus*. 45, *Molinia caerulea*. 46, *Deschampsia cespitosa*.

In spite of the recent discovery (1978) of *M. extrusa,* we have been able to collect quite a lot of data for this species, from which a distribution map could be produced (Fig. 42). The available data suggest that the distribution of *Me* is closely correlated with that of the host-plant *Molinia caerulea.* In Northern Europe *Me* is widespread in bogs, moors and related habitats and presumably extends far to the east. In S. Europe the species is probably restricted to mountainous habitats with a humid climate. Former data of "*M. fairmairei*" from Finland, E. Germany, the Eastern Baltic and Russia refer to *Me.* Material from Sakhalin (E. Russia) is very similar to *Me* (VILBASTE pers. comm.). More material from Siberia and Japan should be examined to see to what extent *Me* is distributed in the boreal and temperate zones of the Eastern Palaearctic.

For *M. brevipennis* many reliable records are given in literature (KUNTZE 1937, KONTKANEN 1952, LOGVINENKO 1975, TORMÄLÄ and RAATIKAINEN 1976, LE QUESNE 1960, BITTNER and REMANE 1977, OSSIANNILSSON 1978). The distribution of *M. brevipennis* is closely similar to that of *M. extrusa* (Fig. 43). The species is widely distributed in temperate and boreal regions of Europe. In southern Europe the species is restricted to higher elevations.

*M3.* Since triploid forms are mainly associated with *Mf,* the distribution of *M3* is closely correlated with that of *Mf* (Fig. 41). It has been shown, however, that in samples of *Mf* from Spain, Portugal and Greece no *M3* females were found. Thus it seems that *M3* extends less southwards than *Mf.* On the other hand M3 is occasionally found in association with *Mb* in areas where *Mf* is absent, like in Denmark and in submountainous areas of Germany. It is not likely that *M3* extends much farther NE since *H. lanatus* becomes rarer there. it can be concluded that the distribution of *M3* is somewhat intermediate between *Mf* and *Mb.* The ecological and geographic relations between *Mf, Mb* and *M3* will be more fully discussed in another paper.

When the bisexual species are compared on a wider geographic scale it can be concluded that *Mb* and *Me* are sympatric, and that *Mf* is partly sympatric with *Mb* and *Me.* The degree of sympatry between *Mf* and both other species is less than suggested by the distribution maps since they occur, like their hostplants, at different altitudes in S. Europe. Syntopic populations of *Mb* and *Mf* or *Me* and *Mf* are mainly found in NW Europe (Ireland, Great Britain, the Netherlands, Denmark, NW Germany).

## DISCUSSION

In this paper it was shown that the species of the genus *Muellerianella* can be distinguished by several small but reliable differences, most of

which are non-genital in females. The importance of ecological and ethological characters in systematics is illustrated by the fact that *M. extrusa* and *M. fairmairei* were found to be distinct species because they differ in ecology and acoustic behaviour (Booy in prep.). Only afterwards morphological differences between these species were found.

The minor differences between *M. extrusa* and *M. fairmairei* suggest that these species are closely related, although morphological differences are not necessarily correlated with genetic differentiation. *M. brevipennis* is morphologically much more diverged, and can hardly be regarded as sibling species of *M. fairmairei* or *M. extrusa*, as was done before (Drosopoulos 1977). Populations of *Muellerianella* from Eastern Europe, Siberia and Japan should be further studied to elucidate the taxonomic structure of this genus completely.

As has been shown in this paper, the morphological variation of triploid gynogenetic females, which is at least partly genetic, is just as wide as in the bisexual females of *M. fairmairei*. This suggests that different clones are present in the field. Although these clones might have differentiated by mutation (recombination is absent), it seems unlikely that they all originated from one single individual and differentiated to such an extent that they could adapt to the variety of habitats and climates in all parts of Europe where they occur now. Presumably they arose several times.

The extensive variation found between triploid forms, makes it very unlikely that morphological characters can be used to distinguish *M3* from *Mf*. At this moment cytological examination is indispensable for the recognition and discovery of *M3* females in samples of *Muellerianella*.

It seems likely that most of the thelytokous reproducing animals are of hybrid origin (White 1978). From crossing experiments, Drosopoulos (1978) concluded that the triploid gynogenetic forms of *Muellerianella* arose by hybridization between *M. fairmairei* and *M. brevipennis*. The fact that some morphological characters are intermediate between these species supports this hypothesis, but most other characters are not intermediate. Much stronger evidence for a hybrid origin is formed by the distribution of the triploid forms. They occur mainly in the region where the bisexual species are sympatric, and they extend their range somewhat NE by accepting *M. brevipennis* as host species, where *M. fairmairei* is absent. If these triploid forms are of hybrid origin indeed, it seems likely that they arose when *M. fairmairei* and *M. brevipennis* became sympatric after the extension of *Holcus lanatus* to the North. Since this extension is closely correlated with the evolution of anthropogenic grasslands in Europe (Scholz 1975), it is possible that these forms are not older than a few thousand years. The ecological and geographic relations between *M. brevipennis*, *M. fairmairei* and *triploid forms* will be further discussed in later papers.

23

REFERENCES

BITTNER, C. & REMANE, R., 1977. Beiträge zur Kenntnis der Zikadenfauna (Homoptera, Auchenorrhyncha, Cicadina) des Roten Moores/Rhön.—Beitr. Naturk. Osthessen **11/12**: 141–162.

DROSOPOULOS, S., 1976. Triploid pseudogamous biotype of the leafhopper *Muellerianella fairmairei*.—Nature **263**: 499–500.

DROSOPOULOS, S., 1977. Biosystematic studies on the *Muellerianella* complex (Delphacidae, Homoptera, Auchenorrhyncha).—Meded. Landbouwhogeschool Wageningen **77**: 1–133.

DROSOPOULOS, S., 1978. Laboratory synthesis of a pseudogamous triploid "species" of the genus *Muellerianella* (Homoptera, Delphacidae).—Evolution: **32**: 916–920.

FLOR, 1861. Die Rynchoten Livlands in systematischer Folge beschrieben. Zweiter Teil: *Rhynchota gulaerostria* Zett. (Homoptera) Cicadina und Psyllodea.—Arch. Nat. Kurklands. **4**: 1–567.

HIEBSCH, H., EMMERICH, R., KRAUSE R., 1978. Zur Fauna einiger Arthropodengruppen des Flächennaturdenkmals "Saugartenmoor" in der Dresdender Heide.—Ent. abh. Staatl. Mus. Tierk. **42**: 212–249.

KONTKANEN, P., 1947. Beiträge zur Kenntnis der Zikadenfauna Finnlands II.—Ann. Ent. Fenn. **13**: 170–175.

KONTKANEN, P., 1950. Quantitative and seasonal studies on the leafhopperfauna of the

fieldstratum on open areas in North Karelia.—Ann. Zool. Soc. Zool. Bot. Fenn. "Vanamo" **13**: 1–91.

KONTKANEN, P., 1952. Beiträge zur Kenntnis der Zikadenfauna Finnlands.—Ann. Ent. Fenn. **18**: 26–24.

KUNTZE, H. A., 1937. Die Zikaden Mecklenburg, eine faunistisch-ökologische Untersuchung.—Arch. für Naturgesch. N.F. **6**: 299–388.

LEQUESNE, W. J., 1960. Handbook for the identification of British insects.—Vol. II, part 3, Hemiptera, Fulgomorpha. Royal Entomological Society of London. 68 pp.

LEQUESNE, W. J. & WOODROFFE, G. E., 1976. Geographical variation in the genitalia of three species of Cicadellidae (Hemiptera).—Syst. Entom. **1**: 169–172.

LOGVINENKO, V. M., 1975. Fauna Ukrainii.—Vol. 20, part 2, Kiev: 1–141.

LOGVINENKO, V. M., 1976. New species of leafhoppers of the superfamily Fulgoroidae (Auchenorrhyncha) from the Caucasus.—Rev. d'Entom de l'URSS, **55**: 602–609.

MARCHAND, H., 1953. Die Bedeutung der Heuschrecken und Schnabelkerfe als Indikatoren verschiedener Grasslandtypen.—Beitr. Ent. **3**: 116–162.

MASLIN, T. P. 1968. Taxonomic problems in parthenogenetic vertebrates.—Syst. Zool. **17**: 219–231.

MAYR, E., 1969. Principles of Systematic Zoology.—McGraw-Hill New York. 428 pp.

MORRIS, M. G., 1974. Auchenorrhyncha (Hemiptera) of the Burren, with special reference to species associations of the grasslands.—Proc. R. Ir. Acad. **74**, Section B: 7–30.

MÜLLER, H. J., 1965. The taxonomic value of the male genetalia in the light of new studies on the seasonal forms of *Euscelis*.—Proc. 10$^{th}$ Intern. Congr. Entom. **1**: 357–362.

NAST, J., 1972. Palaeartic Auchenorrhyncha (Homoptera), an annotated checklist.—Polish Scientific Publishers Warsaw. 550 pp.

NAST, J., 1979. Auchenorrhyncha (Homoptera) Wyzyny Lubelskiej i Roctocza, Czesc I–Fulgoroidea.—Frag. Faun. **25**: 1–13.

OSSIANNILSSON, F., 1978. Fauna entomologica Scandinavia 7, part 1, The Auchenorrhyncha (Homoptera) of Fennoscandia and Denmark.—Scandinavian Science Press Ltd., Klampenborg Denmark. 222 pp.

PERRIS, 1857. Nouvelles excursions dans les Grandes Landes.—Ann. Soc. Linn. Lyon, **4**: 83–180.

REMANE, R., 1958. Die Besiedlung von Grünlandflächen verschiedener Herkunft durch Wanzen und Zikaden im Weser-Ems Gebiet.—Z. Angew. Entom., **42**: 353–400.

SCHIEMENZ, H., 1971. Die Zikadenfauna (Homoptera, Auchenorrhyncha) der Erzgebirgshochmoore.—Zool. Jb. Syst. **98**: 397–417.

SCHIEMENZ, H., 1975. Die Zikadenfauna der Hochmoore im Thüringer Wald und Harz (Homoptera, Auchenorrhyncha).—Faun. Abh. Staatl. Mus. Tierk. Dresden **5**: 215–233.

SCHIEMENZ, H., 1976. Die Zikadenfauna von Heide- und Hochmoore des Flachlandes der DDR.—Faun. Abh. Staatl. Mus. Tierk. Dresden **6**: 39–54.

SCHOLZ, H., 1975. Grassland evolution in Europe.—Taxon **24**: 81–90.

SCOTT, 1871. On certain British Hemiptera-Homoptera. Revision of the family Delphacidae and description of several new species of the genus *Delphax* and others.—Ent. Month. Mag. **7**: 193–196.

SERVADEI, A., 1968. Contributo alla corologia dei Rhynchota Homoptera Auchenorrhyncha d'Italia.—Est. Ann. Mus. di storia Nat. Genova **77**: 138–183.

TÖRMÄLÄ, I. & RAATIKAINEN, N., 1976. Primary production and seasonal dynamics of the flora and the fauna of the fieldstratum in a reserved field in Middle Finland.—Maataloust. Aikakousk. **48**: 363–385.

VILBASTE, J., 1974. Preliminary list of Homoptera-Cicadina of Latvia and Lithuania.—ENSV TA Toimet, Biol. **23**: 131–163.

WAGNER, W., 1963. Dynamische Taxionomie, angewandt auf die Delphacidae Mitteleuropas.—Mitt. Hamburg. Zool. Mus. Inst. **60**: 111–180.

WHITE, M. D., 1978. Modes of speciation.—W. H. Freeman & Company San Francisco. 455 pp.

C. J. H. Booij, Department of Entomology, Agricultural University, Binnenhaven 7, 6709 PD Wageningen, The Netherlands.

# Biosystematics of the *Muellerianella* complex (Homoptera, Delphacidae): host-plants, habitats and phenology

C. J. H. BOOIJ  Department of Entomology, Agriculture University, Wageningen, The Netherlands

ABSTRACT. 1. Ecological differences between closely related species and forms of the planthopper genus *Muellerianella* (Delphacidae) were studied.

2. In the field *M.brevipennis* and *M.fairmairei* are restricted to *Deschampsia cespitosa* and *Holcus lanatus/H.mollis*, respectively. *M.extrusa* is mainly found on *Molinia caerulea*, but has also been recorded from other grass-species. Triploid all-female forms which reproduce by gynogenesis usually live on *Holcus* together with *M.fairmairei*. Other gynogenetic populations were found on *Bromus ramosus*, *Deschampsia flexuosa* and *Calamagrostis canescens*.

3. Breeding experiments showed that the diploid species cannot be maintained on each other's host-plants, but accept other grass-species.

4. Due to overlapping habitat requirements of the host-plants, ecological isolation between the *Muellerianella* species is incomplete.

5. *M.extrusa* lives in bogs, moors and forests on poor acid soil. *M.brevipennis* is found in forests, carr, fens and in extensively managed grasslands. *M.fairmairei* and associated triploid forms occur mainly in grasslands and other man-made habitats. The habitats of all three species are usually wet.

6. In most parts of Europe *M.brevipennis* and *M.extrusa* are monovoltine and *M.fairmairei* is mostly bi- or polyvoltine.

Key words. Delphacidae, *Muellerianella*, ecological isolation, hostplants, habitats, *Molinia*, *Deschampsia*, *Holcus*, gynogenesis.

## Introduction

The genus *Muellerianella* (Homoptera, Delphacidae) consists of three bisexual species: *M.brevipennis* Boheman 1847, *M.fairmairei* Perris 1857 and *M.extrusa* Scott 1871. In addition to these species, triploid all-female forms (referred to as *M3*) occur, which usually coexist with *M.fairmairei* on which they depend for sperm (Drosopoulos, 1976). In a recent paper (Booij, 1981) the taxonomy of the genus has been revised, morphological differences between the species described and distributional data evaluated.

Correspondence: Dr C. J. H. Booij, Department of Entomology, Agriculture University, Binnenhaven 7, 6709 PD Wageningen, The Netherlands.

The ecology of *M.fairmairei* and *M.brevipennis* has been extensively studied by Drosopoulos (1977), particularly reproduction, larval development and diapause. Although the primary host-plants were clearly established by his data, information on habitats and alternative host-plants is poor, and restricted to a few, mainly Dutch, localities. Moreover, *M.extrusa*, which was not distinguished at that time, was not included in his studies.

The species and forms of the genus *Muellerianella* are confined to grasses which grow in moist to wet habitats. They complete one or more generations per year and overwinter in the egg stage. The primary host-plants are *Deschampsia cespitosa* for *M.brevipennis*, *Holcus lanatus* or *H.mollis* for *M.fairmairei*

and triploid forms, and *Molinia caerulea* for *M.extrusa*. Overwintering eggs of *M.fairmairei* are laid in *Juncus*. *M.extrusa* and *M.brevipennis* always oviposit in their host-plants. The life history of triploid forms resembles that of *M.fairmairei*, from which they can only be distinguished by cytological methods.

To see how the closely related species were reproductively isolated, ecological differentiation between them was determined by studying habitats in different parts of Europe and by screening a number of grasses for suitability in the laboratory. A study of ecological differences between the species was considered to be important because the species can hybridize in the laboratory (Drosopoulos, 1977; Booij, unpublished data). From crossing experiments it has also been suggested that the triploid gynogenetic forms arose through hybridization between *M.fairmairei* and *M.brevipennis* (Drosopoulos, 1978). Insufficient ecological isolation may still result in recurrent production of such forms or in introgression between the species.

## Methods

To determine general host-plant suitability, ten females and ten males (all less than 1 week old) were placed in a cage with the grass-species to be tested (see also Drosopoulos, 1977). After 20 days the surviving animals were removed and counted. Cages were checked daily for hatched larvae. The period between hatching of first larvae and the appearance of adults was taken as a measure of larval development time. One week after the appearance of first adults all animals were removed and counted in order to estimate reproduction. Both animals and grasses were kept in the glasshouse at $20-23°C$ and long day conditions (LD 18 : 6). More accurate data on larval development were obtained by rearing single animals in tubes under constant conditions $(20\pm0.5°C,$ LD 16 : 8, 90—100% humidity, see also Drosopoulos, 1977). All experimental animals were taken from mass-rearings of Dutch populations.

Habitat descriptions were made at more than 100 sites all over Europe. Information on landscape, general habitat, soil type, soil moisture, vegetation composition and management of the vegetation were included in these descriptions. A list of the localities with notes on habitat and collected material can be obtained on request at the author's address.

## Results

### Host-plant experiments

The suitability of several grass-species was tested by Drosopoulos (1977) for *M.brevipennis*, *M.fairmairei* and triploid females. His conclusion, that *M.fairmairei* is potentially more polyphagous than *M.brevipennis*, is correct. However, most grass-species on which the species could be successfully reared (apart from *Holcus* and *Deschampsia*) are ecologically irrelevant, because they do not occur in habitats where *Muellerianella* species live. Furthermore, *M.extrusa* was not included in his studies.

Therefore a number of grass-species were screened using the three *Muellerianella* species and a triploid all-female clone, all originating from the Netherlands. For these experiments grass-species were chosen which are ecologically or taxonomically related to the primary host-plants of the *Muellerianella* species. Other grass-species were tested because they had been found in association with *Muellerianella* species. Grass-species recorded as hostplant for '*M.fairmairei*' in Japan (Mochida & Okada, 1971) have not been used in this study because the taxonomic status of this Japanese *Muellerianella* species is unknown (see below).

The results of the breeding experiments are compiled in Table 1. Suitability of the grass-species has been defined relative to survival, larval development and reproduction on the primary host-plants. In this way the success of the species on different grasses could be directly compared, and data obtained by different methods could be included, as well as the data of Drosopoulos (1977).

Table 1 shows that the species cannot be reared on each other's host-plants, except for *M.extrusa* which produces few progeny on *Deschampsia cespitosa*. The particular clone of *M3* follows the general pattern of *M.fairmairei*, but can be bred in low numbers on *D.cespitosa* as well. Furthermore, *M3, M.fairmairei* and *M.extrusa* are potentially more polyphagous than *M.brevipennis*, which can be reared only on a few grass-species.

TABLE 1. Breeding results of the *Muellerianella* species on different grass-species. The figures in the columns for each species indicate survival of adults (S), larval development (L), and reproduction per female (R). The values have been defined as follows: Survival 5: 80–100%, 4: 60–80%, 3: 40–60%, 2: 20–40%, 1: 5–20%, 0: 0%; Larval development 5: 20–25 days, 4: 25–30 days, 3: 25–30 days + some mortality, 2: 30–40 days + mortality, 1: more than 40 days + heavy mortality; Reproduction 5: as on primary host, 4: 50–75%, 3: 20–50%, 2: 10–20%, 1: less than 10%, 0: no progeny.

| | Triploids (M3) | | | M.fairmairei | | | M.extrusa | | | M.brevipennis | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | L | R | S | L | R | S | L | R | S | L | R |
| *Lolium perenne* | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 4 | 4 | 4 | 5 | 4 |
| *Calamagrostis canescens* | 4 | 5 | 4 | 4 | 5 | 5 | 5 | 4 | 4 | 4 | 4 | 2 |
| *Festuca arundinacea* | 3 | 3 | 5 | 3 | 3 | 5 | 3 | 4 | 1 | 4 | 4 | 2 |
| *Arrhenatherum elatius* | 3 | 5 | 4 | 2 | 4 | 3 | 4 | 5 | 4 | 0 | — | 0 |
| *Holcus lanatus* | 5 | 5 | 5 | 5 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Holcus mollis* | 5 | 5 | 5 | 5 | 5 | 5 | — | — | — | 0 | 0 | 0 |
| *Trisetum flavescens* | — | — | — | 4 | 4 | 4 | — | — | — | — | — | — |
| *Phleum pratense* | — | — | — | 4 | 4 | 4 | 4 | 3 | 2 | 2 | 2 | 0 |
| *Molinia caerulea* | 0 | — | 0 | 0 | — | 0 | 5 | 5 | 5 | 0 | — | 0 |
| *Brachypodium pinnatum* | — | — | — | 0 | 0 | 0 | 4 | 4 | 3 | 0 | 0 | 0 |
| *Agrostis tenuis* | — | 0 | 0 | — | 0 | 0 | 5 | 3 | 2 | — | — | 0 |
| *Deschampsia flexuosa* | 0 | 0 | 0 | 0 | — | 0 | 2 | 4 | 3 | 0 | 0 | 0 |
| *Deschampsia media* | — | — | — | 1 | — | 0 | 4 | 4 | 4 | 5 | 5 | 5 |
| *Deschampsia cespitosa* | 4 | 2 | 2 | 0 | 0 | 0 | 3 | 4 | 1 | 5 | 5 | 5 |
| *Cynosurus cristatus* | 0 | 1 | 1 | 0 | 1 | 0 | — | — | — | 3 | 4 | 1 |
| *Poa pratensis* | — | 1 | 0 | — | 1 | 0 | 4 | — | 0 | 0 | — | 0 |
| *Sesleria caerulea* | — | — | — | 0 | — | 0 | 0 | — | 0 | 0 | — | 0 |
| *Anthoxanthum odoratum* | 0 | — | 0 | 0 | — | 0 | 0 | — | 0 | 0 | — | 0 |

Quite remarkable is the success of *M.extrusa* on three species of *Deschampsia*, especially *D.flexuosa* which is often associated with *Molinia caerulea*. Moreover, a triploid form has been found on *D.flexuosa* (see below). Table 1 shows that *M.extrusa* can be reared on *Arrhenatherum elatius* and *Brachypodium pinnatum*. This is interesting because an *M.extrusa*-like population in England (see below) was found associated with these grasses.

*Calamagrostis canescens* and *Lolium perenne* seem to be suitable for all *Muellerianella* species. *L.perenne* is an important component of intensively managed grasslands. *C.canescens* occurs in alluvial woods, carr, fens and marshy meadows all over Europe. In all these habitats *Muellerianella* species occur.

### Host-plants in the field

Despite intensive searching, the bisexual species were never found living on plants other than their primary hosts, *Deschampsia cespitosa* (*M.brevipennis*), *Holcus lanatus* and *H.mollis* (*M.fairmairei*) and *Molinia caerulea* (*M.extrusa*). However, at one locality in the

Netherlands, where *Molinia caerulea* and *Arrhenatherum elatius* grow together, several animals of *M.extrusa* were found feeding on *A.elatius*. Samples from the Castor Hanglands (England) taken by M. G. Morris (personal communication) in great numbers, contained many specimens of a species which strongly resembles *M.extrusa* (Booij, 1981). The vegetation at this locality is dominated by *Arrhenatherum elatius* and *Brachypodium pinnatum*, and *Molinia caerulea* is absent. As mentioned above, *M.extrusa* can be reared on both grass-species.

In Ireland, Morris (1974) collected *Muellerianella* from calcareous grassland at sixteen localities in the Burren. He suggested an association with *Sesleria caerulea* which was frequent there. The material closely resembles *M.extrusa*, but *Molinia caerulea* is not common at these localities (Morris, personal communication). *Sesleria caerulea* was tested as a food plant for *M.extrusa* but appears to be unsuitable (Table 1). Finally an *extrusa*-like species was recently found on *Carex divulsa* by S. Drosopoulos (personal communication) in northern Greece. More detailed information on host-plant choice, acoustic behaviour and breeding com-

patibility is needed to clarify the taxonomic status of these *extrusa*-like populations from Ireland, England and Greece.

The same taxonomic uncertainty surrounds records of *Muellerianella* species from Japan (Mochida & Okada, 1971). *M.extrusa* is probably the only *Muellerianella* species which has been recorded from the Eastern Palaearctic (Sakhalin) (Booij, 1981), but it is not known whether it is the same species which has been recorded from Japan. Recently a female larva of an *M.extrusa* like species was collected on *Calamagrostis canescens* near Kyoto in Japan by R. H. Cobben. This record was not very surprising, since all *Muellerianella* species (except *M.brevipennis*) maintained well on this grass-species in the laboratory (Table 1).

During the screening of alternative host-plants in the field, we found triploid *M3* clones on *Calamagrostis canescens* in the Netherlands and in a mixed vegetation of *Bromus ramosus* and *Brachypodium sylvaticum*, for which the former is the probable host-plant, in eastern Yugoslavia. In the autumn of 1979, R. Remane (Marburg) found females of *M3* on *Deschampsia flexuosa* in West Germany. In the laboratory these clones could be reared easily on *C.canescens, Bromus racemosus* (*B.ramosus* was not available) and *D.flexuosa* respectively. The host-plants of the bisexual species, *H.lanatus, D.cespitosa* and *M.caerulea*, were tested for all three clones, but they generally failed on these grasses. The *Bromus* clone produced a few progeny on *D.cespitosa*, and the *Calamagrostis* clone had very low reproductive success on *H.lanatus*. All three specialized host-plant clones can, like the bisexual species, be reared with reasonable success on *Lolium perenne* and *Calamagrostis canescens* as well. The maintenance of these clones in the field must be problematic, since they reproduce by gynogenesis and their sperm donors live on other host-plants which must grow in close proximity.

The sperm donor of the Yugoslavian *M3* clone on *Bromus* or *Brachypodium* is especially puzzling. Since neither bisexual *Muellerianella* species nor their host-plants were found there, I suggest that a species from quite another genus might serve as a sperm donor!

The sporadic occurrence of triploid forms on *Deschampsia cespitosa* together with *M.brevipennis* is easier to understand. Some *M3* females were found in *M.brevipennis* populations from three sites in the Netherlands and at one locality in north-west Yugoslavia. Since the distinction between *M3* and *M.brevipennis* females in the field is difficult, the *M3* females are easily overlooked.

## Host-plant differences and ecological isolation

The strict confinement of the *Muellerianella* species to different host-plants might serve as an important isolating mechanism, especially because most of the animals are brachypterous and move only over small distances. Ecological isolation may be reduced, however, when the host-plants regularly grow intermixed. Ecological data for the host-plants were gathered from literature, to see in which kind of situations and how often they will be found together. *Holcus mollis* is left out of further discussions, because *M.fairmairei* and *M3* are found only occasionally on this grass-species under special conditions. In Table 2 a number of environmental factors are compared between the host-plants. All three grass-species occur mainly in moist to wet habitats where management is extensive. *H.lanatus* is generally more tolerant of cultivation, fertilization and drainage than *M.caerulea* and *D.cespitosa*. The latter species are common in natural habitats in temperate and northern Europe, whereas *H.lanatus* is mostly found in semi-natural habitats. Due to their overlapping requirements, mixtures of these grass-species can be found in the field. *M.caerulea* and *D.cespitosa* can be found together in mesotrophic fens and carr and in slightly disturbed or drained bogs. In eastern Europe they also grow together in unfertilized, extensively used wet meadows (Mueller-Dombois & Ellenberg, 1974; Balátová-Tuláčková, 1972; Kovacs, 1962). *H.lanatus* and *D.cespitosa* are more frequently found together in poorly drained grasslands and fenlike habitats of central and north-west Europe (Ellenberg, 1963; Mueller-Dombois & Ellenberg, 1974; Larsson, 1976). In south-west Europe, *D.cespitosa* and *H.lanatus* occur at different altitudes. The ecological optima of *H.lanatus* and *M.caerulea* differ markedly with regard to nutrient levels and soil-acidity. Therefore this combination is less common. Mixtures of *H.lanatus* and *M.caerulea*

TABLE 2. Ecological characteristics of the host-plants of the *Muellerianella* species. Sources: Ellenberg (1963), Westhoff & den Held (1975), Westhoff *et al.* (1970, 1971, 1973), Mueller-Dombois & Ellenberg (1974), Horvat *et al.* (1974), Larsson (1976), Passarge (1964) and others.

|  | *Molinia caerulea* | *Deschampsia cespitosa* | *Holcus lanatus* |
|---|---|---|---|
| Soil type | Sand/peat | Sand/peat/sandy clay | Sand/peat |
| Soil moisture | Very wet—moist | Very wet—fresh | Wet—fresh |
| Soil acidity (pH) | 3—5 | 4—5 | 4—6 |
| Nutrient supply | Very low—low | Low—rather high | Rather low—high |
| Exploitation | Absent—extensive | Extensive | Extensive—rather intensive |
| Fertilization | Absent | Absent—moderate | Low—rather high |
| Natural habitats | Bogs, moors, wet scrubland, birchcarr coniferous woods | Alluvial forests, carr and fens | Flushes |
| Preferred grassland | Unfertilized meadows, mown once a year | Extensively used pasture, haypasture and meadows | Haymeadows, haypasture and pasture |
| Optimal distribution | NW and NE Europe | NW and NE Europe | W and S Europe |
| Altitudes (C Europe) | Lowland—high montane | Lowland—subalpine | Lowland—submontane |

occur sometimes in slightly disturbed bogs and in unfertilized wet meadows which are mown once a year.

It should be kept in mind that the ecological requirements of the *Muellerianella* species are more restricted than those of their host-plants; in particular the insects are less tolerant of frequent mowing and dryness than their host-plants. Moreover, populations of *M.fairmairei* and triploid forms can only persist in places where *H.lanatus* is associated with *Juncus effusus* (for laying winter eggs).

During the investigations mixtures of *Muellerianella* species were found at several places. *M.brevipennis*, *M.fairmairei* and *M3* were found together in Ireland (one site), the Netherlands (three sites) and in Sweden (one site). In Denmark and central Germany, at places where *H.lanatus* and *D.cespitosa* grew together, but where *M.fairmairei* was absent *M3* was found in association with *M.brevipennis* (eight sites). In another three Dutch and one Yugoslavian *M.brevipennis* population a few triploid females were found on *D.cespitosa*. Mixtures of *M.extrusa* and *M.brevipennis* were found at four sites (in Great Britain, in Denmark, in West Germany and in the Netherlands). Presumably mixtures of *M.brevipennis* and *M.extrusa* are more common in Scotland, Scandinavia and northeast Europe, but information is lacking. Syntopic occurrence of *M.fairmairei* and *M.extrusa* was found at two localities in the Netherlands. Although *M.extrusa* can serve as sperm donor for *M3* in the laboratory, we

have no certain record of *M3* in association with *M.extrusa* without the presence of *M.fairmairei*.

*Habitats*

*Muellerianella* species have been recorded in several faunistic and ecological studies. Habitat descriptions given in the literature, however, are often very brief or only indicating the type of landscape. Drosopoulos (1977) evaluated habitat descriptions from the literature, but because *M.fairmairei* and *M.extrusa* were not discriminated at that time, the habitats of these species were confused. Furthermore, very little information was available on the habitats of *M.brevipennis*.

Detailed habitat descriptions for *M.fairmairei*, *M3* and *M.brevipennis* were given by Drosopoulos (1977) for his study areas. Other habitat descriptions which apparently refer to *M.fairmairei* and *M3* have been given by Remane (1958) and Marchand (1953). The habitat of what appeared to be *M.extrusa* has been described by Schiemenz (1971, 1975, 1976). He collected numerous individuals of this species in bogs and moors and recognized the association with *M.caerulea*. Less precise ecological data for *Muellerianella* species have been published by Kuntze (1937), Kontkanen (1950) and Vilbaste (1974). From many habitat descriptions, made all over Europe during the last few years, we now have a much better picture of the habitat differences between the *Muellerianella* species. The

TABLE 3. Habitat classification of localities where *Muellerianella* species have been recorded. Some data of Remane (1958), Kuntze (1937), Marchand (1953) and Schiemenz (1971, 1975, 1976) have been included.

| | *M.fairmairei* | *M.brevipennis* | *M.extrusa* |
|---|---|---|---|
| Trenches, ditches and roadsides | 13 | 1 | 2 |
| Ecotones between wet and dry | 6 | 1 | – |
| Pastures and haypastures | 18 | 5 | – |
| Extensively used meadows | 15 | 7 | 3 |
| Fens and fen-like habitats | 6 | 10 | 1 |
| Alluvial forests, carr and clearings | 3 | 11 | 9 |
| Bogs, moors, margins of oligotrophic pools | – | 1 | 40 |

habitat descriptions were categorized into a number of general habitat types (Table 3). The plant species which are most frequently associated with the different *Muellerianella* species are given in Table 4.

Table 3 shows that *M.fairmairei* and the associated *triploid* forms occur mainly in grasslands and other man-made habitats. Most of the plant species associated with *M.fairmairei/M3* populations (Table 4) are characteristic of moist to wet grasslands of various sorts. They are usually situated on poor to moderately rich soils (peat, sand and sandy clay) and not or only poorly fertilized. *M.fairmairei* and *M3* may occur in all such grasslands as long as *Juncus effusus* and *Holcus lanatus* are present. They are absent, however, from meadows which are mown more than once a year and from pastures which are intensively grazed. Furthermore, *M.fairmairei* and *M3* occur in disturbed fens, fen-like habitats and at flushes. In many habitats of *M.fairmairei* and *M3*, plant species were found which indicate dryness, disturbance and eutrophication.

*M.brevipennis* also occurs in moist to wet grasslands, but prefers less disturbed habitats than *M.fairmairei* and *M3*. Most of the grasslands where *M.brevipennis* is found are more extensively grazed and/or mown less than once a year. All the meadow records originate from Scandinavia, where *D.cespitosa* often forms dense stands. Apart from grasslands *M.brevipennis* is also widespread in various natural habitats, like forests, fens and other swampy vegetations along streams. Since we concentrated on grassland habitats during the

investigations, these more natural habitats have been undersampled. *M.brevipennis* certainly occurs more often in forest habitats than is suggested by Table 3 and the associated plant species given in Table 4. The host-plant of *M.brevipennis*, *Deschampsia cespitosa*, grows in forests on rather rich soils which are permanently moist to wet or subjected to regular flooding (carr, alluvial forests and moist oak–ash–hornbeam woods).

The habitats of *M.extrusa* are quite different from those of *M.brevipennis*, *M.fairmairei* and *M3*. *M.extrusa* is mainly found in more or less natural habitats on poor acid soils. These include moors, bogs, birch-carr and moist coniferous woods. The associated plant species given in Table 4 are typical of such environments. Finally *M.extrusa* occurs at low densities in unfertilized wet meadows, where formation of *M.caerulea* tussocks is not completely prevented by yearly mowing. Such meadows were widespread in Europe in the past, but most of them have disappeared after fertilization and drainage. In the Netherlands we found *M.extrusa* in two such meadows.

The habitats of all *Muellerianella* species and forms are often characterized by plant species which indicate high and/or fluctuating ground water levels. Indeed the host-plants of the species are characteristic of such conditions.

*Phenology*

The phenologies of *M.brevipennis* and *M.fairmairei* have been studied by Drosopoulos (1977). He demonstrated that both

TABLE 4. The most frequent plant species associated with species of the *Muellerianella* complex. Under *M.fairmairei* and *M.brevipennis*, names printed in italics refer to species which are characteristic of moist to wet grasslands. Italicized names under *M.extrusa* refer to species which characterize bogs and moors.

| *Muellerianella fairmairei* | *Muellerianella brevipennis* | *Muellerianella extrusa* |
|---|---|---|
| *Holcus lanatus* | *Deschampsia cespitosa* | *Molinia caerulea* |
| *Juncus effusus* | *Juncus effusus* | *Sphagnum* sp. |
| Agrostis tenuis | *Holcus lanatus* | *Eriophorum angustifolium* |
| *Ranunculus acris* | *Ranunculus acris* | *Erica tetralix* |
| *Anthoxanthum odoratum* | Urtica dioica | *Calluna vulgaris* |
| *Festuca rubra* | Agrostis tenuis | *Potentilla erecta* |
| *Cirsium palustre* | *Cirsium palustre* | Holcus lanatus |
| Lolium perenne | *Galium uliginosum* | Deschampsia flexuosa |
| Holcus mollis | *Alopecurus pratensis* | *Empetrum nigrum* |
| *Rumex acetosa* | *Phleum pratense* | Juncus acutiflorus |
| *Plantago lanceolata* | *Vicia cracca* | Calamagrostis canescens |
| *Juncus acutiflorus* | Brachypodium sylvaticum | *Drosera* sp. |
| *Molinia caerulea* | Chamaenerion angustifolium | *Oxycoccus palustris* |
| *Deschampsia cespitosa* | *Molinia caerulea* | Calamagrostis epigeios |
| *Poa pratensis* | *Rumex acetosa* | Myrica gale |
| *Lysimachia vulgaris* | Rubus caesius | *Narthecium ossifragum* |
| Ranunculus repens | *Filipendula ulmaria* | Juncus effusus |
| *Filipendula ulmaria* | *Lotus uliginosus* | Salix aurita |
| Stellaria media | Mentha aquatica | Salix cinerea |
| *Galium uliginosum* | Alnus glutinosa | Betula pubescens |
| *Trifolium pratense* | Fraxinus excelsior | |

species hibernate in the egg stage and are bivoltine in the Netherlands. *M.brevipennis* is on average 1—2 weeks later than *M.fairmairei*, and in cold years the second generation of *M.brevipennis* is only partly completed. Since there is a wide overlap between the two species, they are not seasonally isolated. The phenologies of *M.brevipennis* and *M.fairmairei* are probably similar in lowland areas of Ireland, Great Britain, the Netherlands, Belgium and Germany.

From samples taken in 1977 and 1978, however, it appeared that *M.brevipennis* has only one generation in Denmark, Sweden and Finland. In these samples larvae and young adults of the first generation were found in late summer. Univoltinism has also been found for many populations of *M.brevipennis* in the submontane areas of central Europe. This is indicated by samples taken in July and August in Hessen (West Germany) and in the Hautes Fagnes (Belgium), at altitudes between 300 and 700 m. Even farther south, where *D.cespitosa* is restricted to higher altitudes, *M.brevipennis* often has only one generation. For example, a sample taken in the Pyrenees at 1500 m in the end of June contained only first and second instar larvae. The climatic conditions under which *D.cespitosa* grows

seems to be correlated with the fact that *M.brevipennis* completes only one generation a year.

For *M.fairmairei* two or more generations seem usual. Although we found some populations with one generation in Denmark and Sweden, *M.fairmairei* is absent from most regions where the season is too short to produce two generations. In this respect *M3* differs from *M.fairmairei* because *M3* is also found together with *M.brevipennis* in central parts of Germany and in the Hautes Fagnes, where only one generation is completed.

If the season is longer or if the climate is mild, *M.fairmairei* tends to be polyvoltine. This is not only true for mediterranean populations, but even in mild places in western Europe and partial third generation is indicated. The most clear example of this was found in Glengarriff (south-west Ireland). The climate at this locality is extremely mild and frost or snowfall are rare. While in other places the second generation had already vanished, we found all developmental stages from larvae to adults on 2 October. Continuous breeding is theoretically possible at that site. Three or more generations per year for *M.fairmairei* have also been recorded for populations in Greece (S. Drosopoulos, personal communica-

tion), and Portugal where adults are present in April. However, the phenology in southern Europe can be retarded at higher elevations. In southern Yugoslavia first and second instar larvae of the first generation were found at 800 m at the end of May, which indicates that the season starts later there than, for example, in the Netherlands at sea level.

From samples taken in 1979 and 1980 it became clear that *M.extrusa* is univoltine. In the Netherlands first adults do not appear before the beginning of July. Maximum abundance of adults is observed at the end of August. Because hatching of overwintering eggs and larval development are widely spread in time, adults are present over a very long period. This explains why two generations have been suggested for this species by Schiemenz (1971). One long generation from the end of July until the end of October has also been reported by Hiebsch *et al.* (1978) for a '*M.fairmairei*' population from a bog area in East Germany. Their samples clearly refer to *M.extrusa*.

The phenology of the *Muellerianella* species at different latitudes is illustrated in Fig. 1. As can be seen, the long generation of *M.extrusa* overlaps with the second generations of *M.fairmairei* and *M.brevipennis* in areas where the latter species have two generations. In regions where all three species complete only one generation the phenology overlaps in another way. Thus the phenological differences are nowhere sufficient for a complete seasonal isolation between the species.

## Discussion

Morphological similarities between the *Muellerianella* species (Booij, 1981) and small differences in acoustic behaviour which do not prevent interspecific mating in the laboratory (Booij, unpublished data) suggest that these species are genetically closely related. Therefore it was expected that the species would show clear ecological differences which prevent competition and hybridization.

In this paper it has been shown that differences in host-plants and habitats exist which are of primary importance in ecological isolation of the species. However, the overlapping ecological requirements of the host-plants lead to local mixtures of the *Muellerianella* species. In particular *M.brevipennis* and *M.fairmairei* are regularly found together in grasslands. Since these grasslands are man-made (Ellenberg, 1963), and *M.fairmairei* is almost completely restricted to such habitats, the present syntopic occurrence of these species is presumably largely anthropogenic. Moreover, the host-plant of *M.fairmairei*, *Holcus lanatus*, is probably not native in central and north-west Europe (Scholz, 1975). It seems likely that the ecological isolation between *M.fairmairei* and *M.brevipennis* broke down after the development of grasslands which started a few thousand years ago (Ellenberg, 1963; Scholz, 1975). Hybridization between these species may have resulted in the evolution of triploid forms as suggested by Drosopoulos (1977, 1978). Further evi-
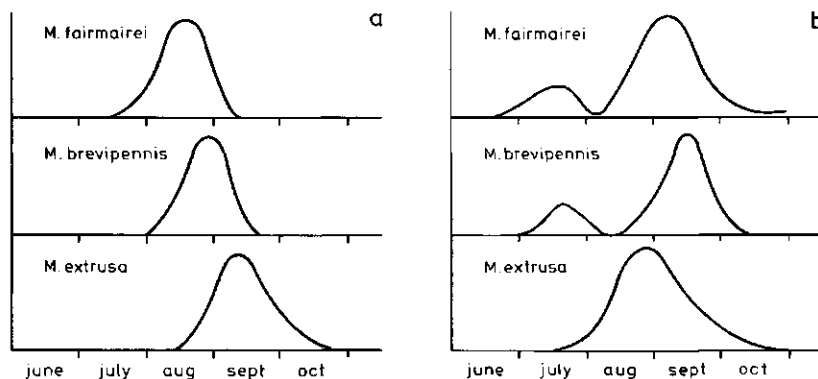


FIG. 1. Schematic presentation of the adult phenology of *Muellerianella* species in Denmark (a) and in the Netherlands (b).

dence for a hybrid origin of these forms is provided by their distribution which is intermediate between *M.fairmairei* and *M.brevipennis* (Booij, 1981). Moreover triploid forms coexist at low densities with *M.brevipennis* in borderline areas where *M.fairmairei* is absent (Booij, 1981, and this paper), and they can be reared on *D.cespitosa* in the laboratory.

The occurrence of triploid forms on host-plants other than *Holcus lanatus* suggests that host-plant preferences can be easily changed by a number of mutations (recombination is absent). A similar situation exists in parthenogenetic moths, where electrophoretically distinct clones are associated with different tree species (Mitter *et al.*, 1979). However, in both cases the existence of different host-plant clones might also be explained by a polyphyletic origin of these forms. These clones could have arisen from bisexual species which live, or may have lived, on other host-plants in unexplored areas.

The overlapping habitat requirements of *M.brevipennis* and *M.fairmairei* might give the impression that these species are ecologically (and possibly genetically) closely related. However, the present ecological overlap seems to be largely anthropogenic. Actually there are more ecological similarities between *M.brevipennis* and the third species, *M.extrusa*. They both occur in natural habitats on grasses with a similar tussock structure. Both lay their overwintering eggs in their host-plants, whereas *M.fairmairei* does not. Moreover, *M.brevipennis* and *M.extrusa* are usually monovoltine and adapted to similar climatic regions. Despite this ecological resemblance between *M.extrusa* and *M.brevipennis*, the closest genetic relation in the genus *Muellerianella* seems to be between *M.extrusa* and *M.fairmairei*, based on a consideration of morphology (Booij, 1981), acoustic behaviour and hybridization studies (Booij, unpublished data). Present information on host-plants and habitats suggests that the ecological isolation between *M.extrusa* and *M.fairmairei* is rather strong, but the interaction between them should be further studied.

The records of *M.extrusa*-like populations from host-plants other than *Molinia caerulea* suggest that this species is oligophagous and that it readily adapts to other host-plants at

certain localities. It is also possible that these populations represent further siblings in the *Muellerianella* complex.

## References

Balàtová-Tuláčková, E. (1972) Flachmoorwiesen im mittleren und unteren Opava-Tal (Schlesien). In: *Vegetace CSSR A4.* Verlag der Tschechoslowakischen Akademie der Wissenschaften, Prag.

Booij, C.J.H. (1981) Biosystematics of the *Muellerianella* complex (Homoptera, Delphacidae), taxonomy, morphology and distribution. *Netherlands Journal of Zoology,* 31, 572–595.

Drosopoulos, S. (1976) Triploid pseudogamous biotype of the leafhopper *Muellerianella fairmairei. Nature,* 263, 499–500.

Drosopoulos, S. (1977) Biosystematic studies on the *Muellerianella* complex (Delphacidae, Auchenorrhyncha, Homoptera). *Mededelingen Landbouwhogeschool Wageningen,* 77, 1–133.

Drosopoulos, S. (1978) Laboratory synthesis of a pseudogamous triploid 'species' of the genus *Muellerianella* (Homoptera, Delphacidae). *Evolution,* 32, 916–920.

Ellenberg, H. (1963) *Vegetation Mitteleuropas mit den Alpen* (2nd edition 1974). Verlag Eugen Ulmer, Stuttgart.

Hiebsch, H., Emmerich, R. & Krause, R. (1978) Zur Fauna einiger Arthropodengruppen des Flächennaturdenkmals 'Saugartenmoor' in der Dresdener Heide. *Entomologische Abhandlungen Staatliches Museum für Tierkunde in Dresden,* 42, 212–249.

Horvat, J., Glavac, V. & Ellenberg, H. (1974) *Vegetation Südosteuropas.* Gustav Fischer Verlag, Stuttgart.

C. J. H. Booij

Kontkanen, P. (1950) Quantitative and seasonal studies on the leafhopperfauna of the fieldstratum on open areas in North Karelia. *Annales Zoologici Societas Zoologicae Botanicae Fennicae 'Vanamo'*, 13, 1–91.

Kovacs, M. (1962) *Die Moorwiesen Ungarns.* Die Vegetation Ungarischen Landschaften III. Boedapest.

Kuntze, H.A. (1937) Die Zikaden Mecklenburgs, ein faunistisch-ökologische Untersuchung. *Archiv für Naturgeschichte*, N.F. 6, 299–388.

Larsson, A. (1976) Wet meadows in southern Sweden: vegetation, succession and management (in Swedish). *Meddelanden från Avdelningen för Ekologisk Botanik, Lunds Universitet*, 31, 1–107.

Marchand, H. (1953) Die Bedeutung der Heuschrecken und Schnabelkerfe als Indikatoren verschiedener Grasslandtypen. *Beiträge zur Entomologie*, 3, 116–162.

Mitter, C., Futuyma, D.J., Schnieder, J.C. & Hare, J.D. (1979) Genetic variation and host-plant relations in a parthenogenetic moth. *Evolution*, 33, 777–790.

Mochida, O. & Okada, T. (1971) A list of the Delphacidae (Homoptera) in Japan with special reference to host-plants, transmission of plant diseases, and natural enemies. *Bulletin of the Kyushu Agricultural Experimental Station*, 15, 737–843.

Morris, M.G. (1974) Auchenorrhyncha (Hemiptera) of the Burren, with special reference to species associations of the grasslands. *Proceedings of the Royal Irish Academy*, 74, Section D, 7–30.

Mueller-Dombois, D. & Ellenberg, H. (1974) *Aims and Methods of Vegetation Ecology.* John Wiley & Sons, New York.

Passarge, H. (1964) Pflanzengesellschaften des nordostdeutschen Flachlandes I. *Pflanzensociologie*, 13.

Remane, R. (1958) Die Besiedlung von Grünlandflächen verschiedener Herkunft durch Wanzen und Zikaden im Weser-Ems Gebiet. *Zeitschrift für Angewandte Entomologie*, 42, 353–400.

Schiemenz, H. (1971) Die Zikadenfauna (Homoptera, Auchenorrhyncha) der Erzgebirgschochmoore. *Zoologisches Jahrbuch für Systematik*, 98, 397–417.

Schiemenz, H. (1975) Die Zikadenfauna der Hochmoore im Thüringer Wald und Harz (Homoptera, Auchenorrhyncha). *Faunistische Abhandlungen Staatliches Museum für Tierkunde, Dresden*, 5, 215–233.

Schiemenz, H. (1976) Die Zikadenfauna von Heide- und Hochmoore des Flachlandes der DDR. *Faunistische Abhandlungen des Staatliches Museum für Tierkunde in Dresden*, 6, 39–54.

Scholz, H. (1975) Grassland evolution in Europe. *Taxon*, 24, 81–90.

Vilbaste, J. (1974) Preliminary list of Homoptera-Cicadina of Latvia and Lithuania. *Eesti NSV Teaduste Akadeemia Toimetised*, 23, Koide Bioloogia 1974, no. 2, 131–163.

Westhoff, V., Bakker, P.A., Leeuwen, C.G. van & Voo, E.E. van der (1970, 1971, 1973) *Wilde Planten*, Pars 1, 2 and 3. Vereniging voor Natuurmonumenten, The Netherlands.

Westhoff, V. & Den Held, A.J. (1975) *Plantengemeenschappen in Nederland.* W. J. Thieme & Cie, Zutphen, The Netherlands.

36

*Department of Entomology, Agricultural University, Wageningen*

# Biosystematics of the *Muellerianella* Complex (Homoptera, Delphacidae), Interspecific and Geographic Variation in Acoustic Behaviour

By C. J. H. Booɪj

*With 13 figures*

## Abstract

Acoustic behaviour was studied in three closely related planthopper species of the genus *Muellerianella* (Homoptera, Delphacidae). In order to establish interspecific differences, which are important in reproductive isolation, behavioural interactions between males and females were observed and the associated acoustic signals were recorded and analysed. In addition, geographic variation in male calling songs of two species was studied in populations originating from different parts of Europe.

## 1. Introduction

The taxonomy of the genus *Muellerianella* (Homoptera, Delphacidae) has been obscure for a long time, because the species of this genus are very similar (Drosopoulos 1977). Investigations of the last few years showed that the genus in W. Europe consists of three bisexual species, *M. fairmairei*, *M. extrusa* and *M. brevipennis*, and a number of *triploid gynogenetic* forms (Booɪj 1981). The bisexual species are more or less specific feeders on *Holcus lanatus*, *Molinia caerulea* and *Deschampsia cespitosa* respectively. Because the ecological requirements of the hostplants overlap, the *Muellerianella* species are regularly found together in the field (Booɪj 1982). Therefore the confinement to different hostplants does not completely prevent interspecific contacts and additional mechanisms are necessary to reduce the chances of hybridization.

37

Among the premating isolating mechanisms, ethological barriers are regarded as very important in many animal species (MAYR 1970). In the reproductive behaviour of insects, sound-production often plays an important role (BUSNEL 1963). OSSIANNILSSON (1949) was the first to describe the very faint sounds produced by many small species of leafhoppers and plant-hoppers. Technical progress in recording and analysis of sounds greatly stimulated further research in this field. Acoustic signals have been described now for a great number of Auchenorrhyncha species and they appear to be very useful for the discrimination of closely related species (STRÜBING 1960, 1963, 1965, 1966, 1970; CLARIDGE and REYNOLDS 1973; SHAW et al. 1974; ICHIKAWA et al. 1975; SHAW 1976).

Preliminary investigations have shown also that the *Muellerianella* species emit characteristic signals (HOUWINK, intern. report). Therefore the acoustic behaviour associated with pair formation was further studied to establish interspecific differences and to assess intraspecific (geographic) variation. Sympatric and allopatric populations could be compared, because many *Muellerianella* strains were cultured in our laboratory.

## 2. Material and Methods

Material from the following populations was used for recording (for detailed information on populations, see BOOIJ 1981):

*Muellerianella fairmairei*

| | | | | |
|---|---|---|---|---|
| Spain 1 | Mf 049 | Orgiva, Sierra Nevada | 36° 54′ N | 2° 55′ W |
| Spain 2 | Mf 050 | Capifeira, Sierra Nevada | 36° 55′ N | 2° 53′ W |
| S. France 1 | Mf 047 | Lavalle, Pyrénées Orient. | 42° 29′ N | 3° 02′ E |
| S. France 2 | Mf 045 | Privas, Ardèche | 44° 40′ N | 4° 30′ E |
| Andorra | Mf 048 | Sant Julia de Lòria | 42° 28′ N | 1° 29′ W |
| C. France | Mf 044 | Nevers, Nièvre | 47° 00′ N | 3° 20′ W |
| Yugoslavia | Mf 057 | Bitola, Macedonia | 41° 08′ N | 21° 19′ E |
| Ireland | Mf 011 | Glengarrif, Co Cork | 51° 45′ N | 9° 34′ W |
| The Netherlands | Mf 026 | Leersum, Utrecht | 52° 00′ N | 5° 24′ E |
| The Netherlands | Mf 025 | Rhenen, Utrecht | 51° 57′ N | 5° 36′ E |
| S. Sweden | Mf 001 | Krankesjön, Skåne | 55° 42′ N | 13° 26′ E |

*Muellerianella brevipennis*

| | | | | |
|---|---|---|---|---|
| S. France | Mb 042 | Formiguères, Pyrénées Orient. | 42° 37′ N | 2° 06′ E . |
| Yugoslavia | Mb 044 | Sisak, Croatia | 45° 30′ N | 16° 28′ E |
| The Netherlands | Mb 021 | Rhenen, Utrecht | 51° 57′ N | 5° 36′ E |
| S. Sweden | Mb 001 | Krankesjön, Skåne | 55° 42′ N | 13° 26′ E |
| C. Sweden | Mb 002 | Filipstad, Värmland | 59° 46′ N | 14° 09′ E |
| N. Sweden | Mb 005 | Umeå, Västerbotten | 63° 52′ N | 20° 15′ E |
| S. Finland | Mb 010 | Näkkilä, Turku-Pori | 60° 24′ N | 23° 30′ E |
| C. Finland | Mb 009 | Alajärvi, Vaasa | 63° 00′ N | 23° 50′ E |

*Muellerianella extrusa*

| | | | | |
|---|---|---|---|---|
| The Netherlands | Me 010 | Kralo, Drente | 52° 47′ N | 6 ° 25′ E |

*Triploid gynogetic forms.* Same localities as listed under *M. fairmairei* (S. France 1, Andorra, C. France and The Netherlands). The geographic origin of the foregoing populations is indicated in Fig. 1, which also gives the area of sympatry.

In order to prevent any loss of variation due to inbreeding, animals were used which had been collected as larvae from the field or which had been bred in the greenhouse (LD 18 : 6, 20 °C) for only one or two generations. Since the *Muellerianella* species sing most actively when not mated — especially females —, sexes were separated in the fifth larval instar. Most recordings were made from animals one week after final ecdysis.

ICHIKAWA and ISHII (1974) demonstrated that sounds produced by planthoppers are mainly transmitted as substrate vibrations. Although the sounds can be detected by means of a sensitive microphone (STRÜBING 1960, 1963, 1966), it appeared that the substrate vibrations can be easily recorded using a gramophone cartridge (ICHIKAWA and ISHII 1974, and others), or by means of an accelerometer attached to the substrate.

In our studies we used both the cartridge technique (Philips GP 390 with a 22 RH 551 amplifier), and the accelerometer technique (Brüel and Kjaer 8307 with Charge amplifier Type 2625). The accelerometer was preferred because of high sensitivity to substrate vibra-
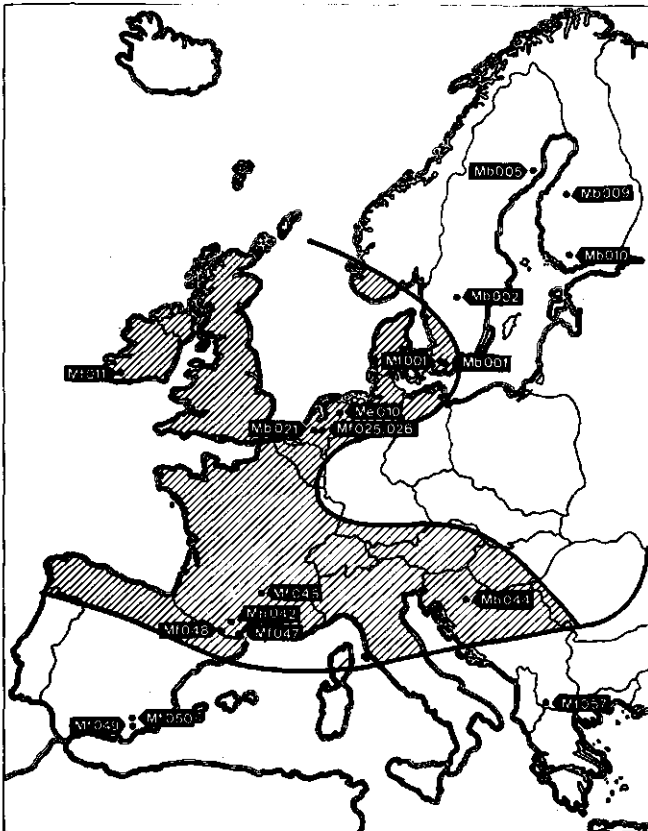


*Fig. 1:* Distribution of populations of *Muellerianella brevipennis* (Mb), *M. fairmairei* (Mf), and *M. extrusa* (Me), used for studies of acoustic behaviour. The shaded area indicates the geographic region where *M. brevipennis* and *M. fairmairei* are sympatric

tions in combination with low sensitivity to background-noise. Moreover, the frequency response of the accelerometer is linear. If necessary frequencies below 100 Hz were filtered out before or after recording using a 3 dB filter (Tektronix AM 502), especially to eliminate 50 Hz hum. The detected signals were recorded on tape (Agfa Gevaert PEM 268, tape speed 19.5 cm/s) using a tape recorder (Revox A77). Oscillograms of the recorded signals were obtained by means of an ink-jet oscillograph (Siemens Oscillomink), after playback at half tape-speed (9.75 cm/s).

All recordings were made in the laboratory at ambient temperature between 19 °C and 26 °C, measured with a simple thermometer.

For recording of calling songs, individuals were placed on a grass-stem (usually *Agrostis tenuis*), which was placed firmly in a vial with moist sand. The cartridge stylus or the accelerometer was attached to the top of the grass-stem. For recording of courtship-sounds, one virgin female was placed together with a mature male on a grass-stem. Recording was started, as soon as one of the animals started to call and recording was continued for about 3 min in case of calling, or until copulation when courtship was studied.

## 3. Terminology

Acoustic signals emitted by insects fall into a number of categories which are defined in relation to the behavioral context in which they are produced. Most terms used for acoustic behaviour in crickets and grasshoppers (ALEX-ANDER 1962, 1967; DUMORTIER 1963; MATTHEWS and MATTHEWS 1978), can be easily applied also to planthoppers. The following categories are distinguished in the present study (function given in parentheses):

*calling:* spontaneously produced sounds by single males or females (advertisement, pair-forming).

*alternate calling:* sounds produced alternately between male and female at a distance from each other. (Orientation of male to female, pair-forming.)

*courtship-sounds:* sounds emitted by males and females in close proximity to each other, engaged in precopulatory behaviour (synchronization and timing of copulatory behaviour).

*attraction-song:* signals emitted by females in response to male sounds during approach and courtship (orientation of male to female).

*copulatory sounds:* sounds emitted by males during copulation (pairmaintainance and insemination-facilitating).

*rivalry-song:* sounds emitted by males in close proximity to each other (competition for mates).

In the past, many terms have been used to describe the structure and physical properties of the enormous variety of sounds produced by animals in general (BROUGHTON 1963; ALEXANDER 1967). Several widely used terms which are not strictly defined but generally accepted, are appropriate to describe the acoustic signals produced by the *Muellerianella* species. These structural terms used in this paper are defined here as follows:

*pulse:* physically unitary sound, composed of a brief succession of sine waves (Fig. 2).

*chirp:* sound which appears unitary to the human ear and which is composed of one or several pulses (Fig. 2).

*roll:* new term, introduced to describe signals composed of one two-pulsed chirp and some extra pulses (2—13) emitted at a rather low rate (Fig. 2), which are characteristic for the *Muellerianella* species.

*trill:* succession of pulses emitted at a fast rate and which is too long to call it a chirp (Fig. 2).

*sequence:* succession of chirps or rolls delimited by pauses or other signals (Fig. 2).
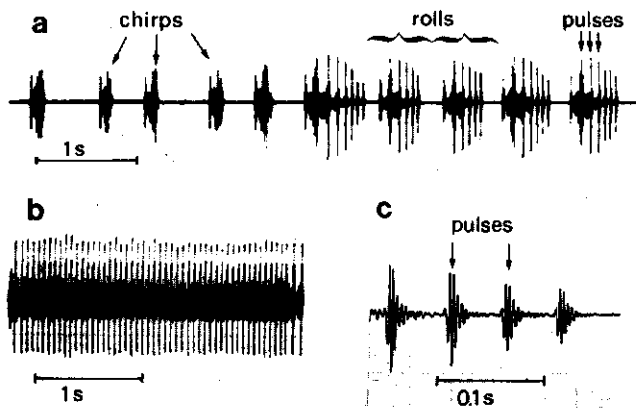


*Fig. 2:* Structural components of acoustic signals emitted by *Muellerianella* species: (a) male calling sequence with chirps and rolls, (b) trill, (c) pulses

## 4. Analysis of Song Patterns and Frequency Spectra

In order to analyse intra- and interspecific variation of calling songs, several variables were chosen which could be easily measured from the oscillograms. For each individual the characteristic variables were calculated by taking the average value measured over a number of sound-units. The following variables were determined:

rolls/sequence: average number of rolls per roll-sequence.

rolls/s: mean number of rolls/s averaged over all recorded roll-sequences.

chirps/sequence: average number of chirps emitted between roll-sequences.

pulses/roll: mean number of pulses per roll averaged over all rolls emitted by an individual.

pulses/s: (pulse repetition rate): number of pulses per second in one roll averaged over ten rolls for each individual (first rolls in roll-sequences were excluded).

Because insects can usually not emit pure tones and most insect-sounds are probably not frequency-modulated, differences in sound-patterning are thought to be more important for species recognition than frequency-spectra (MATTHEWS and MATTHEWS 1978; HASSE 1974; POPOV et al. 1975). Sounds produced by Auchenorrhyncha have broad frequency-spectra (between 100

41

and 4000 Hz) without clear dominant frequencies (Moore 1961; Strübing 1967; Fleming 1975; Purcell and Loher 1976). However, differences in frequency-spectra have been found between *Javesella* species (Strübing and Hasse 1975; de Vrĳer, pers. comm.), which might be important in species recognition, at least as a secondary factor reinforcing the specificity of the signals.

Therefore we studied frequency spectra of the male calling songs of some individuals in each species. Detailed frequency spectra were obtained by subjecting signals to a computerized Fourier-analysis.

## 5. Interspecific Differences between Male Calling Songs

Differences between male calling songs of *M. fairmairei*, *M. extrusa* and *M. brevipennis* were determined by recording and analysis of spontaneously produced sounds. For each male the patterns of 5 to 20 calling sequences were studied. In this way calling songs of 60 *M. brevipennis* males and 92 *M. fairmairei* males originating from several European populations could be characterized. For *M. extrusa* recordings of only five males from the Netherlands were studied in detail, because this species was only recently discovered (Booĳ 1981).

The calling song patterns are characteristic for each species, and can be easily distinguished by ear and from the oscillograms (Fig. 3). The most obvious differences are found in the structure of the rolls (Fig. 4). Moreover the calling song of *M. brevipennis* differs from both other species by the absence of chirps between roll-sequences.

The differences in song structure are reflected in the variables which characterize the signals (Fig. 5). The data presented in these figures give a
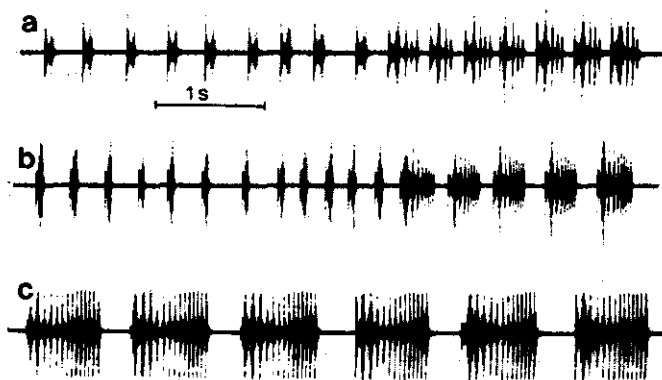


*Fig. 3:* Calling song patterns produced by males of *Muellerianella fairmairei* (a), *M. extrusa* (b) and *M. brevipennis* (c)
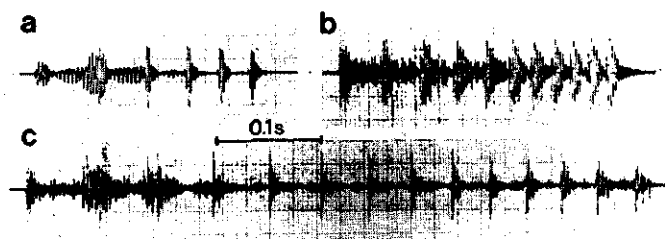
*Fig. 4:* Roll-structure in calling songs of *Muellerianella fairmairei* (a), *M.extrusa* (b), and *M.brevipennis* (c)
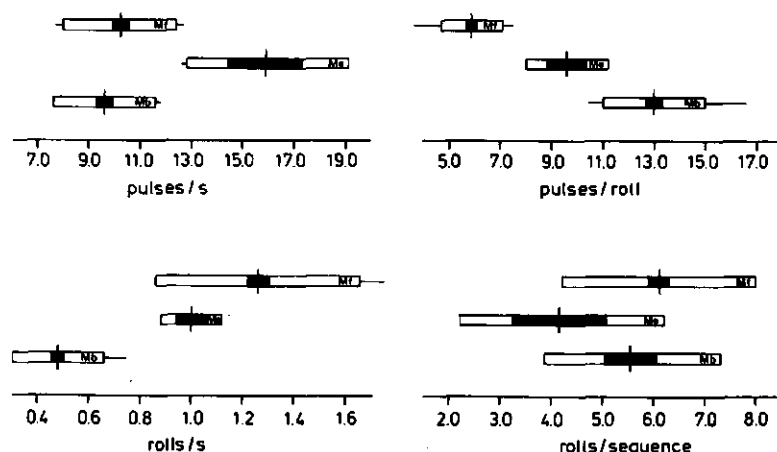


*Fig. 5:* Interspecific differences in four variables which characterize calling songs of *M. fairmairei* (Mf), *M.extrusa* (Me), and *M.brevipennis* (Mb). For each variable the mean, the range, the 95 % confidence interval (black blocks) and the standard deviation (black + open blocks) is given. The data are not corrected for temperature

good impression of the overall variation, since individuals from many populations are included and variables are not corrected for temperature. Despite this variation, interspecific differences are very clear. The most distinct difference is in the number of pulses per roll which is 5—7 in *M. fairmairei*, 8—11 in *M. extrusa* and 11—15 in *M. brevipennis*. Apart from this, the calling song of each species differs in at least one other variable from both other species.

The emission of chirps between roll-sequences in *M. extrusa* and *M. fairmairei* varies strongly between individuals. The mean number of chirps per sequence varies from 0—15 between individuals with a mean of about 5 chirps per sequence in both species. In calling of *M. brevipennis* chirps were never recorded, but they do form an important element in the courtship of this species.

With regard to the frequency spectra of the calling songs, it is difficult to draw any conclusions at the moment. During the analysis of rolls produced by nine males in each species, it appeared that the dominance of certain frequencies in the recorded signals seems to depend on the quality of the recording. Moreover some unexpected variation was found between individuals in each species. My tentative conclusion for *Muellerianella* is that the frequency spectra are not very characteristic at the species level. In all three species most individuals show one peak somewhere between 100 and 200 Hz and one, usually lower peak, between 700—1000 Hz (Fig. 6). The relative intensity of certain frequencies varies strongly between individuals. It seems likely that different frequencies are transmitted differentially by various substrates. Better experimental designs are required to clarify the specificity in frequency spectra and their contribution to species recognition.
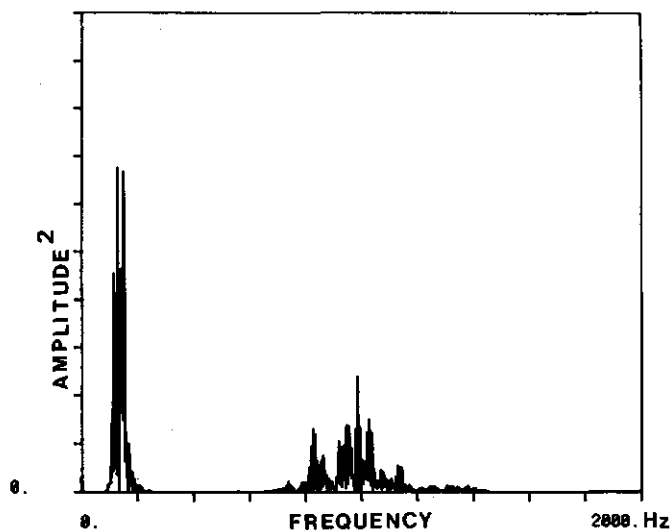


*Fig. 6:* Representative example of a frequency spectrum of a male calling signal, as it is produced by *Muellerianella* species. The spectrum is based on a computerized Fourier analysis.

## 6. Geographic Variation in Male Calling Song Structure

Geographic variation in acoustic behaviour was studied by structural comparison of male calling songs in a number of remote populations of *M. brevipennis* and *M. fairmairei*. For each population recordings of 6—15 males were analysed by methods described earlier. Because temperature during recording varied between 19 °C and 26 °C, and it is known that temperature can strongly affect sound-characteristics in insects (DUMORTIER 1963; VON HELVERSEN 1972; and McINTYRE 1977), the measured variables had to be corrected. Therefore the obtained data were pooled for each species and the

effect of temperature on each variable was determined by regression analysis. As shown in Fig. 7, the influence of temperature can be rather strong. This is especially true for the number of rolls/s and the number of pulses/s, but the other variables were affected too. To eliminate these effects, the measured variables were corrected to 20 °C using the regression coëfficient from the pooled data.
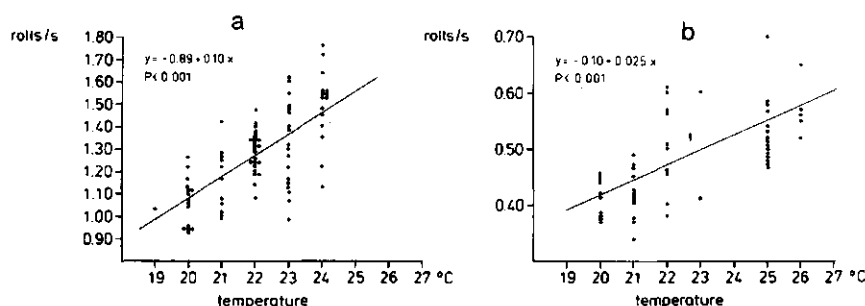


*Fig. 7:* Influence of temperature on the number of rolls/s in male calling songs of *Muellerianella fairmairei* (a) and *M. brevipennis* (b)

From the corrected data, means, standard deviations, 95 % confidence intervals and ranges of the variables were calculated for each population. These values are presented in Fig. 8. By means of analysis of variance, it appeared that significant geographic differences exist with regard to three out of four variables in both species (Table 1). However, the differences between populations are small compared with those between the species. Moreover, most of the variation is rather irregular and without any geographic trend. Only for the number of rolls/s and for the number of pulses/roll a trend is apparent from SW to NE Europe in *M. brevipennis*, leaving out the Yugoslavian population (Fig. 8).

*Table 1:* Results of a one-way analysis of variance to test differences in calling song variables between populations of *M. brevipennis* and *M. fairmairei* from different parts of Europe

|  | M. brevipennis | | M. fairmairei | |
|---|---|---|---|---|
| rolls / sequence | N = 6 | n = 47 | N = 8 | n = 80 |
| rolls / sequence | F = 1.37 | P > 0.05 | F = 1.25 | P > 0.05 |
| rolls / s | F = 2.44 | P < 0.05 | F = 12.48 | P < 0.001 |
| pulses / roll | F = 11.63 | P < 0.001 | F = 9.14 | P < 0.001 |
| pulses / s | F = 3.86 | P < 0.01 | F = 7.94 | P < 0.001 |

N = number of populations; n = number of individuals.

When the variables are compared from regions where *M. fairmairei* and *M. brevipennis* are sympatric with those where they are allopatric, there is some evidence for character displacement. The number of rolls/s and the number of pulses/s in the allopatric northern populations of *M. brevipennis*
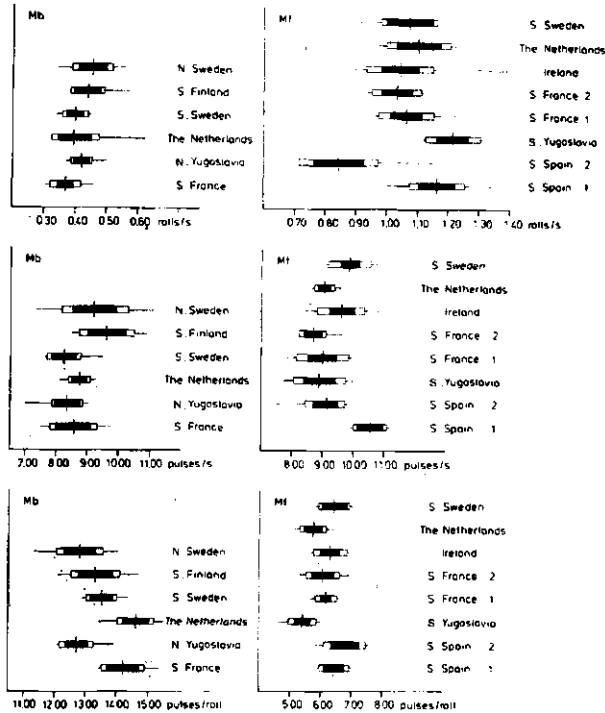
*Fig. 8:* Differences in variables characterizing male calling songs in remote populations of *Muellerianella brevipennis* (Mb) and *M. fairmairei* (Mf). Populations are arranged in latitudinal order. All data are corrected for temperature. Symbols as in Fig. 5

*Table 2:* Differences in male calling song variables between populations of *M. brevipennis* from regions where they are allopatric or sympatric with *M. fairmairei*. The same variables are given for *M. fairmairei* from the area of sympatry to show evidence for character displacement. For each parameter confidence intervals (95 %) are given

|  | M. brevipennis sympatric N = 4 | M. brevipennis allopatric N = 2 | significance of difference | M. fairmairei sympatric N = 5 |
|---|---|---|---|---|
| rolls / s | 0.40 ± 0.02  (n = 36) | 0.45 ± 0.03  (n = 18) | p < 0.01 | 1.06 ± 0.03  (n = 54) |
| pulses / s | 8.48 ± 0.20  (n = 36) | 9.37 ± 0.50  (n = 18) | p < 0.01 | 9.23 ± 0.20  (n = 54) |

N = number of populations; n = number of individuals; p values are two-tailed.

is higher than in populations from sympatric regions (Table 2). Both differences are significant (t-test, two-tailed, p < 0.01). Regarding the allopatric populations of *M. fairmairei* in S. Europe (Spain 1, Spain 2 and Yugoslavia), there is no evidence for reinforcement of differences, but the parameters in these populations vary in an irregular manner. The increased variation in these allopatric populations could be interpreted as "ethological release". Because no other *Muellerianella* species are present in these regions, selection on highly

specific sounds may be less intense. Another indication for decreased specificity in allopatric *M. fairmairei* populations is that males from these populations incorporate on average less chirps in their calling songs ($\bar{x} = 3.2$ chirps/sequence) than males from sympatric populations ($\bar{x} = 6.5$ chirps/sequence), although this difference is not significant.

## 7. Calling Songs of Females

In many insects the ability to discriminate between potential mates is less developed in males than in females (MAYR 1970; OTTE 1974; BLUM and BLUM 1979). The usual explanation for this phenomenon is that females usually mate only once and invest much more energy per offspring than males, which have the ability to mate many times. Therefore acoustic signals which are important in species recognition are mostly less differentiated in females than in males. The same holds for the acoustic signals emitted by females of the *Muellerianella* species. The spontaneous female calling songs in all three species consist of simple pulse-series of which the length and the intensity vary considerably between individuals. Similar pulse-series are characteristic for many delphacid species (STRÜBING 1960; ICHIKAWA and ISHII 1974; ICHIKAWA et al. 1975).

Females of the *Muellerianella* species do not very often sing spontaneously. Consequently the number of recordings which could be analysed is rather low. For each species the average value and the variation of each variable measured is given in Table 3. As can be seen in this table the ranges of the parameters strongly overlap between the species due to much variation. Although the calling songs cannot be reliably distinguished by the parameters used, some general interspecific differences can be indicated. These differences are apparent in some selected oscillograms given in Fig. 9. *Triploid gynogenetic* females and females of *M. fairmairei* mostly emit relatively short pulse-series (4—7 pulses/series). The pulse-series of *M. brevipennis* are often (but not always) longer (5—12 pulses/series). Another typical feature for *M. brevipennis* females is the occurrence of irregular pulses between the pulse-series and a variable intensity (Fig. 9e). Spontaneous calling of *M. extrusa* females seems to be rare. The only recording analysed showed a rather high pulse rate and long pulse-series.

*Table 3:* Interspecific differences in sound variables of female calling songs of the *Muellerianella* species and forms

| | rolls / s | | | pulses / pulse series | | | pulses / s | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\bar{x}$ | $s_x$ | n | $\bar{x}$ | $s_x$ | n | $\bar{x}$ | $s_x$ | n |
| Triploid forms | 1.48 ± 0.31 | | (11) | 4.48 ± 0.66 | | (11) | 12.04 ± 1.18 | | (11) |
| M. fairmairei | 1.35 ± 0.15 | | (6) | 4.42 ± 1.06 | | (6) | 11.20 ± 1.42 | | (6) |
| M. brevipennis | 0.75 ± 0.50 | | (6) | 7.00 ± 2.61 | | (6) | 10.70 ± 2.20 | | (6) |
| M. extrusa | 1.31 | | (1) | 6.94 | | (1) | 16.60 | | (1) |

$\bar{x}$ = mean; $s_x$ = standard deviation; n = number of individuals.
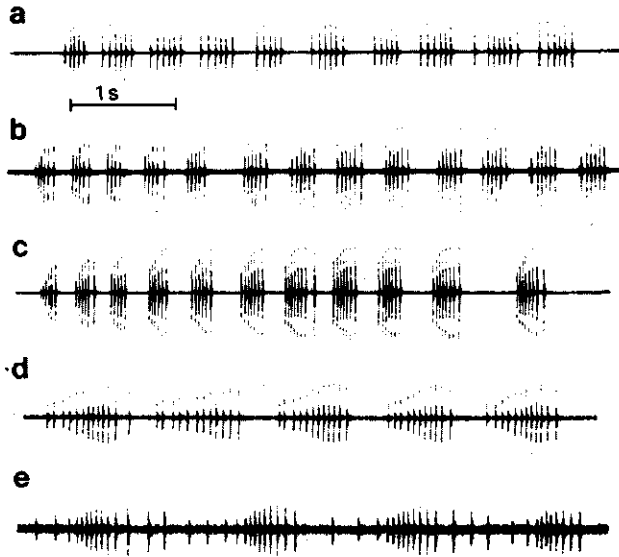
*Fig. 9:* Female calling song patterns of *Muellerianella fairmairei* (a),
*triploid gynogenetic* forms (b), *M. extrusa* (c), and *M. brevipennis* (d, e)

## 8. Sexual Behaviour Patterns

Strictly speaking calling behaviour, as discussed in sections 5—7, can be regarded as sexual behaviour since it functions in pairformation. In this section, however, the term sexual behaviour will be defined as those activities in which both females and males are engaged and which lead as a rule to copulation. It begins when the female responds to the calling male (or vice versa) and it ends with insemination.

For reasons given above, males usually show more distinct and well developed courtship behaviour than females. The sounds emitted by *Muellerianella* females during sexual interaction are very similar to spontaneous calling and all variation may be regarded as variation of pulse repetition rate or pulses/pulse-series. In order to prevent confusion with calling, these signals are called attraction-song here. Males, however, are very active in sexual behaviour and produce a variety of sounds associated with different actions.

The behavioral sequence which results in copulation can be divided into three phases: a) distant communication between potential mates. b) orientation and approach of male to female (searching behaviour). c) courtship in the strict sense.

Although the exact course of this sequence depends on several factors, e.g. the ease with which the male finds the female and the receptive state of the female, a general basic pattern can be distinguished in all three *Muellerianella* species (Fig. 10). The behavioral sequence is started when one animal
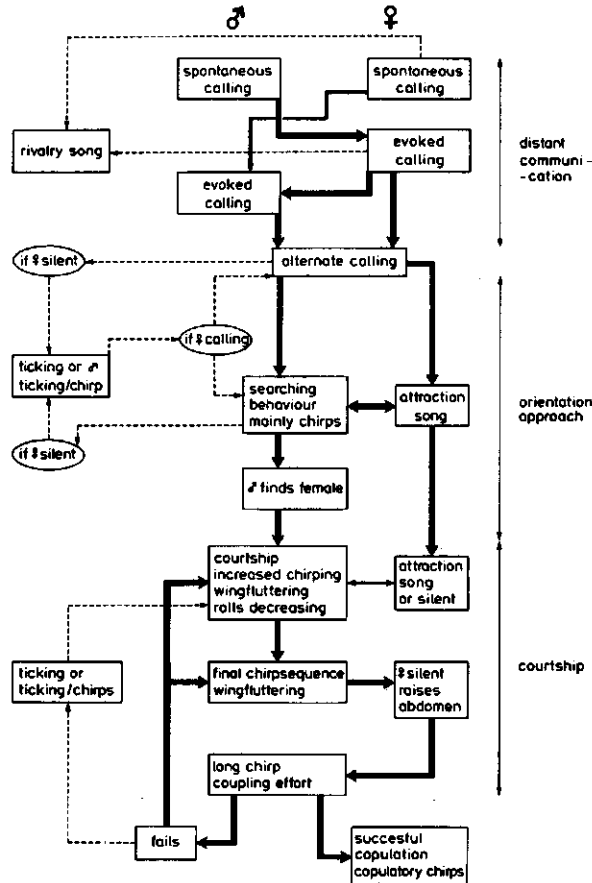
*Fig. 10:* Sexual behaviour patterns of the *Muellerianella* species. The left part of the figure refers to male behaviour, the right part to female behaviour. Continuous arrows indicate basic patterns found in all three species. Dashed arrows indicate alternative behavioural pathways performed by *M. brevipennis* only. Blocks indicate behavioural or acoustic elements, ellipses indicate conditions for certain behavioural responses

(usually the female) responds to a sound emitted by an animal of the opposite sex (usually calling of a male). Thereafter male and female call alternately (Fig. 11a) for a short while before the male starts searching. During searching it orientates itself towards the calling female and emits mainly chirps which are synchronously answered by the female (Fig. 11 b). At this stage the signals emitted by the female are often very short (1—4 pulses/pulse-series).

As soon as the male has found the female, courtship in the strict sense starts. The courtship sounds produced by the *Muellerianella* males differ distinctly from the calling songs with regard to their intensity and the relative importance of chirps to rolls (Table 4). In general the number of chirps in-

49

creases during courtship, whereas the number of rolls decreases. In this way typical courtship patterns are formed by long chirp-sequences alternated by a few or single rolls (Fig. 11c). In *M. brevipennis* these rolls are often much shorter than in calling.

When the male is intensively courting the female, it raises its wings at each chirp. This wing fluttering behaviour seems to be widespread in sexual behaviour of leaf- and planthoppers (STRÜBING 1960; FLEMING 1975; ICHI-KAWA 1976; PURCELL and LOHER 1976; SHAW 1976).
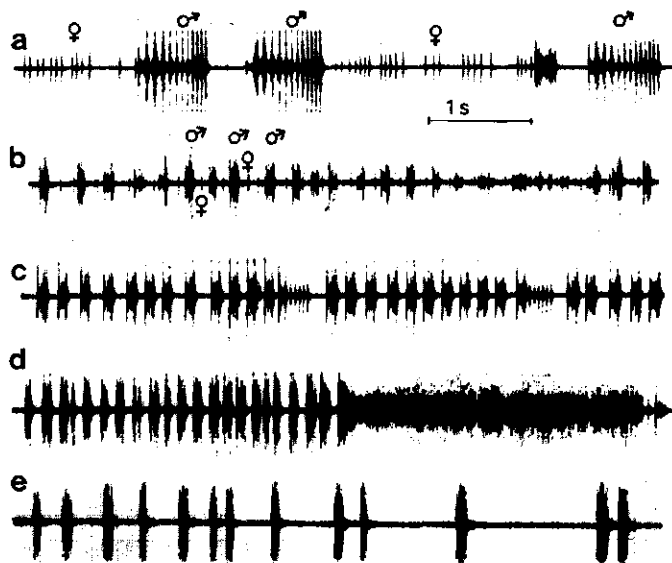


*Fig. 11:* Acoustic signals associated with sexual behaviour (courtship) of the *Muellerianella* species. a: alternative calling between males and females (*M. brevipennis*), b: acoustic signals during approach phase, c: chirp sequences altered by single rolls (*M. fairmairei*) during intensive courtship, d: final chirp-sequence just before copulation which ends in a long chirp emitted at coupling efforts, e: chirps emitted during copulation

The final part of courtship consists of a long chirp-sequence (with wing-fluttering) which ends in a long chirp (Fig. 11 d). At this moment the male turns its position and bends its abdomen under the raised abdomen of the female to couple genitalia. During copulation distinct copulatory sounds are emitted by the male (Fig. 11e). In all *Muellerianella* species the duration of the copulation is rather long (20—60 min), compared with other delphacid species like *Javesella* and *Ribautodelphax* which copulate for only a few s to a few min (DE VRIJER and DEN BIEMAN, pers. comm.).

The described basic pattern of sexual behaviour is performed in a comparable manner in each *Muellerianella* species, and no significant differences are found except for the structure of the rolls which is similar to the differences found in calling.

However, apart from the usual pattern some distinct behavioral elements were observed which do not occur in all three species. First there are the double chirps with three instead of two pulses (Fig. 12 b). These double chirps are regularly produced by courting *M. brevipennis* males (Table 4). Comparable but structurally different double chirps are emitted by *M. fairmairei* during courtship.

*Table 4:* The presence of different acoustic signals in courtship of the *Muellerianella* species. Figures indicate the number of recordings in which certain signals were found

| signals | M. brevipennis | M. extrusa | M. fairmairei |
|---|---|---|---|
| chirp-sequences alternated by one or two rolls | 29 | 4 | 5 |
| double chirps | 15 | 1 | 8 |
| rivalry song | 14 | 4 | 1 |
| ticking or tick / chirp | 18 | - | - |
| total number of recordings | 31 | 5 | 13 |

Furthermore males of *M. brevipennis* and *M. extrusa* often emit signals resembling rivalry song, during first stages of sexual interaction (Table 4). This type of sound, which is usually emitted among competing males, is produced here in reaction to female calling song (Fig. 12 a). Finally some acoustic signals are only produced by males of *M. brevipennis*, namely "ticking" and "tick-chirp" sounds. These sounds are characterized by a very regular emission of weak pulses which can be alternated by a special kind of chirps (Figs. 12 c and 12 d). This behaviour is shown when the normal behaviour sequence is interrupted or when copulation efforts fail. Courtship interruption sounds
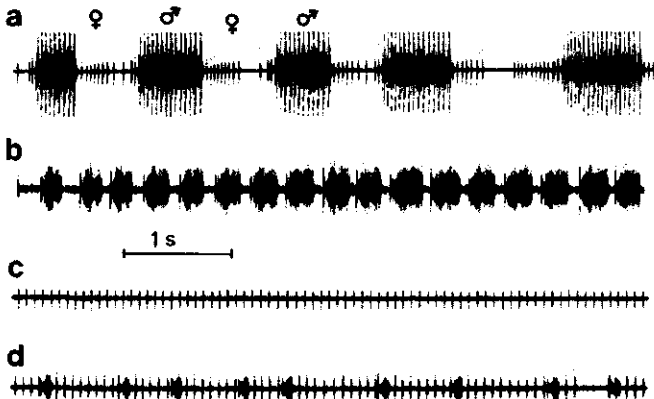


*Fig. 12:* Acoustic signals produced specifically by *M. brevipennis* during courtship. a: rivalry-song like signal produced in response to female attraction song, b: double chirps, c: ticking, d: tick-chirp signal

have also been reported in grasshoppers (ALEXANDER 1962). The complicated interactions and patterns which may occur in the sexual behaviour of the *Muellerianella* species are illustrated in Fig. 10.

From the foregoing it might be concluded that the acoustic behaviour of *M. fairmairei* and *M. extrusa* can be regarded as a transformation of the calling songs with addition of some elements. The courtship behaviour of *M. brevipennis* is more different from its calling song. Chirps are not present in its calling song but they prevail during courtship. Moreover, several additional acoustic signals are produced by this species.

## 9. Rivalry Songs

When *Muellerianella* males are near each other and one is calling or courting a female, the other male often responds by emitting a loud trill which can be sustained for a long time. In turn, the first male answers with similar trills. This mutual emission of so called rivalry-song is often continued until one male gives up or walks away. The pattern of these signals is clearly distinct from other signals produced by males. Rivalry songs have been described for several other insect species like grasshoppers (ALEXANDER 1967), crickets (HILL et al. 1972), and leafhoppers (STRÜBING 1970).

Characteristic patterns of rivalry songs as they are emitted by the *Muellerianella* species are given in Fig. 13. It can be seen that these signals are
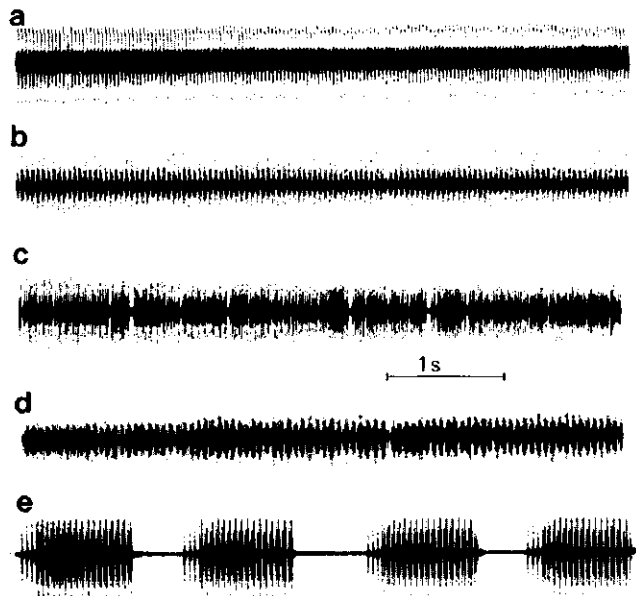


*Fig. 13:* Rivalry songs emitted among males of *M. fairmairei* (a), *M. extrusa* (b), and *M. brevipennis* (c, d, e)

only little differentiated between the species. This might be explained by the fact that agonistic signals do not play any role in reproductive isolation (OTTE 1974). It is not known whether these signals are also used between males of different species.

Although in *M. extrusa* and *M. fairmairei* only one kind of rivalry song was recorded, *M. brevipennis* males produce at least three kinds of rivalry songs (Figs. 13 c, d, e) of which one is also emitted by single males, especially during early stages of sexual behaviour (see previous section). The latter type is differentiated from the other rivalry songs by interruptions and regular patterning (Fig. 13 e). Such an incorporation of aggressive elements in sexual behaviour has also been reported in other insects (DUMORTIER 1963; OTTE 1974).

## 10. Discussion

The present study revealed clear differences in acoustic behaviour between the *Muellerianella* species. As in other Auchenorrhyncha the acoustic signals of males are more differentiated and characteristic than those of females. This agrees well with theories on sexual selection, which predict greater divergence between male signals because females usually discriminate between males, and males are less selective (MATTHEWS and MATTHEWS 1978; OTTE 1974; BLUM and BLUM 1979).

The species are better characterized by their calling songs than by morphological differences, which are very slight (BOOIJ 1981). Actually the specific status of *M. extrusa* was based primarily on its acoustic behaviour, its hostplant and its phenology (BOOIJ 1982). This illustrates the value of non-morphological characters in the systematics of complex species groups.

It is likely that the observed differences in acoustic behaviour are important in reproductive isolation between the *Muellerianella* species. From mating experiments and hybridization studies we know that interspecific matings regularly occur in single choice situations, but the number and rate of inseminations is certainly much lower in interspecific than in intraspecific interactions (DROSOPOULOS, pers. comm., BOOIJ, in prep.). The relative contribution of different behavioral elements (calling, approach and courtship) to reproductive isolation, however, should be further studied. Although the small differences in female signals are probably of minor importance for reproductive isolation, it is desirable to determine the response of males to different female signals, because discriminating abilities of males have also been reported in other planthoppers (ICHIKAWA 1976) and leafhoppers (STRÜBING 1966).

Comparative studies on acoustic behaviour of related taxa might provide insights to their phylogenetic relationships. In the literature general trends have been suggested from simple calling songs in primitive genera, to more complicated acoustic behaviour in more advanced genera (ALEXANDER 1962; STRÜBING 1960). In a similar way information on acoustic behaviour might be used to study phylogenetic relations between closely related species.

With regard to *Muellerianella*, the general similarity in acoustic behaviour between *M. fairmairei* and *M. extrusa* suggests that these species are very

53

closely related and that their divergence is of rather recent origin. *M. brevipennis* is acoustically most differentiated. Similar taxonomic relations have also been suggested from morphological studies (Booij 1981).

The calling songs of *M. extrusa* and *M. fairmairei* both consist of roll-sequences alternated by chirp-sequences, whereas *M. brevipennis* produces only roll-sequences. Since a bipartite structure of calling-songs (short chirps and longer rolls or buzzes) occurs in several other genera related to *Muellerianella* (Strübing 1960), the patterns found in *M. extrusa* and *M. fairmairei* might be regarded as original. The complicated sexual behaviour of *M. brevipennis*, and the general acoustic differences from both other species, indicate that this species split rather long ago from an *M. extrusa*- or *M. fairmairei*-like ancestor species. The absence of chirps in the calling of *M. brevipennis* could be explained by reduction.

It is possible that the geographic variation in male calling songs presented in this paper is influenced by interactions between the species where their distributions overlap. The present sympatry between *M. fairmairei* and both other species probably dates back not more than a few thousand years (Booij 1981, 1982). If the interspecific differences were small before the species made secondary contact, one would expect reinforcement of differences (character displacement) in the area of sympatry (Brown and Wilson 1956; Mayr 1970). However there is not much evidence for character displacement in calling songs between *M. brevipennis* and *M. fairmairei*. This might indicate that their acoustic differences were already large enough to prevent hybridization at the moment of secondary contact.

On the other hand it is remarkable that no clear instances of character-displacement have been found at all in insects (Walker 1974), although some species have been extensively studied (e.g. Hill et al. 1972). The same holds for birds (Thielcke 1969) which have been even more extensively studied. The only convincing examples for acoustic character displacement were found in tree-frogs of the genus *Hyla* (Littlejohn 1965; Snyder and Jameson 1965; Ralin 1977).

There are several reasons why character displacement in acoustic behaviour does not often occur, or, at least why it is difficult to demonstrate. One reason may be that — in case of allopatric speciation — the acoustic signals diverge sufficiently before the species become sympatric. Another reason could be that, when species become sympatric, selection on ecological divergence will be stronger than on ethological divergence. The reason for this is that ecological isolation is a more efficient mechanism (in terms of time and energy) to prevent hybridization than ethological isolation. Moreover because it is important to reduce the probably severe competition between the weakly diverged species, selection on ecological differentiation will be strong. Quickly established ecological differences may prevent hybridization sufficiently and selection on behavioural divergence will be less intense afterwards.

The third reason might be that geographic variation patterns built up due to character displacement in sympatric areas, disappear rather rapidly because of gene flow. This will be the case when there is no counter-selection in the allopatric regions which stabilize existing patterns.

Finally it is not only a matter of absolute differences which accounts for reproductive isolation. Mating errors can also be prevented by reducing the variability of acoustic signals and increasing the selectivity of the females. In that case character displacement finds its expression in a reduced variability of calling songs in the area of sympatry. The high variation of male calling songs of *M. fairmairei* in allopatric populations, which I termed "ethological release", compared with the relative constancy in sympatric populations, might be explained in this way.

Unfortunately we have not been able to study geographic variation in calling songs of *M. extrusa*. Comparison of this species with *M. fairmairei* would be highly interesting, because these species are more closely related than *M. fairmairei* and *M. brevipennis*, their ranges widely overlap, and they can be easily hybridized in the laboratory (BOOIJ, in prep.). Selection probably favours divergence between these species because the differences in acoustic behaviour are still very small and do not prevent interspecific mating.

## Summary

Sounds emitted by males and females of three closely related plant-hopper species of the genus *Muellerianella* were recorded and analysed. The species are characterized by clear differences in the structure of male calling songs, which are supposed to be important in reproductive isolation. Female signals appear to be less distinct.

Courtship songs of males differ from calling-songs mainly with regard to intensity and the relative importance of different sound elements. The court-ship of *M. brevipennis*, in which several additional sounds are produced, appeared to be most complex. Males of all species produce comparable rivalry songs during aggressive interactions.

The differences and similarities in acoustic behaviour between the species suggest the same phylogenetic relations as those proposed earlier on morpho-logical grounds.

Finally some geographic variation was found in the structure of male calling songs of *M. brevipennis* and *M. fairmairei* originating from remote European populations. Character-displacement could not be convincingly shown, but the reduced variation of calling songs of *M. fairmairei* in the area of sympatry may be due to the interaction with *M. brevipennis*.

## Zusammenfassung

Die Laute der Männchen und Weibchen dreier verwandter Zikadenarten der Gattung *Muellerianella* wurden aufgenommen und analysiert. Die Arten lassen sich leicht an der Struktur des Rufgesanges der Männchen unterscheiden. Diese Rufgesänge sind wahrscheinlich wichtig für die reproduktive Isolierung der Arten.

Der Werbegesang der *Muellerianella*-Männchen unterscheidet sich von dem Rufgesang durch erhöhte Intensität und durch eine Verschiebung im relativen Anteil verschiedener Lautelemente. Der Werbegesang der *M. brevipennis*-Männchen ist wesentlich komplizierter wegen mehrerer zusätzlicher Lautelemente. Männchen aller Arten erzeugen während Auseinandersetzungen vergleichbare Rivalengesänge.

Die Differenzen und Ähnlichkeiten im akustischen Verhalten führen zur Annahme derselben phylogenetischen Beziehungen zwischen den Arten wie früher morphologische Befunde.

Die Rufgesänge von *M. brevipennis* und *M. fairmairei*, Abkömmlinge abgelegener europäischer Populationen, variieren geographisch. Obwohl die Unterschiede im Gebiet der Sympatrie nicht deutlich größer sind als außerhalb, weist die verminderte Variabilität im Rufgesang von *M. fairmairei* im Gebiet der Sympatrie auf eine mögliche Interaktion mit *M. brevipennis*.

## Acknowledgements

## Literature Cited

ALEXANDER, R. D. (1962): The role of behavior study in cricket classification. Syst. Zool. 11, 53—72 • ALEXANDER, R. D. (1967): Acoustical communication in arthropods. Ann. Rev. Ent. 12, 495—526.

BLUM, M. S., and N. A. BLUM (eds.) (1979): Sexual Selection and Reproductive Competition in Insects. Acad. Press, New York, San Francisco, London • BOOIJ, C. J. H. (1981): Biosystematics of the *Muellerianella* complex (Homoptera, Delphacidae), taxonomy, morphology and distribution. Neth. J. Zool. 31, 572—595, in press • BOOIJ, C. J. H. (1982): Biosystematics of the *Muellerianella* complex (Homoptera, Delphacidae), hostplants, habitat, and phenology. Ecol. Entom. 7, in press • BROUGHTON, W. B. (1963): Method in bioacoustic terminology. In: Acoustic Behavior of Animals. (BUSNEL, R. G., ed.) Elsevier, Amsterdam, London, New York • BROWN, W. L., and E. O. WILSON (1956): Character displacement. Syst. Zool. 5, 49—64 • BUSNEL, R. G. (ed.) (1963): Acoustic Behavior of Animals. Elsevier, Amsterdam, London, New York.

CLARIDGE, M. F., and W. J. REYNOLDS (1973): Male courtship songs and sibling species in the *Oncopsis flavicollis* species group (Hemiptera, Cicadellidae). J. Entomol. 42, 29—39.

DROSOPOULOS, S. (1977): Biosystematic studies on the *Muellerianella* complex (Delphacidae, Homoptera, Auchenorrhyncha). Meded. Landbouwhogeschool Wageningen 77, 1—133 • DUMORTIER, B. (1963): Ethological and physiological study of sound emissions in Arthropods.

In: Acoustic Behaviour of Animals. (BUSNEL, R. G., ed.) Elsevier, Amsterdam, London, New York.

FLEMING, C. A. (1975): Acoustic behaviour as a generic character in New Zealand Cicadas (Hemiptera; Homoptera). J. Roy. Soc. New Zealand 5, 47—64.

HASSE, A. (1974): Lauterzeugungsmechanismus und Lautrezeption von Kleinzikaden. Naturwissenschaften 61, 81—82 • HELVERSEN, D. VON (1972): Gesang des Männchens und Lautschema des Weibchens bei der Feldheuschrecke *Chorthippus biguttulus* (Orthoptera, Acrididae). J. Comp. Physiol. 81, 381—422 • HILL, K. G., J. J. LOFTUS-HILLS and D. F. GARTSIDE (1972): Premating isolation between the Australian fieldcrickets *Teleogryllus commodus* and *T. oceanicus* (Orthoptera: Gryllidae). Austral. J. Zool. 20, 153—163.

ICHIKAWA, T. (1976): Mutual communication by substrate vibrations in the mating behavior of planthoppers (Homoptera: Delphacidae). Appl. Ent. Zool. 11, 8—21 • ICHIKAWA, T., and S. ISHII (1974): Mating signal of the brown planthopper, *Nilaparvata lugens* Stål (Homoptera: Delphacidae): vibration of the substrate. Appl. Ent. Zool. 9, 196—198 • ICHIKAWA, T., M. SAKUMA and S. ISHII (1975): Substrate vibrations: mating signals of three species of planthoppers which attack the rice plant. Appl. Ent. Zool. 10, 162—171.

LITTLEJOHN, M. J. (1965): Premating isolation in the *Hyla ewingi* complex (Anura: Hylidae). Evolution 19, 234—243.

MATTHEWS, R. W., and J. R. MATTHEWS (1978): Insect Behavior. John Wiley & Sons, New York, Chicester, Brisbane, London • MAYR, E. (1970): Populations, Species, and Evolution. Belknap Press of Harvard Univ. Press, Cambridge, Mass., London. 2nd ed. 1970 • McINTYRE, M. (1977): Acoustical communication in the field crickets *Pteromobius nibrovus* and *P. bigelowi* (Orthoptera: Gryllidae). New Zealand J. of Zool. 4, 63—72 • MOORE, T. E. (1961): Audiospectrographic analysis of sounds of Hemiptera and Homoptera. Ann. Ent. Soc. Am. 54, 273—291.

OSSIANNILSSON, F. (1949): Insect drummers. Opusc. Ent. Suppl. X, 1—145 • OTTE, D. (1974): Effects and functions in the evolution of signalling systems. Ann. Rev. Ecol. Syst. 5, 385—417.

POPOV, A. V., V. F. SHUVALOV, I. D. SVETLOGORSKAYA and A. M. MARKOVICH (1975): Acoustic behaviour and auditory systems in insects. Rheinland-Westfalen, Akad. Wiss. 53, 281—306 • PURCELL, A. H., and W. LOHER (1976): Acoustical and mating behavior of two taxa in the *Macrosteles fascifrons* species complex. Ann. Ent. Soc. Am. 69, 513—518.

RALIN, D. B. (1977): Evolutionary aspects of mating call variation in a diploid-tetraploid species complex of treefrogs (Anura). Evolution 31, 721—736.

SHAW, K. C. (1976): Sounds and associated behavior of *Agallia constricta* and *Agalliopsis novella* (Homoptera: Auchenorrhyncha: Cicadellidae). J. Kans. Ent. Soc. 49, 1—17 • SHAW, K. C., A. VARGO and O. V. CARLSON (1974): Sounds and associated behavior of some species of *Empoasca* (Homoptera: Cicadellidae). J. Kans. Ent. Soc. 47, 284—307 • SNYDER, W. F., and D. L. JAMESON (1965): Multivariate geographic variation of mating call in populations of the Pacific tree frog (*Hyla regilla*). Copeia 1965, 129—142 • STRÜBING, H. (1960): Paarungsverhalten und Lautäußerung von Kleinzikaden, demonstriert an Beispielen aus der Familie der Delphacidae (Homoptera—Auchenorrhyncha). Verh. XI. Int. Kongr. Entomol., Wien, 3, 12—14 • STRÜBING, H. (1963): Lautäußerungen von *Euscelis*-Bastarden (Homoptera—Auchenorrhyncha). Verh. Dtsch. Zool. Ges. Münster 1963, 268—281 • STRÜBING, H. (1965): Das Lautverhalten von *Euscelis plebejus* Fall. und *Euscelis ohausi* Wagn. (Homoptera—Cicadinae). Zool. Beitr. N. F. 11, 289—341 • STRÜBING, H. (1966): Ein Vergleich von Lautäußerungen verschiedener *Euscelis*-Arten (Homoptera—Cicadina). Dtsch. Entomol. Z. N. F. 13, 351—358 • STRÜBING, H. (1967): Zur Untersuchungsmethodik der Lautäußerungen von Kleinzikaden (Homoptera—Cicadina). Zool. Beitr. 13, 264—284 • STRÜBING, H. (1970): Zur Artberechtigung von *Euscelis alsius* Ribaut gegenüber *Euscelis plebejus* Fall. (Homoptera—Cicadina). Ein Beitrag zur Neuen Systematik. Zool. Beitr. 16, 441—478 •

57

STRÜBING, H., and A. HASSE (1975): Ein Beitrag zur Neuen Systematik — demonstriert am Beispiel zweier *Javesella*-Arten (Homoptera—Cicadina: Delphacidae). Zool. Beitr. **21**, 517—543.

THIELCKE, G. (1969): Geographic variation in bird vocalizations. In: Bird Vocalizations. (R. HINDE, ed.) Cambridge Univ. Press.

WALKER, T. J. (1974): Character displacement and acoustic insects. Am. Zool. **14**, 1137—1150.

Author's address: C. J. H. BOOIJ, Agricultural University, Department of Entomology, Binnenhaven 7, 6709 PD Wageningen, The Netherlands.

# Biosystematics of the Muellerianella complex (Homoptera, Delphacidae), hybridization studies

C. J. H. Booij

*Department of Entomology, Agricultural University, Binnenhaven 7, 6709 PD Wageningen, The Netherlands*

## Abstract

Crosses were made between three closely related species of the planthopper genus *Muellerianella, M. brevipennis, M. extrusa* and *M. fairmairei*. Most hybrids are produced in crosses between *M. extrusa* and *M. fairmairei*. Most of the hybrid females in these crosses and some of the hybrid males are fertile and can be successfully backcrossed with the parental species. Crosses of *M. brevipennis* with either *M. fairmairei* or *M. extrusa* yield less progeny, of which all males are sterile. Backcrosses of the hybrid females with the parental species are partly successful. The possible evolutionary consequences of mating readiness and interfertility between the three species under field conditions are discussed. Efforts to resynthesize triploid gynogenetic forms by backcrossing hybrid females of *M. fairmairei* and *M. brevipennis* with males of *M. fairmairei*, as was reported by Drosopoulos (Evolution 32: 916–920, 1978), failed. To produce offspring, the existing gynogenetic forms have to mate with males of one of the three bisexual species.

## Introduction

In Western Europe the genus *Muellerianella* is represented by three closely related bisexual species and a number of triploid forms which reproduce by gynogenesis (pseudogamy). The taxonomy of the species, which are morphologically difficult to distinguish, has recently been revised (Booij, 1981). Further studies have shown that the species differ with regard to host-plant, habitat and phenology (Drosopoulos, 1977; Booij, 1982a) and in acoustic behaviour (Booij, 1982b). Due to these ecological and ethological differences, one may expect that the species are reproductively isolated under natural conditions. However, in certain situations the isolating mechanisms break down, possibly resulting in hybridization. In the field, the ecological isolation between the species is not complete, because their host plants have overlapping habitat requirements and the species have regularly been found together (Booij, 1982a). Preliminary studies of

Drosopoulos (1977) showed that *M. fairmairei* and *M. brevipennis* mate rather easily in the laboratory, in spite of ethological differences. Since the sexual behaviour patterns of *M. extrusa* and *M. fairmairei* are even more similar, we expected these species to mate even more readily.

The subject of this paper is to describe the possible consequences of failing premating isolating mechanisms. If the species would successfully hybridize, this might lead to introgression or eventually to the origin of new hybrid forms (White, 1963). The arisal of new forms is particularly interesting in this context, since a hybrid origin was suggested for the triploid gynogenetic *Muellerianella* forms by Drosopoulos (1976, 1978). His conclusion was based on one cross in which triploid females were obtained by backcrossing female hybrids of *M. fairmairei* and *M. brevipennis* with males of *M. fairmairei*. One of our aims was to confirm the synthesis of such forms by repeated crosses. Furthermore hybridization studies between the *Muelleri-*

*anella* species might give insight in the genetic and phylogenetic relationships between them.

## Material and methods

Experimental material was provided by mass-rearings of the *Muellerianella* species which were kept in the greenhouse at 18–24 °C and long-day conditions (LD 18:6). Inbreeding in these rearings was prevented by exchange of males between parallel series at each generation. The material used for crosses originated from various populations which had been bred in the laboratory for at most six generations.

To obtain virgin females, sexes were separated in the fifth larval instar. One week after final ecdysis most females are receptive and readily mate with conspecific males.

Laboratory populations used in this study originate from samples taken at the following localities:

*M. fairmairei* – Spain: Orgiva, Sierra Nevada; S. France: Gorges de Lavall, Pyrénées Orientales; C. France: Nevers, Nièvre; The Netherlands: Leersum, Utrecht; Ireland: Glengarriff, Co Cork; S. Sweden: Krankesjön, Skåne; Yugoslavia: Bitola, Macedonia; Greece: Skaloula, Fokis.

*M. brevipennis* – France: Formiguères, Aude; The Netherlands: Rhenen/Leersum, Utrecht; S. Sweden: Kranesjön, Skåne; C. Sweden: Filipstad, Värmland; N. Sweden: Umeå, Västerbotten; Ireland: Dungarvan, Co. Waterford; Yugoslavia: Sisak, Croatia.

*M. extrusa* – The Netherlands: Dwingeloo, Drenthe and Nijmegen, Gelderland; Ireland: Killarney, Co. Kerry.

All interspecific crosses were made with groups of animals in cages (see also Drosopoulos, 1977). The host plants of both species were provided. In most cases 10 or 20 virgin females (one week after final ecdysis) were placed together with at least 10 mature males. After three weeks of reproduction all the animals were removed and females were dissected to test insemination. The spermatheca of each female was examined under the microscope, in physiological solution (Levy). The presence of motile sperm has been used as an indication for insemination. It has been assumed that sperm survives several weeks after insemination, although accurate data are lacking.

By daily inspection of the cages information was

obtained about the larval development and host-plant preference of the hybrids. When most of the hybrid offspring had reached the adult stage, they were removed and counted. Backcrosses were made in a comparable manner.

Fertility of hybrid and backcross males was tested by examination of the testis in physiological solution. Observations were made on spermatid development and on the presence of motile sperm. Since hybrid males are usually sterile (see also Drosopoulos, 1977), backcrosses were made by pairing hybrid females with males of one of the parental species.

Meiosis in hybrid and backcross females was studied by chromosome preparations of (semi) mature eggs. The chorion of the eggs was removed by treatment in 50% propionic acid and the eggs were stained in lacto-acetic orcein. A detailed description of this method will be given elsewhere (Booij in prep.). Meiosis in males has not been studied.

## Results

Most attention was paid to crosses between *M. fairmairei* and *M. brevipennis* because these species occur most frequently together in the field and because we liked to confirm the synthesis of triploid gynogenetic forms through hybridization and backcrossing, as was reported by Drosopoulos (1978). Fewer crosses were made between *M. extrusa* and either *M. brevipennis* or *M. fairmairei* because *M. extrusa* was only recently collected and recognized as a distinct species. (Booij, 1981).

### Interspecific mating and insemination

Despite differences in acoustic behaviour (Booij, 1982b), the *Muellerianella* species may mate interspecifically when they are kept long enough together in no-choice situations. In the experimental crosses, the number of inseminated females was determined three weeks after males of the other species had been provided.

In other experiments it had been found that 50 to 80% of the females mate already within one day when conspecific males are provided, and usually all females are inseminated within one week. Table 1 shows that, even after three weeks, the insemination frequency in interspecific crosses is much

Table 1. Frequency of interspecific insemination between species of the genus *Muellerianella* in no-choice experiments after a period of three weeks. Data were obtained from group crosses in cages. Intraspecific insemination is assumed to be 100%. The material originates from various European localities.

| Cross ♀♀ | ♂♂ | No. of ♀♀ tested | No. and % inseminated | |
|---|---|---|---|---|
| *M. fairmairei* × *M. extrusa* | | 15 | 10 | 67% |
| *M. extrusa* × *M. fairmairei* | | 20 | 16 | 80% |
| *M. fairmairei* × *M. brevipennis* | | 104 | 20 | 19% |
| *M. brevipennis* × *M. fairmairei* | | 22 | 7 | 31% |
| *M. brevipennis* × *M. extrusa* | | 21 | 3 | 14% |
| *M. extrusa* × *M. brevipennis* | | 55 | 10 | 18% |

lower. This is particularly true for crosses of *M. brevipennis* with either *M. extrusa* or *M. fairmairei*. Apparently the behavioral differences are strong enough to reduce mating in these crosses. The mating barrier between *M. fairmairei* and *M. extrusa*, however, appears to be much lower. The majority of females in these crosses were inseminated after three weeks. In an additional experiment it was found that 5 of 19 *M. extrusa* females (26%) already mated with *M. fairmairei* males within one week.

*Hybridization of* M. fairmairei *and* M. brevipennis

Although *M. fairmairei* and *M. brevipennis* do not easily mate with each other (see above), hybrid offspring was obtained in several of the crosses made (Tables 2 and 3). In crosses of *M. fairmairei* females with *M. brevipennis* males (Table 2) it appeared that crosses between remote populations, of which one originates from outside the area of sympatry, are generally more successful than crosses between populations from the same region. It should be noted, however, that the success of different crosses in both groups is very variable. Since the insemination frequency was about the same in the two groups of crosses, it seems likely that the difference in the number of hybrid offspring is caused by stronger genetic barriers in the area of sympatry. In the reciprocal crosses the number of crosses is too low to draw any conclusions in this respect.

As Table 3 shows the average production of hybrids in crosses of *M. brevipennis* females with *M. fairmairei* is higher than in the reciprocal crosses (Table 2). This might partly be explained by the fact that *M. brevipennis* females mate more easily with

Table 2. Results of crosses between females of *M. fairmairei* and males of *M. brevipennis*. Crosses were made between populations from the same region (A) and between remote populations of which at least one is from outside the area of sympatry (B). Crosses 1, 7, 8, 9 and 10 were made by Drosopoulos (1977). For each cross the number of hybrid females and males and the offspring per female are given.

| Nr. | Cross ♀♀ | ♂♂ | No. of ♀♀ used | Hybrid progeny ♀♀ | ♂♂ | n/♀ |
|---|---|---|---|---|---|---|
| | A Sympatric | | | | | |
| 1 | Netherlands × Netherlands | | 1 | 3 | 4 | 7.0 |
| 2 | Netherlands × Netherlands | | 40 | 9 | 10 | 0.5 |
| 3 | S. France × S. France | | 10 | 0 | 0 | 0.0 |
| 4 | S. France × S. France | | 10 | 1 | 0 | 0.1 |
| 5 | S. Sweden × S. Sweden | | 10 | 0 | 0 | 0.0 |
| 6 | S. Sweden × S. Sweden | | 25 | 0 | 4 | 0.2 |
| | Total group A | | 96 | 13 | 18 | 0.3 |
| | B Non-sympatric | | | | | |
| 7 | Greece × Netherlands | | 11 | 0 | 0 | 0.0 |
| 8 | Greece × Netherlands | | 11 | 4 | 3 | 0.6 |
| 9 | Greece × Netherlands | | 11 | 10 | 5 | 1.4 |
| 10 | Greece × Netherlands | | 9 | 6 | 6 | 1.3 |
| 11 | Greece × Netherlands | | 20 | 120 | 139 | 12.9 |
| 12 | Greece × Netherlands | | 20 | 39 | 47 | 4.3 |
| 13 | S. France × C. Sweden | | 10 | 0 | 0 | 0.0 |
| 14 | S. France × N. Sweden | | 10 | 30 | 39 | 6.9 |
| 15 | Netherlands × S. France | | 12 | 0 | 0 | 0.0 |
| | Total group B | | 102 | 209 | 239 | 4.3 |
| | Total group A + B | | 198 | 222 | 257 | 2.4 |

Table 3. Results of crosses between females of *M. brevipennis* and males of *M. fairmairei*. Crosses 16 and 18 were made by Drosopoulos (1977). Cf. Tab. 2.

| No. | Cross ♀♀ | ♂♂ | No of ♀♀ used | Hybrid progeny ♀♀ | ♂♂ | n/♀ |
|---|---|---|---|---|---|---|
| 16 | Netherlands × Netherlands | | 43 | 298 | 33 | 7.6 |
| 17 | S. Sweden × S. Sweden | | 10 | 8 | 0 | 0.8 |
| 18 | Netherlands × Greece | | 27 | 21 | 5 | 1.3 |
| 19 | S. France × Netherlands | | 11 | 64 | 6 | 6.4 |
| 20 | C. Sweden × C. France | | 10 | 0 | 0 | 0.0 |
| 21 | C. Sweden × Netherlands | | 10 | 45 | 7 | 5.2 |
| | Total | | 111 | 436 | 51 | 4.4 |

*M. fairmairei* males than *M. fairmairei* females with *M. brevipennis* males (Drosopoulos, unpubl. data, and Table 1). In both cases the average production of hybrid offspring is only a minor fraction (5–10%) of the production in conspecific crosses.

Table 3 also shows that there is a great excess of females in *M. brevipennis* × *M. fairmairei* crosses, whereas there are slightly more males in the reciprocal crosses (Table 2). In both types of crosses all hybrid males were sterile, and larval development of hybrids was often retarded, indicating lower viability. Larval mortality was insignificant. Hybrid larvae were observed feeding more often on *Deschampsia caespitosa* (host plant of *M. brevipennis)* than on *Holcus lanatus* (host plant of *M. fairmairei)* (see also Drosopoulos, 1977). However, the preferences became less clear when larvae grew older, and most of the adults were observed to feed and oviposit mainly on *Holcus lanatus*.

Although the development of ovaries in hybrid females appeared to be normal in most cases, meiosis was always abnormal. In normal diploid females 14 units are observed. In all of the 28 hybrid females studied, more than 14 units were seen at metaphase I, indicating a mixture of bivalents and univalents. (compare Fig. 1a and 1b, 1c). In some eggs only univalents were observed, which indicates complete failure of pairing between homologous chromosomes. Similar meiotic unbalance in hybrid females was reported by Drosopoulos (1978).

Because *Muellerianella* species are very similar, it is very difficult to detect hybrids in the field. Among about 100 females from sites where both species occurred, and which were studied cytogenetically (Booij and Guldemond, unpubl. data), two females were found in which metaphase patterns strongly resembled that of laboratory hybrids (Fig. 1d). Both females originated from Krankesjön (S. Sweden), where *M. fairmairei* and *M. brevipennis* live very close together and chances for hybridization seem to be optimal.

The hybrid females of crosses 2, 11, 12, 14, 19, 21
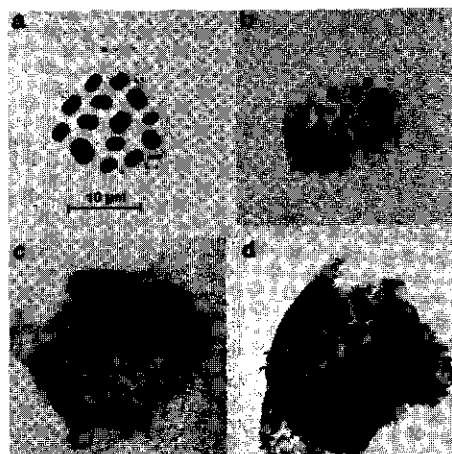


*Fig. 1.* Meiotic configurations in eggs of normal and aberrant *Muellerianella* females: (a) metaphase plate with 14 bivalents of a normal female *(M. extrusa)*, polar view; – (b) metaphase plate in a hybrid female *M. fairmairei* × *M. brevipennis* containing mainly univalents; – (c) the same with a mixture of bivalents and univalents; – (d) a possible hybrid female from the field with a mixture of bivalents and univalents. Arrows in (c) and (d) indicate some of the univalents.

(Tables 2 and 3) were backcrossed with males of the parental species. As found earlier by Drosopoulos (1977), most backcrosses of hybrid females with *M. brevipennis* males fail to produce any offspring. In the present study 53 hybrid females were backcrossed with *M. brevipennis* males. Only one female was obtained. This female failed to reproduce. Backcrosses of hybrid females with *M. fairmairei* males are usually more successful. Tables 4,

*Table 4.* Production of adult offspring in successive backcross generations (B1, B2, B3 and B4) of hybrids of *M. fairmairei* and *M. brevipennis*, backcrossed with males of *M. fairmairei (Mf)*. For each cross the number of females used (n) and the offspring produced are given. Cross numbers from Tables 2 and 3.

| Cross Nr | n | B₁ (= F₁ × Mf) ♀♀ | ♂♂ | prog/♀ | n | B₂ (= B₁ × Mf) ♀♀ | ♂♂ | prog/♀ | n | B₃ (= B₂ × Mf) ♀♀ | ♂♂ | prog/♀ | n | B₄ (= B₃ × Mf) ♀♀ | ♂♂ | prog/♀ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 9 | 9 | 3 | 1.3 | 3 | 59 | 39 | 33 | 5 | ca 200 | | ca 40 | 10 | ca 500 | | ca 50 |
| 11 | 15 | 39 | 24 | 4.2 | | – | | | | – | | | | – | | |
| 12 | 15 | 96 | 56 | 10.1 | | – | | | | – | | | | – | | |
| 14 | 16 | 71 | 29 | 6.7 | 30 | 103 | 84 | 6 | 10 | ca 1400 | | ca 140 | | – | | |
| 19 | 10 | 4 | 2 | 0.6 | 4 | 61 | 34 | 24 | 10 | 36 | 26 | 6.2 | 20 | 370 | 210 | 24 |
| 21 | 10 | 2 | 0 | 0.2 | | – | | | | – | | | | – | | |
| Total | 75 | 221 | 114 | 4.5 | 37 | 223 | 157 | 10.2 | 25 | ca 1660 | | ca 66 | 30 | ca 1080 | | ca 36 |

Table 5. Fertility of hybrid males of crosses between *M. fairmairei* and *M. brevipennis* and of males in successive backcross generations with *M. fairmairei*. The number of males tested and the percentage of them with motile sperm are given.

| Cross nr. | F₁ | | B₁ (F₁ × Mf) | | B₂ (B₁ × Mf) | | B₃ (B₂ × Mf) | | B₄ (B₃ × Mf) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | tested | fertile | tested | fertile | tested | fertile | tested | fertile | tested | fertile |
| 2 | 6 | 0% | 3 | 0% | 14 | 7% | 8 | 37% | | |
| 11 | 10 | 0% | 14 | 7% | | | | | | |
| 12 | 10 | 0% | 18 | 6% | | | | | | |
| 14 | 10 | 0% | 24 | 0% | 41 | 34% | | | | |
| 19 | 3 | 0% | 2 | 0% | 18 | 0% | 5 | 20% | 10 | 90% |
| 21 | 7 | 0% | | | | | | | | |
| Total | 46 | 0% | 61 | 3% | 73 | 20% | 13 | 30% | 10 | 90% |

Table 6. Bivalent and univalent formation at metaphase I as an indication for meiotic (un-)balance in hybrid females of *M. fairmairei* and *M. brevipennis* and in females of backcross generations. The number of studied females in each category is given. Material from crosses 2, 11, 14, 19 and 21.

| Genera-tion | 14 bivalents (normal) | 7-13 bivalents + univalents | 0-6 bivalents + univalents |
|---|---|---|---|
| FI | 0 | 10 | 4 |
| BI | 28 | 38 | 6 |
| B2 | 42 | 6 | 0 |

5 and 6 show that after recurrent backcrossing, reproductive success per female may increase again, meiosis is stabilized and fertility of males is restored. Consequently females and males of the B₂ and B₃ generation can be successfully crossed inter se (Crosses 2, 14 and 19). All backcross products resemble *M. fairmairei* morphologically and feed mainly on *Holcus lanatus*.

As can be seen in Table 4, there is an excess of females is most backcross generations, but no evidence was found for the occurrence of gynogenetic triploid forms among the backcross females. All females of the first backcross generation examined cytologically (n = 76) appeared to be diploid and had a bisexual reproduction indicated by a normal to slightly irregular meiosis. None of them had an ameiotic oogenesis with only univalents at first metaphase which is characteristic of gynogenetic forms. Thus the synthesis of triploid forms by back-crossing of hybrids with *M. fairmairei* males, as reported by Drosopoulos (1978), could not be repeated.

## Hybridization of M. fairmairei *and* M. extrusa

Only recently was it discovered that *M. extrusa*, formerly regarded to be identical with *M. fairmairei*, represents a distinct species. The two species are characterized by small morphological differences (Booij, 1981), by their ecology (Booij, 1982a) and acoustic behaviour (Booij, 1982b). Without doubt these species are very closely related. Crosses were carried out to assess their ability to hybridize and to check their specific status.

In addition to the low mating barriers discussed above, crosses between *M. fairmairei* and *M. extrusa* result in many offspring (Table 7). The average production, however, is much lower (15-30%) than in conspecific crosses. Moreover, the sex ratio of the hybrid F₁ is strongly skewed in favour of females.

In 72 hybrid males spermatogenesis was studied. In 47 of them only spermatids were found and in 18 others little motile sperm was observed and many spermatozoids were deformed. Only 7 males seemed to be fully fertile having large quantities of motile sperm.

In each cross the meiosis of two hybrid females was studied. In all these females (6) meiosis seemed to be regular having 14 bivalents at metaphase I.

In each cross, hybrid females and males were crossed inter se, but only from cross 23 could an F₂ generation be obtained. In this cross about 40 animals were obtained from 15 F₁ females. Despite this low production both males and females of the F₂ appeared to be fertile.

Although no significant mortality occurred among F₁ larvae, their development was variable

*Table 7.* The number of females and males in the hybrid progeny and the average production of hybrids per female in crosses between *M. fairmairei (Mf)* and *M. extrusa (Me).*

| Cross Nr. | ♀♀ | ♂♂ | No. of ♀♀ used | Hybrid production | | |
|---|---|---|---|---|---|---|
| | | | | ♀♀ | ♂♂ | hybrids/♀ |
| 22 | *Mf* Netherlands × *Me* Netherlands | | 10 | 84 | 44 | 12.8 |
| 23 | *Me* Netherlands × *Mf* Netherlands | | 10 | 88 | 79 | 16.6 |
| 24 | *Me* Ireland × *Mf* Ireland | | 10 | 231 | 66 | 29.7 |
| | Total production | | 30 | 403 | 189 | 19.7 |

and on average much slower than that of the parental species. This might strongly reduce the success of possible hybrids in the field. The hybrid larvae fed on the host plants of both parental species, *Holcus lanatus* and *Molinia caerulea.*

Hybrid females of crosses 22, 23, and 24 were backcrossed with males of *M. fairmairei* and *M. extrusa.* Table 8 shows that the results of the backcrosses vary, but in 4 out of 6 possible backcrosses progeny was obtained. Unfortunately the $B_1$ generation of cross 23 was lost and could not be further studied. Males and females of the $B_1$ in cross 24 appeared to be fully fertile and could be crossed inter se.

Although the data collected thus far are rather scanty, my tentative conclusion is that *M. fairmairei* and *M. extrusa* may readily hybridize in no-choice situations. Moreover, the fertility of possible hybrids and backcross products might lead to some introgression when the premating isolating mechanisms would fail.

### Hybridization of M. extrusa *and* M. brevipennis

Two crosses were made between females of *M. brevipennis* and males of *M. extrusa* (crosses 25 and 26) and three between females of *M. extrusa* and

males of *M. brevipennis* (crosses 27, 28 and 29). As discussed earlier the number of interspecific inseminations between these species is low (see Table 1). This might partly explain the low production of hybrids in these crosses (Table 9). Only cross 28 did yield a considerable number of hybrids. In the latter cross 33% of the females proved to be inseminated.

In crosses 27, 28 and 29 all hybrid males examined (n = 18) were completely sterile. In some males of cross 25 motile sperm was found, but fertility was not further tested. In 7 hybrid females meiosis was studied. At metaphase I mixtures of bivalents and univalents were observed, indicating meiotic irregularities. In none of the females was meiosis regular.

Ten of the hybrid females of cross 28 were backcrossed with males of *M. brevipennis,* and another ten with males of *M. extrusa.* From these backcrosses 7 and 6 larvae respectively were obtained. Thus the production of offspring per hybrid female in back-crosses is extremely low.

The poor results of crosses and back-crosses between *M. extrusa* and *M. brevipennis* are comparable to those between *M. fairmairei* and *M. brevipennis* or even less successful. This indicates that *M. brevipennis* is well isolated from *M. extrusa* and that chances for hybridization and introgression in the field are low.

*Table 8.* Production of adult offspring by female hybrids *M. fairmairei (Mf)* × *M. extrusa (Me)* after backcrossing with the parent species and results of crosses of animals of the $B_1$ generation inter se. Total production and progeny per female are given.

| Cross | $B_1 (F_1 × Mf)$ | | | $B_1 (F_1 × Me)$ | | | $B_1 × B_1$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of ♀♀ used | Progeny | Prog/♀ | No. of ♀♀ used | Progeny | Prog/♀ | No. of ♀♀ used | Progeny | Prog/♀ |
| 22 | 10 | 0 | 0 | 10 | 0 | 0 | – | – | – |
| 23 | 10 | 6 | 0.6 | 10 | 100 | 10 | – | – | – |
| 24 | 5 | 60 | 12 | 10 | 140 | 14 | 10 | ca 650 | 65 |

*Table 9.* Number of hybrid females and males and average progeny per female in crosses between *M. extrusa (Me)* and *M. brevipennis (Mb)*.

| Cross nr | ♀♀ | ♂♂ | No of ♀♀ used | Hybrid progeny ♀♀ | Hybrid progeny ♂♂ | hybrids/♀ |
|---|---|---|---|---|---|---|
| 25 | *Mb* C. Sweden × *Me* Netherlands | | 10 | 4 | 6 | 1.0 |
| 26 | *Mb* Ireland × *Me* Irelands | | 20 | 0 | 0 | 0.0 |
| 27 | *Me* Netherlands × *Mb* N. Sweden | | 20 | 0 | 1 | 0.1 |
| 28 | *Me* Netherlands × *Mb* Netherlands | | 20 | 37 | 44 | 4.1 |
| 29 | *Me* Ireland × *Mb* Ireland | | 20 | 5 | 4 | 0.5 |
| | Total production | | 90 | 46 | 55 | 1.1 |

*Sex ratios in progenies of intraspecific crosses*

Drosopoulos (1976) showed that the excess of females in most populations of *M. fairmairei* is caused by the presence of gynogenetic triploid females which resemble females of the bisexual species. When the bisexual species is reared in the laboratory a normal sex ratio of 1:1 is found (Drosopoulos, 1977). However, predominance of females has also been reported from *M. brevipennis* populations from Finland (Kontkanen, 1952). The prevalence of females in northern *M. brevipennis* populations was confirmed by samples we took in Sweden and Finland in 1977, in total comprising of 399 females and 226 males.

To see whether the sex ratio in individual progenies differed from 1:1, 11 pairs of Swedish *M. brevipennis* were placed in separate cages and the offspring were counted and sexed. The animals were obtained from strains bred for only one generation in the laboratory. On average each pair produced about 200 offspring. In the progeny of 5 of the 11 pairs, significantly more females than males were found (Chi-square, $P < 0.05$). It is not known whether the skewed sex ratios are caused by an unusual sex-determination system or by other factors. It should be mentioned here that sex ratios in subsequent generations of the mass rearing were about 1:1. It is possible that the diapause of the eggs laid in the first generations in the laboratory was more easily broken in females than in males, but this can hardly explain the sex ratios in the field.

Deviating sex ratios may also be found when intraspecific crosses are made between populations which are genetically differentiated. Such effects were apparent in crosses between Greek and Dutch populations of *M. fairmairei* made by Drosopoulos (1977). I found similar effects in crosses between remote populations of both *M. brevipennis* and *M. fairmairei*. Eight pairwise crosses were made between *M. brevipennis* from N. Sweden and from S. France. Although the distance between the populations is about 2 600 km, only in one case were significantly more females than males produced.

The distance effect seems to be clearer in intraspecific crosses of *M. fairmairei*. Females of this species originating from the Netherlands were crossed with males from S. France (4 pairs) and from S. Sweden (4 pairs). In addition Irish females were crossed with males from Yugoslavia (3 pairs). In the offspring of five out of these eleven pairs, significantly more females than males were present. The abnormal sex ratios may be caused by genetic unbalances in the F1 zygotes, apparently resulting in differential mortality.

*Mating relations of gynogenetic females with the bisexual species*

Formerly the gynogenetic *Muellerianella* forms have been regarded as triploid biotypes of the bisexual species *M. fairmairei* (Drosopoulos, 1976, 1977) because they usually coexist with that species in the field, they have similar life histories and both female types are morphologically indistinguishable. However, the term biotype is usually restricted to genetic variants of a species which are characterized by their ability to feed on certain different plant species or varieties (Claridge, 1980). The triploid *Muellerianella* forms, however, feed on the same host plant as *M. fairmairei*. Moreover, although morphologically very similar to *M. fairmairei*

65

females, they are genetically too different to call them biotypes, especially if the triploids are of hybrid origin, as was suggested by Drosopoulos (1978). Another reason not to apply the term biotype to the triploid forms is that the association with *M. fairmairei* is not as close as was previously believed. Drosopoulos (1977) already showed that the gynogenetic females could reproduce in the laboratory after insemination by males of *M. brevipennis.* Later it was shown that gynogenetic populations persist also in the field in association with *M. brevipennis,* on places where *M. fairmairei* is absent (Booij, 1981, 1982a).

A third bisexual species, *M. extrusa,* also mates readily with the gynogenetic females. Thus all three bisexual species may serve as sperm donor for the gynogenetic females. However, as Table 10 shows, the average production of offspring is highest when the triploids are mated with *M. fairmairei* males and lowest when mated with *M. brevipennis.* Although *M. extrusa* seems to be a suitable sperm donor, we have no certain records of such an association in the field. In areas where the triploids have two generations per year and *M. extrusa* only one, such an association may be impossible because of asynchronous phenology. At those places where both complete only one generation (N and C Europe), the phenologies may sufficiently overlap for the triploids to get inseminated.

*Table 10.* Reproductive success of *gynogenetic Muellerianella* females when mated with males of different related bisexual species.

| Sperm donor species | Average offspring/♀ | Nr. of ♀♀ |
|---|---|---|
| *M. fairmairei* | 284 ± 53 | n = 8 |
| *M. extrusa* | 266 ± 57 | n = 5 |
| *M. brevipennis* | 11 ± 9 | n = 8 |

## Discussion

As was shown in this paper interspecific matings between the *Muellerianella* species can be induced by keeping females of one species long enough together with males of another species. In all pairwise combinations hybrids can be produced which have a varying degree of fertility.

The crossability of the species under artificial

conditions confirms the close relationships between the *Muellerianella* species and the results of the crosses might be used to estimate the degree of genetic isolation between them. Although the strength of the genetic isolation cannot be used as an accurate index for the phylogenetic distance or degree of genetic differentiation (White, 1973), the crossability within groups of related taxa is often correlated with the phylogenetic relations based on other evidence (see for examples, Blair, 1972; Bock, 1978; Ae, 1979).

The number of hybrids produced in crosses between *M. fairmairei* and *M. extrusa* and their fertility suggest that these species are genetically much less isolated from each other than each of them is from *M. brevipennis.* Since morphological and behavioral evidence (Booij, 1981, 1982b) points in the same direction, the degree of genetic compatibility between the species probably reflects the phylogenetic relations between them.

The significance of hybridization for the evolution of the *Muellerianella* complex is difficult to determine. It should be realized that all experiments thus far are made under artificial conditions with no choice for the females. It seems likely that in the field, where females can choose between males of their own and of other species, the number of interspecific matings is strongly reduced. In some cases, however, mating errors may lead to some introgression or even to the arisal of persistent hybrid forms.

Among the *Muellerianella* species, chances for introgression are highest between *M. fairmairei* and *M. extrusa.* The ethological and genetic barriers between these species are low and under artificial conditions hybrids can be obtained easily. Backcrosses of hybrid females with males of both parental species are often successful. Although the number of offspring in interspecific crosses and backcrosses is much lower than in intraspecific crosses, exchange of genes between these species seems possible. However, the ecological differences between the species are considerable (Booij, 1982a), and, as far as we know now, the species occur together only occasionally. Therefore it is likely that introgression is negligible in the field.

With regard to *M. fairmairei* and *M. brevipennis* the situation is different. In NW Europe their host plants often grow together in semi-natural habitats (moist to wet grasslands), and the species are regu-

larly found together in the field (Booij, 1982a). Therefore interspecific encounters are probably frequent, but due to ethological differences and strong genetic barriers, hybrids will be produced only in a few cases. Chances for introgression are further decreased because reproductive success and viability of hybrids and backcross-products are low.

From crossing experiments, Drosopoulos (1979) concluded that the triploid gynogenetic *Muellerianella* forms arose by hybridization of *M. fairmairei* and *M. brevipennis*. For the synthesis of triploids, hybrid females should produce unreduced eggs which are fertilized by sperm of *M. fairmairei*. The chromosome set of the resulting triploids consists of two genomes of *M. fairmairei* and one of *M. brevipennis*.

Such a hybrid origin fits well in the general picture that most polyploid thelytokous animals are allopolyploids (White, 1978). Also the distribution and ecology of the triploid *Muellerianella* forms supports the hybrid origin hypothesis (Booij, 1981, 1982a). Unfortunately, the synthesis of triploids, as reported by Drosopoulos (1979), could not be repeated in our extensive crosses. Possibly a particular genetic constitution of Drosopoulos' material might have favoured the synthesis of triploids. On the other hand it cannot be excluded that his rearing was accidentally contaminated by triploids reared in the same greenhouse. There are several reasons which indicate that such a contamination may have occurred. The first is that the triploid females were found in the cages only after four months whereas later generations developed in two months. Furthermore the synthesized triploids happened to be of the same cytotype ($3n = 41$) as the one reared in the greenhouse. Since the bisexual species are $2n = 28$, triploids are expected to be $3n = 42$, which is actually the most common cytotype in the field (Booij, unpubl. data). Finally, Drosopoulos' cross yielded only balanced triploids, whereas in all our crosses many diploid products were produced which had an irregular meiosis.

Probably the best way to elucidate the genetic constitution of the triploid *Muellerianella* forms with more certainty, is to study their allozyme patterns and those of their bisexual relatives. The fact that the triploids, which resemble *M. fairmairei*, can also reproduce after insemination by *M. brevipennis* males cannot be used as an argument for the hybrid origin hypothesis, since this holds true also for *M. extrusa*, which actually is an even better sperm donor, because more offspring is produced in that case. Up till now we have no certain records of triploids in association with *M. extrusa* in the field, but it is possible that they will be found in the future. In that case the gynogenetic forms could maintain themselves by using one out of three sperm donor species in the field. As far as I know, such a complex has not been reported in nature before.

## References

Ae, S. A., 1979. The phylogeny of some Papilio species, based on hybridization data. Syst. Ent. 4: 1–16.

Blair, W. F. (ed.), 1972. Evolution of the genus Bufo – Chapter 11: Evidence from hybridization. – University of Texas Press. Austin and London, 459 pp.

Bock, I. R., 1978. The bipectinata complex: a study in interspecific hybridization in the genus Drosophila (Insecta: Diptera). Aust. J. Biol. Sci. 31: 197–208.

Booij, C. J. H., 1981. Biosystematics of the Muellerianella complex (Homoptera, Delphacidae), taxonomy, morphology and distribution. Neth. J. Zool. 31: 572–595.

Booij, C. J. H., 1982a. Biosystematics of the Muellerianella complex (Homoptera, Delphacidae), hostplants, habitat and phenology. Ecol. Ent. 7: (in press).

Booij, C. J. H., 1982b. Biosystematics of the Muellerianella complex (Homoptera, Delphacidae), interspecific and geographic variation in acoustic behaviour. Z. Tierpsych. (in press).

Claridge, M. F., 1980. Biotaxonomic studies on leafhopper and planthopper pests of rice with special reference to Nilaparvata lugens (Stål). ODA Research Scheme R 3282 Final Report, Cardiff U.K. 54 pp.

Drosopoulos, S., 1976. Triploid pseudogamous biotype of the leafhopper Muellerianella fairmairei. Nature 263: 499–500.

Drosopoulos, S., 1977. Biosystematic studies on the Muellerianella complex (Delphacidae, Homoptera, Auchenorrhyncha). Meded. LandbHoogesch. Wageningen 77: 1–133.

Drosopoulos, S., 1978. Laboratory synthesis of a pseudogamous triploid 'species' of the genus Muellerianella (Homoptera, Delphacidae). Evolution 32: 916–920.

Kontkanen, P., 1952. Beiträge zur Kenntnis der Zikadenfauna Finnlands II. Ann. Ent. Fenn. 18: 26–34.

White, M. J. D., 1973. Animal cytology and evolution, Cambridge University Press, 961 pp.

White, M. J. D., 1978. Modes of speciation. W. H. Freeman and Company San Francisco, 455 pp.

# Chromosome numbers in the *Muellerianella* complex (Homoptera, Delphacidae) with special reference to clonal variation in gynogenetic forms

C.J.H. BOOIJ and J.A. GULDEMOND

INTRODUCTION

    Some years ago, Drosopoulos (1976) discovered that the excess of females in populations of the planthopper *Muellerianella fairmairei* Perris 1857, is caused by triploid gynogenetic (pseudogamic) forms which strongly resemble females of the bisexual species. The eggs of these all-female forms, which are produced parthenogenetically, develop only after activation by sperm, supplied by males of one of the three closely related species *M. fairmairei, M. brevipennis* Boheman 1847 or *M. extrusa* Scott 1871 (Booij, 1981b). In the field the triploid forms are usually associated with *M. fairmairei,* because they live on the same hostplant and have similar life--histories (Drosopoulos, 1976, 1977; Booij, 1981a, 1982). Meiosis in these triploids is reduced to a single equational division (Drosopoulos, 1976).

    Complexes of asexual and related bisexual species, which are found in several animal groups (for a review see White, 1978), are of great interest to evolutionary theory, especially because they enable us to study the evolutionary significance of sexuality and recombination (Maynard Smith, 1978; White, 1978; Williams, 1975). Most studies in this field concentrate on the relation between genetic variation in asexual and bisexual populations and the evolutionary potential to adapt to different or changing environments.

    It has frequently been shown that variation in asexual populations is much wider than generally believed and expected. Most asexual "species" apparently consist of many independent clones which are differentiated genetically, morphologically and ecologically (White, 1973, 1978 and many other references in the present paper).

    Earlier studies on the asexual *Muellerianella* forms showed that the "triploid pseudogamous biotype" (Drosopoulos, 1976, 1977) also represents a complex of morphologically varying clones, some of which are even confined to different hostplants (Booij, 1981a, 1982). The present paper deals with another aspect of clonal diversity, the extensive variation in chromosome

numbers found in asexual *Muellerianella* populations of W. Europe. In
addition, the chromosome numbers of the bisexual species are given, also
including some variation.

MATERIAL AND METHODS

For chromosome studies, samples were taken from 1977-1979 at the
localities listed below. At each site, the number of females of which the
chromosome number was determined, is given in parentheses. Samples of
*M. fairmairei* and of asexual forms are combined because they are usually
associated. Samples of *M. fairmairei* (first figure) and *triploid forms*
(second figure): PORTUGAL, Algarve: Sierra de Monchique (7;0). SPAIN,
Sierra Nevada: Orgiva (5;0) and Capifeira (14;0). ANDORRA, Sant Julia de
Lòria (0;1). FRANCE, Pyrénées Orientales: Gorges de Lavall (1;1), Ardèche:
Privas (11;1), Nièvre: Nevers (6;1), Artois: Cap Gris Nez (14;8). YUGOSLAVIA,
Macedonia: Bitola (7;5) and Otesevo (2;0), Serbia: Bela Palanka (0;4),
Bosnia: Zvornik (5;21), Croatia:Sisak (0;2). GREECE, Lidokhorikion:
Skaloula (4;0). AUSTRIA, Steiermark: Graz (0;15). W. GERMANY, Schwarzwald:
Steinen (0;6) and Emmendingen (0;7), Nordrhein-Westfalen: Dülmen (0;19),
Brilon (0;8), Paderborn (0;7), Laasphe (0;4), Winterberg (0;2). BELGIUM,
Ardennes (Liège): Sourbrodt (0;14) and Francorchamps (0;23). THE NETHERLANDS,
Friesland: Schiermonnikoog (0;22), Terschelling (3;20), Wolvega (5;10),
Drenthe: Drenthse Aa (0;31), Overijssel: Denekamp (15;38), Gelderland:
Renkum (7;19), Utrecht: Kortenhoef (0;15), Veenendaal (0;5), Rhenen (0;67)
and Leersum (13;27), Z-Holland: Langeraar (0;20), N-Holland: Texel (3;17),
Zeeland: Renesse (23;0), Burgh-Haamstede (7;10), Vrouwenpolder (6;0),
N-Brabant: Ulecoten (1;8), Baarle Nassau (1;8), Geldrop (22;5), Limburg:
Elsloo (8;3), Weert (6;40). GREAT BRITAIN Wales (Co Dyfed): St. Davids
(0;24). IRELAND, Co. Mayo: Knock (0;5), Co. Roscommon: Castlerea (0;6),
Fuerty (1;2), Derrywode (0;13), Co. Connemara: Recess (0;7), Letterfrack
(0;12), Co. Clare: Lissycasey (4;15), Co. Cork: Glengarriff (2;7), Co.
Waterford: Dungarvan (3;16). DENMARK, Jutland: Aabenraa (8;19), Rödhus
(0;14), Rebild (0;30), Fröstrup (0;16), Varde (0;18). SWEDEN, Skåne:
Krankesjön (10;4), Uppland: Alunda (0;1).
Samples of *M. extrusa* :
DENMARK, Jutland: Aabenraa (7), Hjöllund (13) and Store Vildmose (7).

IRELAND, Co. Roscommon: Roscommon (2). THE NETHERLANDS, Friesland:
Terschelling (5), Drenthe: Dwingeloo (10), Gelderland: Nijmegen (4),
N-Brabant: Geldrop (5). W. GERMANY, Hessen: Marburg (5).
Samples of *M. brevipennis* :
SWEDEN, Skåne: Krankesjön (3), Småland: Filipstad (6), Västerbotten: Umeå
(2). FINLAND, Vaasa: Alajärvi (2), Turku-Pori: Salo (2).
IRELAND, Co. Waterford: Dungarvan (2). DENMARK, Jutland: Törring (2),
Rebild (12). THE NETHERLANDS, Utrecht: Rhenen (5). BELGIUM, Ardennes
(Liège): Sourbrodt (2). W. GERMANY, Hessen: Marburg (4). FRANCE, Aude:
Formiguères (2). YUGOSLAVIA, Croatia: Sisak (5).

Chromosome preparations were made from material collected directly
from the field. To obtain unbiased samples, eggs, larvae, or adults were
taken at random from the site using a simple pooter. Eggs and larvae were
reared to adulthood in the greenhouse.

For chromosome preparations, 5-15 days old females with swollen abdomens
appeared to be most suitable. Meiosis was studied from mature or semi-mature
eggs stained with lacto-acetic orcein. The following procedure is followed:
the animals are anaesthetized using ethyl acetate. After a few minutes
the abdomen is separated from the thorax with two pairs of forceps and put
into a black dissection-block with 40-50% propionic acid. Immediately
there-after the ovaries are pushed out of the abdomen by pressing the abdomen
dorsally starting from the caudal end. After 3-6 minutes the semi-mature
eggs swell considerably and become loosened from follicle cells and other
tissues. Semi-mature eggs have no chorion but possess a small cap that is
present at the base. Before this cap gets detached, the eggs are taken out
of the acid and transferred to a microscope slide using a capillary glass-
-tube. After the superfluous acid is taken away with a small piece of filter
paper, a drop of lacto-acetic orcein is put on the eggs. The staining is
continued for at least 15 minutes, but not longer than one hour. After that
period a cover slip is gently placed upon the eggs. The eggs are squashed
by the weight of the cover slip without further pressing.

When no semi-mature eggs are available in the animal, mature eggs (with
a chorion) can also be used, although the method is more tedious. In that
case the eggs are left for at least 15 minutes in the propionic acid. When
the chorion hardens and become glossy, it can be carefully broken at one
of the ends using two fine needles. The egg-mass swells and loosens from the
chorion. The swollen undamaged eggs can be taken out of the dissection-block

and treated in the same way as the semi-mature eggs. The method described proved to be suitable for many species of Delphacidae.

If not lost during treatment, the heavily stained nucleus can be found in the basic part of the egg. Nuclei in semi-mature or mature eggs usually contain regular metaphase plates. In diploids bivalents are observed at the first meiotic metaphase. In triploid gynogenetic forms, however, meiosis is reduced to a single equational division and metaphase plates only contain amphitelic univalents (Drosopoulos, 1976). In both diploids and triploids the chromosomes are strongly condensed and metaphase plates remain stable untill fertilization. In general, differences in chromosome numbers between eggs of one individual were not found. For counts only clear metaphase plates were used. In each individual at least two plates were counted. When unusual numbers were observed, more plates were counted to get more certainty. To determine the size of the animals, head-with has been measured using a dissection microscope (Wild M5, 40x).

CHROMOSOME NUMBERS OF THE SEXUAL SPECIES

Two species of the *Muellerianella* complex, *M. brevipennis* and *M. fairmairei* have recently been studied cytogenetically (Drosopoulos, 1976, 1977; Drosopoulos and Sybenga, 1977).

These species have 28 chromosomes and a (neo-) XY sex determination system. At the first meiotic division 14 bivalents are observed. In males the sex-bivalent is strongly heteromorphic, which is somewhat more pronounced in *M. fairmairei* than in *M. brevipennis*.

Observations on the third species of the complex, *M. extrusa*, showed that this species has also 2n=28 (Fig. 1a) and a (neo-) XY system. The heteromorphy of the sex-bivalent is comparable to that of *M. brevipennis*. No detailed comparisons could be made between the karyotypes of the species, because the holokinetic chromosomes lack any visible structural differentiation, and only small gradual differences in size are found.

During the investigations on the *Muellerianella* populations, several individuals were found with unusual chromosome numbers. Of *M. extrusa* meiotic plates were studied in 58 females originating from 9 different populations. In 53 of these females the usual chromosome number (2n=28) was found. In three *M. extrusa* females however, 15 bivalents (2n=30) were

observed at meiosis, and in one female even 16 bivalents were found (Fig. 1b). Two of these females were found at Geldrop (The Netherlands), where *M. extrusa* lives close together with *M. fairmairei* and hybridization may occasionally occur. Another interesting *M. extrusa* female was discovered near Aabenraa (Denmark). The metaphase plates of this female showed 25-28 chromosome bodies, most approximately of the size of single chromosomes, suggesting that most of the chromosomes did not pair (Fig. 1e).

In populations of *M. fairmairei* checked thus far, aberrant configurations seem to be less common. Although 218 females of this species from many localities were examined, only nine deviations from the usual pattern were found. In three of these 1 or 2 supplementary fragments were observed at meiosis (Fig. 1c). These fragments may be regarded as microsomes or small B-chromosomes. Similar fragments have been reported in several other delphacid species (Halkka, 1959). In five other *M. fairmairei* females 1-4 extra chromosomal units were present in metaphase plates (Fig. 1d). We could not determine whether these units were bivalents or univalents. In all five cases the unusual configuration was observed in all eggs examined of one individual. Thus the abnormalities were not due to occasional meiotic
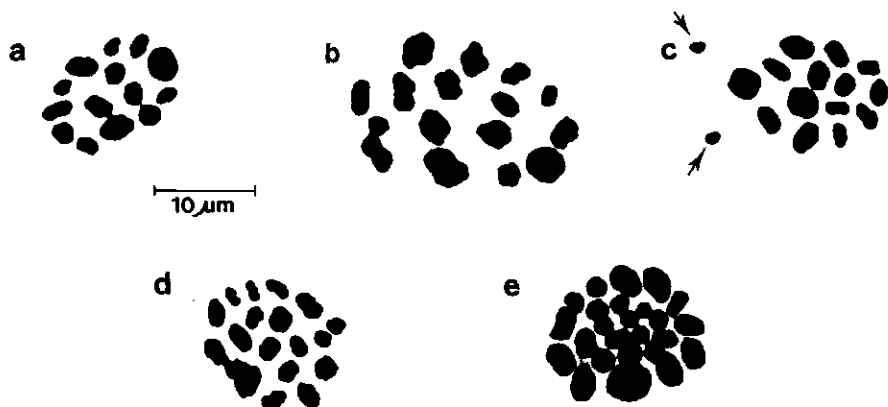


Fig.1. Meiotic metaphase I plates of ovarian eggs of sexual *Muellerianella* females.

(a) normal *M.extrusa* female with 14 bivalents (2n=28), seen in polar view.

(b) aberrant *M.extrusa* female showing 16 bivalents, seen in plain view.

(c) aberrant *M.fairmairei* female with 14 bivalents + 2 microsomes (arrows), polar view.

(d) aberrant *M.fairmairei* female with ca. 18 bivalents, seen in polar view.

(e) aberrant *M.extrusa* female showing 25 bivalents and univalents in polar view.

disturbances in particular eggs.

In the third species, *M. brevipennis*, no abnormalities were found among 49 females studied from various localities. All showed 14 bivalents at metaphase (2n=28).

In spite of the recorded abnormalities, we may safely conclude that all three *Muellerianella* species basically have a chromosome set of 2n=28 with an (neo-) XY sex determining system. More cytological information of *M. extrusa* is desirable, since the material studied was limited.


## CHROMOSOME NUMBERS OF GYNOGENETIC FORMS

Because it was found that chromosome number of gynogenetic *Muellerianella* females often differs from 3n=41, the number reported by Drosopoulos (1976, 1977), special attention was paid to chromosomal variation in these forms.

It should be stressed here that each individual has a given chromosome number which is usually the same in all of its offspring (Booij, unpubl. lab. experiment). Thus chromosomal variation in field populations of *Muellerianella* indicates that different clones are present with characteristic chromosome numbers (cytotypes). More evidence of the clonal inheritance of the chromosome numbers is given below. In this respect chromosomal variation in *Muellerianella* is quite different from that reported in certain weevils (Oliver et al., 1973; Takenouchi, 1969, 1981), where each individual as a rule produces eggs with variable chromosome numbers.

The results of the investigations are compiled in Table 1, which gives the frequency distribution of different cytotypes at various sampling sites. All chromosome counts were made from stable metaphase plates observed in polar view. A selection of photographs of such plates is given in Figs. 2a-2f.

Chromosome numbers varied from 3n=32 to 3n=44 with cytotypes 3n=41 and 3n=42 prevailing in most samples. The large proportion of the latter cytotypes (78%) in the pooled samples supports the idea that the gynogenetic forms are basically triploid, since the bisexual species have 2n=28. All cytotypes other than 3n=42 should be regarded as aneuploid triploids.

Table 1 shows that there is a clear-cut relation between the degree of aneuploidy and the frequency of the cytotype. The more chromosomes are
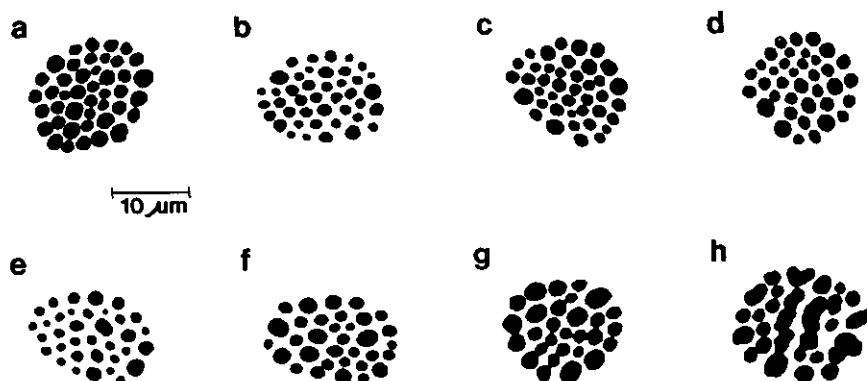
Fig.2. Pseudomeiotic metaphase I plates with different chromosome numbers found in ovarian eggs of triploid gynogenetic *Muellerianella* females. All plates are photographed in polar view. (a) 3n=43, (b) 3n=42 (basic form), (c) 3n=40, (d) 3n=37, (e) 3n=35, (f) 3n=33, (g) gynogenetic female with chromosome number at the diploid level, 2n=29, (h) triploid gynogenetic female with some degree of bivalent formation.

lacking, the less common the cytotype is.

The weak but significant correlation we found between chromosome number and size of the animals in Dutch populations (r=0.21, n=185, P<0.01), suggests that aneuploidy causes a reduction in viability. This might partly explain the frequency distribution of cytotypes found. Laboratory experiments showed that the fecundity of aneuploid clones (cytotypes 3n=41 and 3n=38 were reared for several generations) was not reduced compared with that of euploids (3n=42).

Table 1 also shows that the occurrence of particular cytotypes varies from site to site and between geographic regions, although some of the observed variation may be due to bias in the samples taken. The local variation of cytotypes depends on how many clones colonize the site from the surrounding area and on the rate at which new cytotypes are produced. For example at Burgh-Haamstede (The Netherlands) only one, rather rare, cytotype (3n=43) was detected, indicating a recent establishment of one single clone. This example also shows that aneuploid cytotypes are present as clones which maintain and multiply in the field. At most other sites several cytotypes

Table 1. Clonal diversity in triploid gynogenetic forms of the *Muellerianella* complex, demonstrated by variation of cytotypes with different chromosome numbers found in European populations. For each site the number of females of a certain cytotype is given. Additional small samples are: Sant Julia (Andorra) 1x42, Gorges de Lavalle (F) 1x39, Nevers (F) 1x41, Privas (F) 1x41, Sisak (YU) 2x41, Winterberg (BRD) 2x42, Laasphe (BRD) 4x42, Renesse (NL) 1x43, Ulecoten (NL) 1x42, Geldrop (NL) 5x42, Alunda (S) 1x42, Krankesjön (S) 4x42, Fuerty (IRE) 2x42.

| sites | | chromosome numbers (cytotypes) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 |
| Lissycasey | (IRE) | | 1 | 1 | 1 | | | | 1 | 2 | 3 | 6 | | |
| Letterfrack | (IRE) | | | 2 | | 4 | | 1 | | | 4 | 1 | | |
| Derrywode | (IRE) | | | | | | 3 | | | 5 | 2 | 2 | 1 | |
| Dungarvan | (IRE) | | | | | | | 1 | 2 | 2 | 6 | 5 | | |
| Recess | (IRE) | | | | | 1 | | | | | 5 | 1 | | |
| Glengarriff | (IRE) | | | | | | | | | 1 | 1 | 5 | | |
| Knock/Castlerea | (IRE) | | | | | | | | 2 | | 1 | 8 | | |
| St. Davids | (UK) | | | | | 1 | | | | 2 | 2 | 14 | 5 | |
| Rebild | (DK) | 1 | 1 | 2 | 3 | | | | | | 20 | 3 | | |
| Varde | (DK) | | | 1 | | | | | | | 9 | 6 | 1 | 1 |
| Fröstrup | (DK) | | | | | | | | | | 1 | 14 | 1 | |
| Rödhus | (DK) | | | | | | | | | | 4 | 9 | 1 | |
| Aabenraa | (DK) | | | | | | | | | | 7 | 12 | | |
| Schiermonnikoog | (NL) | | | | | | | | 2 | 6 | 11 | 3 | | |
| Terschelling | (NL) | | | | | | | | | 1 | 6 | 12 | 1 | |
| Texel | (NL) | | | | | | | 1 | 1 | 4 | 11 | | | |
| Wolvega | (NL) | | | | | | | | 1 | 2 | 7 | | | |
| Denekamp | (NL) | | | | | | | | 2 | 4 | 28 | 4 | | |
| Drenthse Aa | (NL) | | | | | | | | | 8 | 19 | 4 | | |
| Renkum | (NL) | | | | | | | | | | 17 | 2 | | |
| Kortenhoef | (NL) | | | | | | | | | 2 | 10 | 3 | | |
| Veenendaal | (NL) | | | | | | | | 1 | 2 | 1 | | 1 | |
| Rhenen | (NL) | | | | | | | | | 12 | 50 | 4 | | 1 |
| Leersum | (NL) | | | | | | | | | 5 | 16 | 6 | | |
| Langeraar | (NL) | | | | | | | | | | 6 | 14 | | |
| Burgh-Haamstede | (NL) | | | | | | | | | | | 10 | | |

| sites | | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Baarle Nassau | (NL) | | | | | | | | | | 6 | 1 | 1 | |
| Weert | (NL) | | | | | | | | | 2 | 33 | 4 | | 1 |
| Elsloo | (NL) | | | | | | | | | | 1 | 1 | 1 | |
| Francorchamps | (NL) | | | | | | | | | | 4 | 19 | | |
| Sourbrodt | (B) | | | | | | | | 1 | | 1 | 12 | | |
| Paderborn | (BRD) | | | | | | | | | | | 2 | 5 | |
| Brilon | (BRD) | | | | | | | | | | | 7 | 1 | |
| Dülmen | (BRD) | | | | | | | | | | | 19 | | |
| Emmendingen | (BRD) | | | | | | | | | | | 5 | 2 | |
| Steinen | (BRD) | | | | | | | | | | 1 | 1 | 4 | |
| Graz | (AUS) | | | | | | | | | | 1 | 10 | 1 | 3 |
| Zvornik | (YU) | | | | | | | | | 1 | 20 | | | |
| Bela Palanka | (YU) | | | | | | | | | 1 | 1 | 2 | | |
| Bitola | (YU) | | | | | | | | | 1 | | | 4 | |
| Cap Gris Nez | (F) | | | | | | | | | | | 4 | 4 | |
| Total Europe | | | | | | | | | | | | | | |
| no. of individuals | | 1 | 2 | 4 | 6 | 6 | 3 | 4 | 13 | 66 | 311 | 245 | 44 | 6 |
| no. of sites | | 1 | 2 | 3 | 3 | 3 | 1 | 4 | 9 | 21 | 35 | 44 | 18 | 5 |

were present, suggesting multiple colonization. In that case the local
cytotype diversity is correlated with the variation in surrounding
populations.

On the other hand, new cytotypes may rapidly evolve at a certain site
after the establishment of one or two triploid clones by loss, breakage,
or non-disjunction of chromosomes (see also discussion). This seems more
likely than an increase by non-disjunction starting from a diploid gyno-
genetic female. Evidence for such a local production of cytotypes is
provided for instance by the sample from Rebild (Denmark). It seems highly
unlikely that the four rare cytotypes present (3n=32, 33, 34 and 35)
colonized the site independently. Presumably cytotypes 3n=32, 33 and 34
evolved from cytotype 3n=35 by recurrent loss of chromosomes. It is possible
that certain clones (genotypes) produce new cytotypes more frequently than
others.

Whatever the source of local clonal variation may be, it is likely to be correlated with the age and stability of the population and with features of the habitat. It is striking for example that at unstable transient sites like Zvornik, Dülmen and Renkum (Table 1) only one or two cytotypes were detected, although sample size was sufficiently large. All three sites can be described as ecotonal and marginal, being roadsides and a trench, while suitable habitat was present only over a few square meters. At sites where more cytotypes were found, like Denekamp, Drenthse Aa, Rhenen, Leersum, Varde, Rebild and several of the Irish sites, the habitat consisted of more natural, extensively managed grasslands and the populations there were usually larger.

In Fig. 3 the proportional occurrence of cytotypes is given for pooled samples from different geographic areas. It appeared that there is much more variation in Ireland where 11 cytotypes were found, than in Central Europe where cytotypes with low chromosome numbers are lacking. The clonal diversity (as measured by cytotype diversity) differs significantly between these regions (Shannon–Weaver index, $H_1=1.80$, $H_2=0.79$, $t=7.14$, $df=212$, $P<0.001$). Another remarkable fact is that cytotype $3n=41$ prevails in Dutch populations
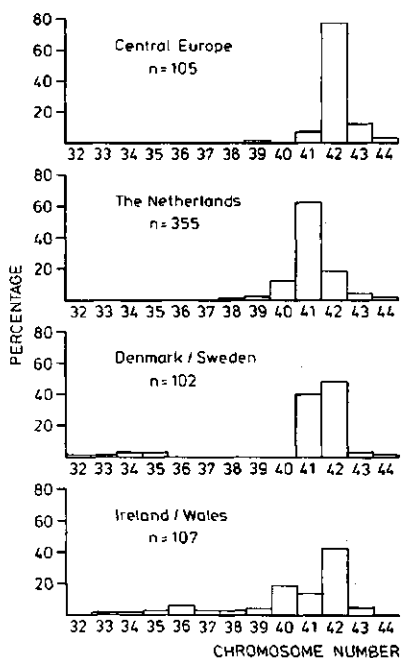


*Fig. 3.* Frequency of cytotypes with different chromosome numbers, found in gynogenetic *Muellerianella* populations, sampled in different parts of Europe. For each site the number of females belonging to a certain cytotype is given.

whereas 3n=42 dominates in populations from Central Europe.

During our study some females were found which did not fit in the picture presented above. Two of these (from Geldrop and Paderborn respectively) showed metaphase plates with 29 univalents (Fig. 2g). Unfortunately no rearings had been set up before dissection, but gynogenetic reproduction seemed likely. Metaphase plates in these females were highly stable, showing regular arrangement of univalents in all eggs examined. The configuration strongly resembled those found in triploid gynogenetic forms. One might suggest that the females mentioned were either hybrids or aberrant females of the morphological similar species *M. fairmairei*. However, in hybrids usually all or at least most metaphase plates are irregular and show mixtures of bivalents and univalents. At the Paderborn site, where one of the females was found, only the bisexual species *M. brevipennis* occurred and *M. fairmairei* was absent nor did it occur in the wide surrounding. Finally it is unlikely that the females represented extreme aneuploid triploids because cytotypes with less than 32 chromosomes were never found. At Paderborn and Geldrop, where the two females were found, even no cytotypes were found with less than 42 chromosomes.

The only interpretation left is that these females should be regarded as diploid gynogenetic clones with one extra chromosomal body. For the evolution of the triploids such clones may be of great significance (see discussion).

Finally four triploid females were found in which meiotic tendencies were not fully suppressed (Weert (2), Rhenen, Cap Gris Nez). In these females part of the eggs showed metaphase plates in which a considerable number of bivalents was formed (Fig. 2h). Other eggs in these females contained regular metaphase plates with 41 or 42 univalents respectively. From one of the females a rearing was set up, from which it appeared that females of the next generation also produced eggs with pairing and eggs with non-pairing chromosomes. This suggests that the mechanism which regulates pairing is easily switched and genetically determined.

DISCUSSION

In evolutionary theory on sexuality it is still a matter of debate whether the twofold reproductive advantage of asexual reproduction compensates

for the absence of recombination (Williams, 1975; Maynard Smith, 1978). In sexual organisms recombination enables the species to spread the risk of an unpredictable and heterogeneous environment over a great number of different genotypes, to exploit a wide range of ecological resources, to reduce competition among offspring, to adapt to changing ecological conditions, and to regain certain genotypes rapidly after heavy population losses.

In order to compete successfully with the bisexual species, asexual organisms should have properties which compensate for the loss of recombination. In addition to the reproductive advantage of not producing males, the ability to preserve well-adapted flexible genotypes and a certain degree of clonal variation within and between populations may account for the persistence of asexual "species". According to White (1978) the great majority of thelytokous forms are of hybrid origin and many of them are polyploid. Due to these properties asexual forms may strongly benefit from heterosis and phenotypic plasticity. Clonal variation may further increase the adaptive potential of many asexual "species" and their success in competition with sexual relatives. The main sources of clonal variation are multiple origin, mutation and chromosomal rearrangements.

## The origin of triploid gynogenetic Muellerianella forms

From cross experiments, Drosopoulos (1978) concluded that the triploid gynogenetic *Muellerianella* forms arose as a hybrid product between *M. fairmairei* and *M. brevipennis*, containing two chromosome sets of the former and one of the latter species. Indeed these species can easily be hybridized in the laboratory (Drosopoulos, 1978; Booij, 1982 ). Hybrid females frequently produce eggs with highly unstable meiotic plates. Non-pairing in these eggs may result in diploid eggs, which, after fertilization by haploid sperm, might give rise to triploids. The geographic distribution of the triploid forms, which corresponds    largely with the area of overlap between the bisexual species, also favours the hybrid origin hypothesis (Booij, 1981a). However, several new attempts to synthesize triploids in the way reported by Drosopoulos (1978) were not successful (Booij, 1981b) .

An alternative explanation for the origin of triploid *Muellerianella* forms may be that they arose by occasional hybridization of a diploid gynogenetic clone with any of the bisexual species. Such an explanation has also been suggested for triploid salamanders of the genus *Ambystoma*

80

(Uzzel and Goldblatt, 1967), for the triploid fish *Poecilia formosa* (Rasch, Prehn and Rasch, 1970) and for triploid lizards of the genus *Chemidophorus* (Cuellar, 1974). Because of stronger heterosis, the newly formed triploids may replace the original diploid thelytokous forms at most places. The existence of diploid gynogenetic *Muellerianella* forms is indicated by two females reported in this paper. Both females were diploid and only univalents were observed at meiotic metaphase. At least for one of the females there was good evidence for gynogenetic reproduction. Since only two such females were found among 1500 gynogenetic females, examined during the whole investigation, the diploid gynogenetic females seem to be extremely rare. Unfortunately it is not known whether these diploid females were autodiploid or allodiploid. If they were autodiploid, this would support the idea of Cuellar (1974) that thelytoky and hybridization are not as closely linked as is thought by most other authors (Schultz, 1969; Uzzel and Goldblatt, 1967; and others).

*Clonal variation in gynogenetic Muellerianella populations.*

In recent years clonal variation has been studied in a great variety of asexual "species", including poecilid fishes (Kallman, 1962; Vrijenhoek et al., 1977, 1978; Angus and Schultz, 1979; Moore and Eisenbrey, 1979; Angus, 1980), whiptail lizards (Cuellar, 1977; Parker and Selander, 1976), black flies (Basrur and Rothfels, 1959), moths (Stalker, 1956; Ochman et al., 1980; Lokki et al., 1975; Mitter et al., 1979), beetles (mainly weevils) (Saura et al., 1976; Suomalainen and Saura, 1973; Lokki et al., 1975, 1976a, 1976b; Suomalainen et al., 1976), cockroaches (Parker et al., 1977), grasshoppers (White, 1973, 1978), and earthworms (Jaenike et al., 1980). All these studies have shown that there is much more genetic variation in asexual populations than previously believed.

The number of genetic variants detected in each asexual species, depends strongly on the sample size and the methods of analysis used. Without doubt tissue graft analysis, as used in studies on asexual fishes (Angus and Schultz, 1979; Moore and Eisenbrey, 1979; Angus, 1980) is the most powerful method, followed by electrophoresis which was applied in most other studies. In this respect, studies on chromosomal polymorphisms seem to be least discriminating.

One of the most spectacular cases of clonal diversity, however, was found by cytogenetic methods. In the parthenogenetic black fly, *Cnephia*

*mutata*, over 700 biotypes were found, being heterozygous for different chromosomal inversions (Basrur and Rothfels, 1959; and see White, 1973). Only a few publications deal with aneuploidy in asexual forms. This is somewhat surprising, since many asexual forms are polyploids, which are regarded to be relative insensitive to aneuploidy. The cases reported mostly concern minor deviations from the usual number (Mikulska, 1960; Roth and Cohen, 1968; Suomalainen, 1940) or variation between eggs within individuals (Oliver et al., 1973; Takenouchi, 1969, 1981). In that respect clonal variation in the asexual *Muellerianella* populations is rather unique, showing extensive aneuploid series ranging from 32 to 44 chromosomes. In contrast to the cases reported from weevils by Takenouchi (1969, 1981), where chromosomal variation occurs within offspring of a single individual, the cytotype variation in *Muellerianella* should be regarded as clonal variation. At most of the sampling sites 2-7 different cytotypes were detected. Since each cytotype probably covers several or many genotypes, much more genetic variation is likely to be found when more discriminative methods are used.

In most other organisms studied thus far, except *Cnephia mutata*, clonal variation per site does not exceed the variation found in *Muellerianella*, although more powerful methods of analysis were used. In asexual fishes the number of clones per site mostly varies from 1-6 (Kallman, 1962; Vrijenhoek et al., 1978; Angus and Schultz, 1979), although in one river 17 clones were detected (Angus, 1980). Extensive variation in electromorphs was also reported in parthenogenetic weevils but no more than 10 clones per site were found (Suomalainen and Saura, 1973; Saura et al., 1976; Lokki et al., 1976).

A feature which characterizes all asexual populations studied thus far, is the predominance of one or two genotypes in most samples, the other clones occurring locally and in low frequencies (Saura et al., 1976; Lokki et al., 1976; Vrijenhoek et al., 1978; Angus and Schultz, 1979; Parker and Selander, 1976; Jaenike et al., 1980; Ochman et al., 1980). This is also true for the asexual *Muellerianella* forms, of which 78% of the animals belong to cytotypes with 3n=41 and 3n=42. Such predominant clones may be regarded as well-adapted generalized clones wich outcompete the less flexible specialized clones at most places (Parker et al., 1977; Vrijenhoek et al., 1978; Angus and Schultz, 1979; Jaenike et al., 1980).

The overall clonal diversity of an asexual "species" is primarily

82

determined by the rate at which new clones are generated and the rate at which they become extinct. The clonal variation at a certain locality or geographic region is affected by factors comparable with those affecting species diversity. In conformity with theories on species diversity, it has been suggested that clonal diversity may be promoted by temporal stability and spatial heterogeneity (Vrijenhoek et al., 1978; Moore and Eisenbrey, 1979; Jaenike et al., 1980; Ochman et al., 1980). However, there are no convincing data favouring these hypotheses. Indirect supportive evidence is constituted by data on clonal diversity in the beetles *Adoxus obscurus* (Lokki et al., 1976a) and *Polydrosus mollis* (Lokki et al., 1976b). The latter species, which lives in climax communities, is much more polymorphic than the former which lives in unstable weedy habitats.

Another factor which affects clonal variation, is the degree of isolation between populations, which on its turn depends on the distribution of suitable habitats and the vagility of the animals. The clonal variation of an asexual form as a whole may be high if it consists of many isolated populations which have had time to differentiate. High vagility in such cases would reduce the diversity of clones because locally evolved genotypes would often be replaced by high fitness genotypes from elsewhere. Such an explanation was given for the fact that several non-flying asexual insect species as a whole are much more polymorphic (Lokki et al., 1975; Suomalainen and Saura, 1973; Saura et al., 1976) than migrating ones (Lokki et al., 1976a). Clonal variation per site, however, was found to be higher in the flying species. Such an effect might be expected, especially when local populations are temporarily, and clonal diversity depends largely on establishment by clones from elsewhere.

Finally population-size is a significant factor affecting clonal diversity. One would expect large populations to sustain more clones than smaller ones, because the random extinction rate is higher in smaller populations (Moore and Eisenbrey, 1979; Vrijenhoek et al., 1978).

From this theoretical framework we may try to interpret clonal diversity in asexual *Muellerianella* populations. The most striking contrast in clonal diversity was found between populations from Ireland and those from Central Europe. Although the pooled sample size and the number of sampling sites were about the same in these regions, eleven cytotypes were detected in Ireland and only five in Central Europe. On average four cytotypes per site were found in Ireland and only two per site in Central

Europe. Moreover, in Central Europe 84% of the animals belongs to the generalized cytotypes 3n=41 and 3n=42, whereas only 65% of the Irish animals belongs to these categories.

In Ireland *Holcus lanatus*, on which both the triploids and the most suitable sperm donor species *M. fairmairei* live, grows optimally and is very common. Consequently the triploids and *M. fairmairei* are widespread and abundant, and they live in a favourable environment which is characterized by a mild and stable climate as far as temperature is concerned (Booij, 1981a), 1982 ). In Central Europe, however, the circumstances are less favourable. These regions are suboptimal for the hostplant *Holcus lanatus* and the bisexual species *M. fairmairei* is very local. At most sites, the triploids use sperm of *M. brevipennis*, which is less suitable (lower offspring) and lives on another hostplant, *Deschampsia cespitosa* (Booij, 1981a,1981b, 1982 ). As a result the asexual triploids are only locally distributed and densities are generally low. Moreover the climate is harsher and less stable than in Ireland. We suggest that these factors are among the most important to explain differences in clonal diversity between the two regions.

An alternative explanation is that the asexual forms originated in Ireland, and that clonal diversity is negatively correlated with the distance from the origin. This implies that the original well established clones 3n=41-43 allready colonized the central parts of Europe, but that the later derived cytotypes with lower chromosome numbers have not reached these areas yet.

The number of cytotypes present at a certain site is influenced by many factors including the age and size of the population, the degree of isolation with, and the clonal variation of surrounding populations, and the stability and heterogeneity of the habitat. It was shown in this paper that most populations from a certain geographic region have comparable clonal diversities and usually the same cytotypes are prevailing. This indicates extensive exchange of animals between sites. Moreover it was found that at transient unstable habitats usually only one or two cytotypes occurred, whereas three or more cytotypes were found at more natural sites.

The range of existing cytotypes in asexual *Muellerianella* populations might have a multiple origin or most of the cytotypes may be derived from one or a few original clones. It is difficult to imagine how a multiple origin could explain the range of cytotypes, because the related bisexual species all have the chromosome number 2n=28 without any chromosomal

84

variation. The triploids should consequently have 3n=42. This does not mean
that they arose only once, since clones with 3n=42 may have evolved several
times. Some of the aneuploid variation may be explained by non-disjunction
or other abnormalities in the hybrids or in the diploid gynogenetic forms
which gave rise to the triploids.

It seems more likely however, that most cytotypes are derived from
cytotype 3n=42 by loss, non-disjunction or breakage of chromosomes. Fusion
of chromosomes seems less likely, since chromosomes of for example
cytotypes 3n=34 or 35 are not visibly larger than those having 3n=41 or 42.
The fact that non-disjunction of single chromosomes was occasionally observed
in triploid eggs further supports the importance of loss. The idea that
the arisal of new cytotypes occurs rather frequently is favoured by the fact
that at many sites where cytotype 3n=x is abundant, also cytotypes 3n=x+1,
3n=x-1 and 3n=x-2 are present. In many cases, however, this may also be
explained by multiple colonization from surrounding populations.

The stability and maintainance of the aneuploid cytotypes is favoured
by their triploid constitution and by the reduction of meiosis to a single
equational division which eliminates negative effects of absence of pairing.

SUMMARY

Chromosome numbers were studied of females of the bisexual planthopper
species *Muellerianella fairmairei, M. brevipennis* and *M. extrusa,* and
especially of triploid gynogenetic forms of this genus. All material was
sampled alive from 70 localities all over Europe.

Chromosome counts were made on first maturation divisions in eggs,
using an improved method of preparation. All three bisexual species appeared
to be diploid with 2n=28, although a few females with additional chromosomes
were found.

Chromosome numbers in the gynogetic forms, which are principally
triploid and have an ameiotic oogenesis, ranged from 32 to 44 representing
an exceptional aneuploid series. The majority of the females (78%) belonged
to cytotypes with 3n=41 or 42. Clonal variation (cytotype diversity) varied
geographically, being highest in Ireland and lowest in Central Europe.
Factors affecting clonal diversity on a local and regional scale are
discussed. Two females were found with 29 univalents at metaphase I. These

might represent gynogenetic clones at the diploid level, from which the triploids could possibly have arisen. This would be an alternative to the hybrid origin hypothesis of Drosopoulos (1978).

LITERATURE CITED

Angus, R.A. 1980. Geographic dispersal and clonal diversity in unisexual fish populations. Amer. Natur. 115: 531-550.
Angus, R.A., and R.J. Schultz. 1979. Clonal diversity in the unisexual fish *Poeciliopsis monacha-lucida*: a tissue graft analysis. Evolution 33: 27-40.
Basrur, V.R. and K.H. Rothfels. 1959. Triploidy in natural populations of the black fly *Cnephia mutata* (Malloch). Can.J.Zool. 37: 571-589.
Booij, C.J.H. 1981a. Biosystematics of the *Muellerianella* complex (Homoptera, Delphacidae), taxonomy, morphology and distribution. Neth.J.Zool. 31: 572-595.
Booij, C.J.H. 1981b. Biosystematics of the *Muellerianella* complex (Homoptera, Delphacidae, hybridization studies. Genetica 57: 161-170.
Booij, C.J.H. 1982. Biosystematics of the *Muellerianella* complex (Homoptera, Delphacidae): hostplants, habitats and phenology. Ecol. Entom. 7: 9-18.
Cuellar, O. 1974. On the origin of parthenogenesis in vertebrates: the cytogenetic factors. Amer.Natur. 108: 625-648.
Cuellar, O. 1977. Genetic homogeneity and speciation in the parthenogenetic lizards *Cnemidophorus velox* and *C. neomexicanus*: evidence from intraspecific histocompatibility. Evolution 31: 24-31.
Drosopoulos, S. 1976. Triploid pseudogamous biotype of the leafhopper *Muellerianella fairmairei*. Nature 263: 499-500.
Drosopoulos, S. 1977. Biosystematic studies on the *Muellerianella* complex (Delphacidae, Homoptera, Auchenorrhyncha). Meded.Landbouwhogeschool Wageningen 77: 1-133.
Drosopoulos, S. 1978. Laboratory synthesis of a pseudogamous triploid

"species" of the genus *Muellerianella* (Homoptera, Delphacidae). Evolution 32: 916-920.

Drosopoulos, S. and J. Sybenga. 1977. Orientation of the heteromorphic sexbivalent in *Muellerianella* (n=14, holokinetic). Caryologia 30: 343-346.

Halkka, O. 1959. Chromosome studies on the Hemiptera Homoptera Auchenorrhyncha. Ann. Acad.Sci, Fenn., A IV, 43: 1-71.

Jaenike, J., E.D. Parker and R.K. Selander. 1980. Clonal niche structure in the parthenogenetic earthworm *Octolasion tyrtaeum*. Amer.Natur. 116: 196-205.

Kallman, K.D. 1962. Population genetics of the gynogenetic teleost *Mollienisia formosa* (Girard). Evolution 16: 497-504.

Lokki, J., E. Suomalainen, A. Saura and P. Lankinen. 1975. Genetic polymorphism and evolution in parthenogenetic animals II. Diploid and polyploid *Solenobia triquetrella* (Lepidoptera: Psychidae). Genetics 79: 513-525.

Lokki, J., A. Saura, P. Lankinen and E. Suomalainen. 1976a. Genetic polymorphism and evolution in parthenogenetic animals V. Triploid *Adoxus obscurus* (Coleoptera: Chrysomelidae). Genet.Res. 28: 27-36.

Lokki, J., A. Saura, P. Lankinen and E. Suomalainen. 1976b. Genetic polymorphism and evolution in parthenogenetic animals VI. Diploid and triploid *Polydrosus mollis* (Coleoptera: Curculionidae). Hereditas 82: 209-216.

Maynard Smith, J. 1978. The Evolution of Sex. Cambridge University Press 1978.

Mikulska, I. 1960. New data to the cytology of the parthenogenetic weevils of the genus *Othiorrhynchus* Germ. (Curculionidae, Coleoptera) from Poland. Cytologia 25: 322-333.

Mitter, C., D.J. Futuyma, J.C. Schneider and J.D. Hare. 1979. Genetic variation and hostplant relations in a parthenogenetic moth. Evolution 33: 777-790.

Moore, W.S. and A.B. Eisenbrey. 1979. The population structure of an asexual vertebrate *Poeciliopsis 2 monacha-lucida* (Pisces: Poeciliidae). Evolution 33: 563-578.

Ochman, H., B. Stille, M. Niklasson, R.K. Selander and A.R. Templeton. 1980. Evolution of clonal diversity in the parthenogenetic fly *Lonchoptera dubia*. Evolution 34: 539-547.

Oliver, J.H., K. Tanaka and M. Sawada. 1973. Cytogenetics of ticks (Acari: Ixodoidea) 12. Chromosome and hybridization studies of bisexual and parthenogenetic *Haemaphysalis longicornis* races from Japan and Korea. Chromosoma 42: 269-288.

Parker, E.D. and R.K. Selander. 1976. The organization of genetic diversity in the parthenogenetic lizard *Cnemidophorus tessulatus*. Genetics 84: 791-805.

Parker, E.D., R.K. Selander, R.O. Hudson and L.J. Lester. 1977. Genetic diversity in colonizing parthenogenetic cockroaches. Evolution 31: 836-842.

Rasch, E.M., L.M. Prehn and R.W. Rasch. 1970. Cytogenetic studies in *Poecilia* (Pisces) II: Triploidy and D.N.A. levels in naturally occurring populations associated with the gynogenetic teleost *Poecilia formosa* (Girard). Chromosoma 31: 18-40.

Roth, L.M. and S.H. Cohen. 1968. Chromosomes of the *Pycnoscelus indicus* and *P. surinamensis* complex (Blatteria: Blaberidae: Pycnoscelinae). Psyche 75: 53-76.

Saura, A., J. Lokki, P. Lankinen and E. Suomalainen. 1976a. Genetic polymorphism and evolution in parthenogenetic animals III: Tetraploid *Othiorrhynchus scaber* (Coleoptera, Curculionidae). Hereditas 82: 79-100.

Saura, A., J. Lokki, P. Lankinen and E. Suomalainen. 1976b. Genetic polymorphism and evolution in parthenogenetic animals IV: Triploid

*Othiorrhynchus salicis* (Coleoptera, Curculionidae). Entomologica
Scandinavica 7: 1-6.

Schultz, R.J. 1969. Hybridization, unisexuality and polyploidy in the teleost
*Poeciliopsis* (Poeciliidae) and other vertebrates. Amer.Natur. 103:
605-619.

Stalker, H.D. 1956. On the evolution of parthenogenesis in *Lonchoptera*
(Diptera). Evolution 10: 345-359.

Suomalainen, E. 1940. Beiträge zur Zytologie der parthenogenetischen Insekten
I: Coleoptera. Ann. Acad. Sci. Fenn. A 54: 1-144.

Suomalainen, E. 1954. Zur Zytologie der parthenogenetischen Curculioniden der
Schweiz. Chromosoma 6: 627-655.

Suomalainen, E. and A. Saura. 1973. Genetic polymorphism and evolution in
parthenogenetic animals I: Polyploid Curculionidae. Genetics 72: 489-508.

Suomalainen, E., A. Saura and J. Lokki. 1976. Evolution of parthenogenetic
insects. Evol. Biol. 9: 209-257.

Takenouchi, Y. 1969. A further study on the chromosomes of the parthenogenetic
weevil, *Listroderes costirostris* Schönherr, from Japan. Cytologia 34:
360-368.

Takenouchi, Y. 1981. A further chromosome survey on a parthenogenetic
weevil, *Callirhopalus bifasciatus* Roelofs (Coleoptera, Curculionidae).
Genetica 55: 141-145.

Uzzel, T.M. and L. Berger. 1975. Electrophoretic phenotypes of *Rana ridibunda,
Rana lessonae* and their hybridogenetic associate *Rana esculenta*. Proc.
Acad. Nat. Sci. Philadelphia 127: 13-24.

Uzzel, T.M. and S.M. Goldblatt. 1967. Serum proteins of salamanders of the
*Ambystoma jeffersonianum* complex, and the origin of triploid species of
this group. Evolution 21: 345-354.

Vrijenhoek, R.C. 1978. Coexistence of clones in a heterogeneous environment.
Science 199: 549-552.

Vrijenhoek, R.C., R.A. Angus and R.J. Schultz. 1977. Variation and hetero-
zygosity in sexually vs. clonally reproducing populations of *Poeciliopsis*.
Evolution 31: 767-781.

Vrijenhoek, R.C., R.A. Angus and R.J. Schultz. 1978. Variation and clonal
structure in an unisexual fish. Amer. Natur. 112: 41-55.

White, M.J.D. 1973. Animal Cytology and Evolution. Cambridge University
Press. 961 pp.

White, M.J.D. 1978. Modes of speciation. W.H. Freeman and Company, San
Francisco. 455 pp.

Williams, G.C. 1975. Sex and Evolution. Princeton University Press.

# Ecological and distributional differentiation between gynogenetic planthoppers and related sexual species of the genus *Muellerianella* (Homoptera, Delphacidae)

C.J.H. BOOIJ and J.A. GULDEMOND

INTRODUCTION

One of the most intriguing questions in evolutionary biology is why
most organisms reproduce sexually and not parthenogenetically. Although
parthenogenetic forms potentially have a great reproductive advantage over
sexual species, they rarely displace their sexual relatives completely.
Apparently sex and recombination give the sexual species some advantage which
compensates for the reproductive disadvantage. These problems have been
discussed extensively by Williams (1975), Maynard Smith (1978) and others.
Most authors agree that parthenogenetic forms are doomed to extinction sooner
or later by lack of evolutionary potential. However, it is still a matter of
debate whether sexuality has also short-term advantages which compensate for
the lower reproductive rate. Since theoretical models developed by population
geneticists cannot give an adequate answer to this question, it seems
important to collect evidence from existing field populations of related
asexual and sexual forms. The question in that case could be what the outcome
is of competition between the two forms, and which ecological conditions
favour asexual or sexual reproduction.

In several animal groups, in which both the asexual forms and sexual
species from which the asexuals arose are known, the asexual forms have
replaced the related sexual species from part of their ecological or
geographical range and do not compete any longer (Suomalainen 1950, Glesener
and Tilman 1978). The situation is quite different, however, in gynogenetic
and hybridogenetic forms, which reproduce parthenogenetically but depend on
sperm of their sexual relatives. In such forms the geographic distribution of
the asexuals is closely linked to that of the sexual species, and they are
forced to compete at least for sperm, but probably also for food, space and
other resources. If competitive relations in such complexes are only
determined by the difference in reproductive capacity of the two forms, the
mixed population would be highly unstable. Because of the twofold reproductive
advantage of parthenogenesis, the frequency of asexual females in mixed

asexual-sexual populations tends to increase rapidly from generation to generation. Finally, the sexual population will go extinct when, by sampling error, only asexual females are inseminated by the few remaining males. Without sperm resource, the demise of the asexual population follows in the next generation.

These problems have been studied in mixed populations of sperm dependent asexual forms and related sexual fish species of *Poeciliopsis* (Moore et al 1970, Moore and McKay 1971, Moore 1975, 1976, McKay 1971, Schultz 1971, Thibault 1978) and in salamanders of the genus *Ambystoma* (Uzzel 1964, Wilbur 1971). The frequency of asexual forms in the populations was found to differ considerably between localities, but at most sites it remained stable from year to year. Apparently there are mechanisms which stabilize the mixed populations and allow the two forms to coexist. Moreover, the level at which the frequency of the asexual forms is stabilized seems to be influenced by environmental factors.

Moore (1976) developed an illuminating model which relates the frequency of asexuals in a mixed population to differences in fitness between asexual and sexual forms. In his model total fitness is partitioned into three components: (i) the probability of a given offspring to be female (which is 1.0 in asexual forms and usually 0.5 in sexual species), (ii) the probability of a given female being pregnant or inseminated, (iii) primary fitness, including fecundity and mortality. In the asexual- sexual fish populations the frequency of asexual females is stabilized, because the tendency of males to discriminate against asexual females increases when the asexual females become more frequent. The level at which the asexual frequency is balanced depends on the mating behaviour of the males and on differences in primary fitness between sexual and asexual females. Thus when the equilibrium frequency of asexuals in a given population is high, this means that the primary fitness of the asexual forms is relatively high at that place compared with that of the sexual species. Consequently it is possible to study ecological conditions which are favourable for sexual or asexual reproduction respectively, by comparing the asexual frequencies of populations in different environments.

Recently an asexual-sexual complex comparable to those in *Poeciliopsis* and *Ambystoma* was discovered by Drosopoulos (1976, 1977) in the planthopper genus *Muellerianella* (Delphacidae). The asexual forms of this genus are triploid and reproduce by gynogenesis. Sperm may be supplied by males of

90

*M. fairmairei, M. extrusa* or *M. brevipennis.* Although it is possible to study competition of the asexual forms with either of the related sexual species, there is valid reason to study the competitive relations between the asexuals and *M. fairmairei* in the first place. The sexual females are usually associated with *M. fairmairei* because they live on the same hostplant, *Holcus lanatus,* and have similar life histories. At some localities the asexuals occur syntopically with *M. brevipennis,* but since they are confined to different hostplants, there is no competition for food, space or oviposition sites. The asexual females only "steal" some copulations of *M. brevipennis* males (Booij 1982).

It was allready noted by Drosopoulos (1977) that the frequency of asexual females in a Dutch population of *M. fairmairei* was quite stable. In some other populations he found much higher or lower frequencies, however. He also suggested that the two forms may have different ecological requirements which enable them to coexist.

To gain further insights in the ecological and geographic distribution. of asexual and sexual forms, we analysed asexual frequencies in a great many populations both on a larger geographic scale (Europe) and a more regional scale (The Netherlands). This paper presents the results of these investigations. It will be shown that the asexual and sexual forms are adapted to different geographic regions and ecological conditions. When relating asexual frequencies with environmental factors, it is necessary that these frequencies are sufficiently stable in a given environment. It will be shown that this prerequisite is fulfilled and factors are discussed which may contribute to the stability in sexual-asexual *Muellerianella* populations.

MATERIAL AND METHODS

To determine the frequency of asexual females in mixed populations, larvae, adults or diapausing eggs in *Juncus* stems were collected. To avoid biased sampling, the animals or stems were taken regularly spaced over the site. Eggs and larvae were reared to adulthood in the laboratory.

To discriminate between asexual triploid females and *M. fairmairei* females, which are morphologically indistinguishable, chromosome preparations were made of ovarian eggs of freshly killed animals (Booij in prep.). To

characterize the population structure, the ratio of asexual females to *M. fairmairei* females + asexual females is used. Following Moore (1976) this ratio is referred to as the asexual frequency.

The samples were taken at 35 sites in Sweden, Denmark, W. Germany, Austria, Yugoslavia, Greece, Ireland, Belgium, France, Andorra, Spain and Portugal (see Fig. 1). Another series of samples was taken at 27 sites in the Netherlands (see Fig. 3). At each site notes were made on general habitat type, vegetation-structure and composition, soil-type, soilwater level, and land-use. For the Netherlands, regional climatic data were obtained mainly from the Dutch climatic atlas (K.N.M.I. 1972).

Experiments, to study reproductive capacity of females and mating capacity of males, were all made in the laboratory at $20^{o}$C and long-day conditions (LD 18:6). In one experiment total egg-production per female was determined by placing single fertilized females in tubes containing suitable stems of *Holcus lanatus*. Every second day the stems were refreshed and the eggs laid were counted. In another experiment the adult progeny per female was determined from single mated females kept in cages with *Holcus* for 4 weeks. To determine mating capacity of *M. fairmairei* males, single males were kept in cages with 25 or 50 asexual females for one or two weeks, respectively. Insemination of females was checked by examining the spermatheca for the presence of mobile sperm.

## STABILITY OF ASEXUAL FREQUENCIES IN MIXED *MUELLERIANELLA* POPULATIONS

The primary source of instability in mixed populations of sperm-dependent asexual forms and sexual species, is the potential capacity of an asexual female to produce two females for every one produced by a sexual female. This twofold reproductive advantage is only realized however if both female types have equal chances to be inseminated and if they can produce the same number of eggs.

For *Muellerianella* females, Drosopoulos (1977) suggested that in the laboratory asexual females produce more eggs than the sexual *M. fairmairei* females. Although his data were inconclusive, new experiments also showed that the asexual females on average can produce as many as, or even more offspring than *M. fairmairei* females (Table 1). Thus, when sperm is not limited and mortality rates are the same, the twofold reproductive advantage

92

Table 1. Reproductive capacity of asexual (gynogenetic) *Muellerianella* females and sexual *M. fairmairei* females in the laboratory. Mean values and ranges are given for egg-production[*] or the adult offspring[**].

| origin of females | | reproduction | |
| --- | --- | --- | --- |
| | | *M. fairmairei* ♀♀ | gynogenetic ♀♀ |
| Nevers | (France) | * 175 (25-399) n=23 | 217 (59-382) n=15 |
| Bitola | (Yugoslavia) | ** 266 (249-275)n=3 | 265 (219-314)n=3 |
| Leersum | (Netherlands) | ** 164 (54-264) n=5 | 296 (239-355)n=5 |

of the asexuals can be realized. As might be expected, the asexual females rapidly outcompete the sexual *M. fairmairei* in mixed laboratory populations (Drosopoulos, 1977).

With regard to discriminative mating behaviour of males as a stabilizing factor in asexual-sexual populations, the situation in *Muellerianella* is different from that in poecilid fishes (Moore 1976) and in salamanders of the genus *Ambystoma* (Uzzel, 1964), where males discriminate against asexual females, especially when there is an excess of females. In *Muellerianella* however, like in most other insects, males are not very selective in courting and mating females. As a rule it is the female that discriminates between males. *M. fairmairei* males readily court the asexual females and preliminary experiments (Guldemond, unpublished data) indicate that there is no significant preference of males to mate with sexual females. The low selectivity of *M. fairmairei* males is partly compensated by the fact that they are able to inseminate about 15 females a week (Table 2). In the laboratory at 20°C males live for about two months. Assuming that they survive on average for two weeks in the field, they may inseminate as many as 30 females. This means that in a mixed population with an asexual frequency of 0.97 still sufficient males are present to inseminate all females. It should be realized, however, that in the field the mean distance between animals is much greater than in the laboratory cages.

Even if males do not discriminate at all, it is still possible that sexual females have better chances to be inseminated than asexuals. This may be the case when the asexuals and sexuals occur in more or less separate clusters in the field. Such a clustering may arise in *Muellerianella* populations because the larvae move only over very small distances, if at

Table 2. Capacity of *M. fairmairei* males to inseminate receptive asexual females in laboratory cages.

| no. of ♀♀ offered/♂ | period | no. of ♀♀ inseminated |
|---|---|---|
| 25 | 1 week | 15, 15, 14 resp. |
| 50 | 2 weeks | 35, 30, 27, 35 resp. |

all. Most adults are brachypterous and not very mobile either. Thus, even when the asexual frequency is high and males are scarce, a sexual female is likely to find herself near one of her brothers by which she may be inseminated. Although such a mechanism may cause severe inbreeding, it may save the sexual population from extinction. Some degree of spatial isolation between asexuals and sexuals may also arise when the two forms prefer different microhabitats. Also in that case the sexual females may have better chances to be inseminated.

In one population with an asexual frequency of 0.78 (Leersum, The Netherlands) we determined the proportion of inseminated females in both the sexuals and the asexuals. Although the population was at the end of its generation, 14 out of 55 females (25%) appeared not to be inseminated. Of 12 sexual *M. fairmairei* females 11 (92%) were inseminated, whereas of the 43 asexual females only 30 (70%) were inseminated. Although the difference is not significant (Fishers exactly probability test, P=0.12), there is a good indication that the sexual females have better insemination chances. More data are needed to confirm this trend, especially from populations with higher asexual frequencies.

The stability of asexual frequencies in several of the populations studied, indicates that the success of the asexual females must be limited in one or another way. For the mixed Dutch population at Leersum (site 33) Drosopoulos (1977) reported that the sex-ratio in suction samples changed only little from year to year (1973: 0.30, 1974: 0.36, 1975: 0.37). Since there is a direct correlation between sex-ratio and the asexual frequency of a population, these data indicate that the asexual frequency is quite constant in this population.

Recurrent sampling at Leersum and at other sites also suggest that the asexual frequency changes only little in most populations (Table 3). Although changes occur, there is no indication for a general increase, which would

Table 3. Year to year changes in the asexual frequencies of *Muellerianella* populations at five sites in The Netherlands.

| Site | Sample-date | Sample-size | asexual frequency |
|------|-------------|-------------|-------------------|
| 17 Leersum | 7-vii-78 | 100 | 0.72 |
| | 3-vii-79 | 23 | 0.74 |
| | 30- ix-81 | 55 | 0.78 |
| 11 Rhenen | 10-xii-78 | 120 | 0.90 |
| | 14-xii-79 | 123 | 0.75 |
| 21 Geldrop I | 2- x -77 | 44 | 0.43 |
| | 18-vii-78 | 18 | 0.44 |
| 25 Geldrop II | 18-vii-78 | 49 | 0.20 |
| | 18-vii-79 | 35 | 0.14 |
| 27 Renesse | 18-vii-77 | 40 | 0.00 |
| | 19- x -78 | 29 | 0.12 |

be expected when the asexuals tend to displace the sexual species. We are fully aware of the fact that at several of our sample sites the asexual frequency may not have been at equilibrium, especially at newly invaded sites, or at places where conditions rapidly change.

ASEXUAL FREQUENCIES IN *MUELLERIANELLA* POPULATIONS FROM DIFFERENT PARTS OF EUROPE - GEOGRAPHIC TRENDS

The overall distribution of asexual *Muellerianella* forms in Europe is given in an earlier paper (Booij 1981). In that publication it was shown that the asexual forms are widespread in a great part of Europe and that the distribution approximately coincides with the area of sympatry between the sexual species *M. fairmairei* and *M. brevipennis*.

Here we will analyse the pattern of asexual frequencies of 62 populations from different regions in Europe. The results of these investigations are presented in Figure 1 and Table 4. Due to varying sample sizes the estimated asexual frequencies are not equally accurate for each population, but the general picture over Europe is quite clear.

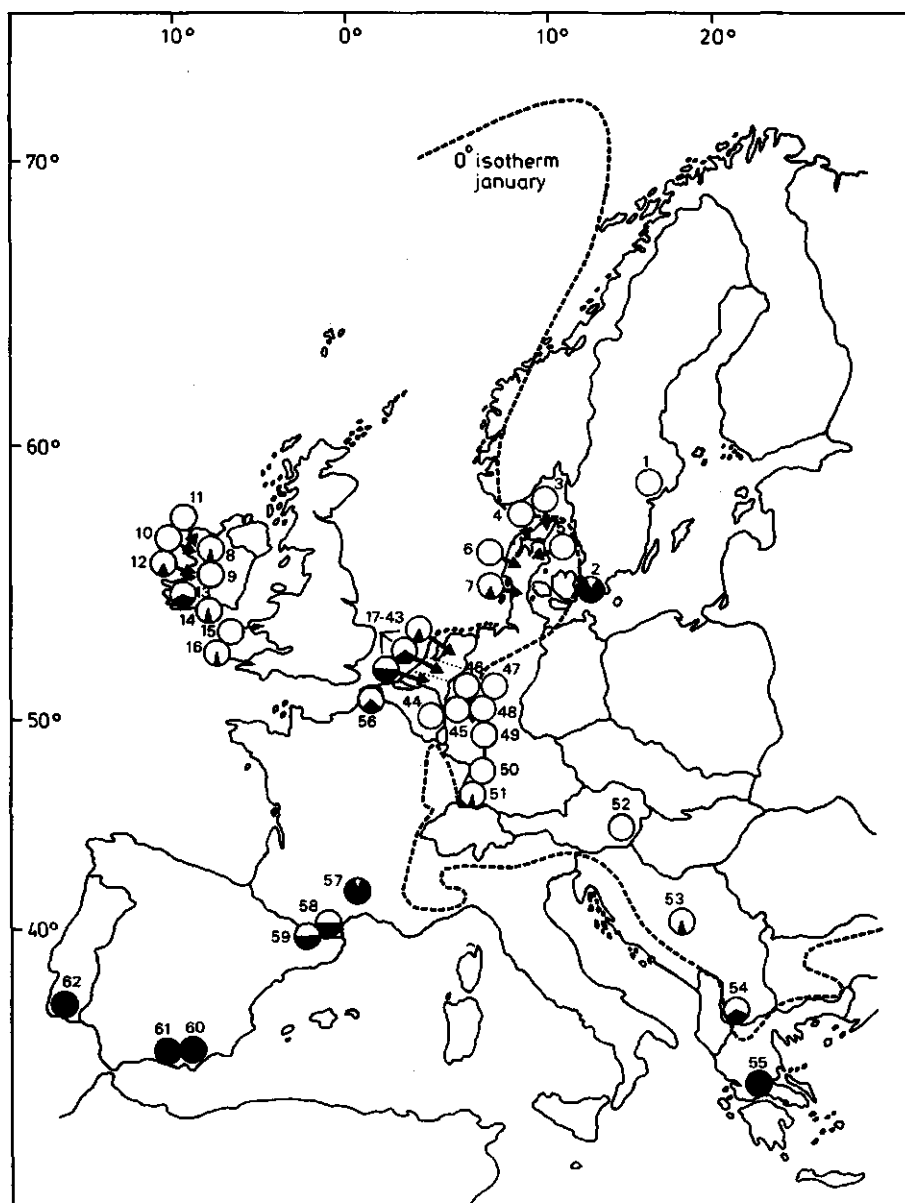From the observed pattern some obvious conclusions may be drawn. First,

*Fig.1.* Frequency of triploid gynogenetic *Muellerianella* forms in mixed populations with the sexual species *M.fairmairei*, in different parts of Europe. At sites 3-6,44,45,47-49 and 52 the asexuals are associated with *M.brevipennis*. *white:*gynogenetic ♀♀ *black: M.fairmairei* ♀♀

Table 4  Asexual frequencies in mixed populations of *M.fairmairei* and *triploid gynogenetic forms* in various parts of Europe. Estimations of asexual frequencies are based on cytogenetic examination of the females, except for sites 16, 58 and 59 where the sex-ratio was used.
Sites where the asexual females are associated with *M.brevipennis* are marked with an asterix.
e: sample of eggs,  l:sample of larvae,  a: sample of adults

| Site | | altitude (m) | sample-date | sample-size | asexual frequency |
|---|---|---|---|---|---|
| 1 Alunda | Sweden | 25 | 15. ix .77 | 7 (e) | 1.00 |
| 2 Krankesjön | Sweden | 50 | 22.viii.77 | 50 (e) | 0.20 |
| 3 Fröstrup | Denmark | 25 | 1.viii.79 | 24 (1,a) | 1.00 * |
| 4 Rödhus | Denmark | 25 | 1.viii.79 | 16 (a) | 1.00 * |
| 5 Rebild | Denmark | 75 | 31. vii.79 | 44 (1,a) | 1.00 * |
| 6 Varde | Denmark | 25 | 1.viii.79 | 26 (1) | 1.00 * |
| 7 Aabenraa | Denmark | 25 | 29. vii.79 | 42 (a) | 0.79 |
| 8 Knock/Castlerea | Ireland | 100 | 27. xi .77 | 49 (e) | 0.98 |
| 9 Derrywode | Ireland | 100 | 28. ix .78 | 15 (e) | 1.00 |
| 10 Recess | Ireland | 50 | 29. ix .78 | 7 (e) | 1.00 |
| 11 Letterfrack | Ireland | 25 | 29. ix .78 | 13 (e) | 1.00 |
| 12 Lissycasey | Ireland | 100 | 30. ix .78 | 24 (e) | 0.79 |
| 13 Glengarriff | Ireland | 25 | 2.  x .78 | 40 (1,a) | 0.68 |
| 14 Dungarvan | Ireland | 0 | 3.  x .78 | 37 (e) | 0.91 |
| 15 St.Davids | Wales (UK) | 50 | 26. ix .78 | 38 (e) | 1.00 |
| 16 Portreath Cornwall (UK) | | ? | 21. vii.70 | - | 0.94 |
| 17 N.Netherlands (10 sites) | | 0 | 1978/1979 | 268 (e,1,a) | 0.94 |
| C.Netherlands ( 7 sites) | | 0 | 1978/1979 | 656 (e,1,a) | 0.64 |
| 43 S.Netherlands (10 sites) | | 0 | 1978/1979 | 445 (e,1,a) | 0.46 |
| 44 Francorchamps | Belgium | 500 | 31. vii.78 | 70 (1,a) | 1.00 * |
| 45 Sourbrodt | Belgium | 600 | 31. vii.78 | 25 (1,a) | 1.00 * |
| 46 Dülmen | W.Germany | 100 | 5. vii.79 | 45 (1,a) | 1.00 |
| 47 Blankenrode | W.Germany | 400 | 5. ix .79 | 10 (a) | 1.00 * |
| 48 Brilon | W.Germany | 300 | 4. ix .79 | 9 (a) | 1.00 * |
| 49 Laasphe | W.Germany | 600 | 28.viii.79 | 4 (a) | 1.00 * |
| 50 Emmendingen | W.Germany | 350 | 29. ix .79 | 14 (a) | 1.00 |
| 51 Steinen | W.Germany | 350 | 29. ix .79 | 20 (a) | 0.95 |
| 52 Graz | Austria | 300 | 3. vi .79 | 25 (1) | 1.00 * |
| 53 Zvornik | Yugoslavia | 300 | 1. vi .79 | 45 (1) | 0.87 |
| 54 Bitola | Yugoslavia | 600 | 26.  v .79 | 12 (1) | 0.58 |
| 55 Skaloula | Greece | 550 | 2. iv .80 | 10 (1,a) | 0.00 |
| 56 Cap Gris Nez | France | 0 | 29. xi .79 | 24 (1,a) | 0.79 |
| 57 Privas | France | 650 | 23.  x .79 | 15 (1,a) | 0.13 |
| 58 Lavalle | France | 300 | vi-75/vi-77 | - | 0.50 |
| 59 Sant Julia | Andorra | 1000 | vi-75/vi-77 | - | 0.50 |
| 60 Orgiva | Spain | 450 | 22. iii.78 | 7 (1) | 0.00 |
| 61 Capifeira | Spain | 1600 | 24. iii.78 | 22 (e) | 0.00 |
| 62 Monchique | Portugal | 300 | 25. iv .80 | 10 (1,a) | 0.00 |

the asexual forms seem to be absent in populations from the southernmost part of the range of *M. fairmairei*. Secondly there is a significant trend for the asexual frequencies to increase with latitude and altitude. Finally the asexual frequency may reach 1.00 (defined in relation to *M. fairmairei*) in the northeastern borderline populations in Denmark, the submontanous areas of W. Germany and Belgium (sites 3-6 and 44-52). Here the asexual females are associated with the more northern species *M. brevipennis*, which lives on another hostplant (see also introduction and discussion).

The pattern of asexual frequencies suggests that there are relations with climatic factors like temperature and humidity. High asexual frequencies are found in the region where *M. fairmairei* is at its northeastern distribution limit, which coincides approximately with the $0^{o}C$ isotherm in January. At these sites the growing season is relatively short and only one generation per year can be completed (Booij 1982). The opposite conditions are found in the mediterranean region where the asexuals are absent (Portugal, Spain and Greece). Here the winter temperatures are much higher and the growing season is much longer. *M. fairmairei* completes often three or more generations per year in these regions.

At the remaining sites of W. Europe usually two generations are completed. Of this category the highest asexual frequencies are found in Ireland. Since the climate of this country is mild but very wet, we suggest that wetness favours the asexual forms. In this context it should be mentioned that a great excess of females in *M. fairmairei* populations has been observed at the Azores (Remane pers.comm.). These islands are situated at the same latitude as the mediterranean sites of *M. fairmairei*, but there is a much more atlantic climate. It is also striking that the lowest asexual frequency in Ireland was found in a population near Glengarriff. This place is well-known for its extremely mild climate. It is the only known Irish site where probably three generations are completed instead of two (Booij 1982). The suggested relations of asexual frequencies with the length of the growing season and wetness are visualized in Figure 2.

It should be kept in mind that deviations from the observed trends may be found due to microclimatic or other ecological conditions at particular sites. Moreover, several geographic regions are insufficiently explored. Additional data are necessary to test the validity of the supposed trends.
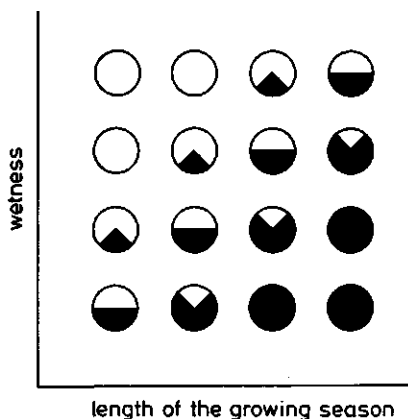
Fig.2. Hypothetical relations of asexual
freqencies in mixed *Muellerianella* popula-
tions with the length of the growing season
and wetness of the climate.
*white:* gynogenetic ♀♀ *black:* M.fairmairei ♀♀

## ASEXUAL FREQUENCIES IN *MUELLERIANELLA* POPULATIONS FROM THE NETHERLANDS — ECOLOGICAL TRENDS

In the Netherlands 27 mixed populations of gynogenetic forms and *M. fairmairei* were traced and screened cytogenetically to determine the asexual frequencies. Since our aim was to study the relation between asexual frequencies and environmental factors (habitat and regional climate), samples were taken from different parts of the Netherlands and from a wide variety of habitats. All sites were situated at altitudes below 50 m.

The results of the investigations are given in Figure 3 and Table 6. Because suitable habitats for *M. fairmairei* and the asexual forms are not very common and only present in certain parts of the Netherlands, sample-sites could not be selected at random or regularly spaced. Some of our data are based on rather small samples, which reduces the accuracy of the estimated asexual frequencies. Despite these limitations several trends could be observed.

It is clear that the average asexual frequency increases slightly but significantly from the southwestern to the northeastern parts of the Netherlands (Spearman rank correlation, $r_s=0.66$, n=27, P< 0.001). The trend
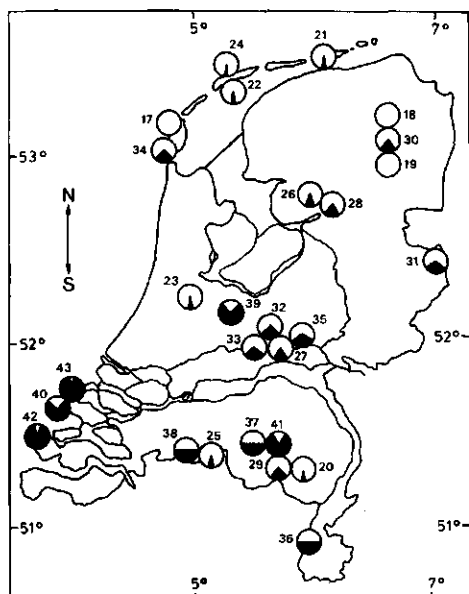
99

Fig.3. Frequency of triploid gynogenetic *Muellerianella* females in mixed populations with the sexual species *M.fairmairei* in the Netherlands.

*white:* gynogenetic ♀♀  *black:M.fairmairei* ♀♀

indicates a relation between regional climatic differences and the asexual frequencies. Therefore correlation coëfficients were calculated between asexual frequencies and 12 climatic variables concerning temperature and humidity (Dutch climatic atlas, KNMI, 1972). The asexual frequencies appeared to be significantly correlated with eight of the variables (Table 5). Although the correlations are weak, they are consistent with two general trends: (i) the asexual frequency increases with decreasing average temperatures, especially with regard to spring-temperatures, (ii) the asexual forms seems to be favoured at places where precipitation or humidity is high. Both trends are in general agreement with the European trends that the asexual frequencies tend to be high with winter-temperatures are low, the growing season is short and where humidity is high. In Figure 4 two of the significant correlations are given in scatter diagrams.

Since regional climatic data can only be a rough measure for the real temperatures and humidities to which the animals are exposed in the field, regional climatic differences can only partly explain the variation between asexual frequencies at different sites. The remaining variation may be

100

Table 5. Significant correlations between asexual frequencies of
*Muellerianella* populations (n=27) and regional climatic factors in the
Netherlands. $r_s$: Spearman rank correlation coëfficient. Levels of
significance: * P<0.05 ** P<0.01

| climatic variable | $r_s$ | |
|---|---|---|
| 1 average precipitation may—october | 0.39 | * |
| 2 precipitation/saturation deficit june—october | 0.45 | * |
| 3 average number of days with snowcover | 0.39 | ** |
| 4 average annual temperature | −0.49 | ** |
| 5 day at which temperature exceeds $10^{\circ}C$ | −0.49 | ** |
| 6 average temperature in april | −0.39 | * |
| 7 average temperature in may | −0.44 | * |
| 8 average temperature in june | −0.41 | * |

explained by differences in microclimate, vegetation structure and other
ecological features of the habitat. Since the range of variation between
asexual frequencies at Dutch sites is almost as wide as the variation in the
whole of Europe, these local factors must be very important.

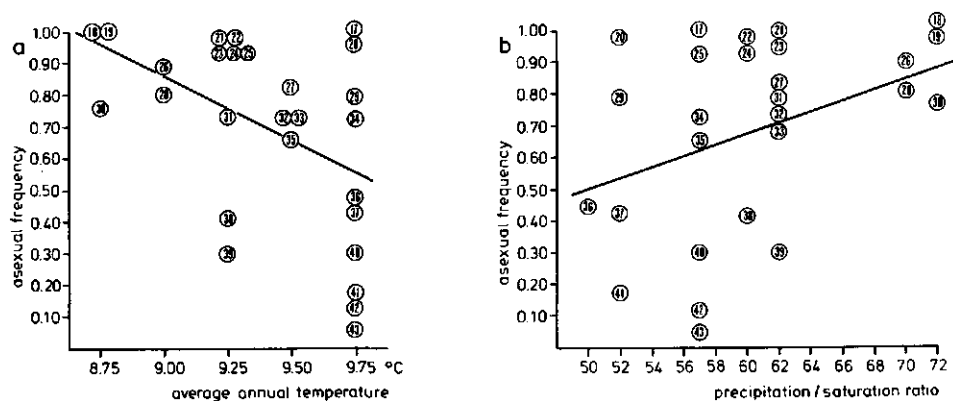When Table 6 is studied more closely, it becomes clear that at all



*Fig.4.* Correlations of the asexual frequency in Dutch asexual-sexual *Muellerianella* populations
with two regional climatic factors. The numbers refer to sites.

Table 7. Predicted and observed asexual frequencies of samples taken in mixed asexual-sexual *Muellerianella* populations in 1979. Predictions were based on ecogeographic trends found in samples taken in 1978 at other sites.

| Site | Asexual frequency | |
| --- | --- | --- |
| | predicted | observed |
| 6 Terschelling II | 0.90 | 0.95 |
| 8 Terschelling I | 0.90 | 0.97 |
| 9 Baarle Nassau | 0.70 | 0.93 |
| 10 Wolvega I | 0.70 | 0.89 |
| 12 Wolvega II | 0.90 | 0.80 |
| 20 Elsloo | 0.40 | 0.45 |
| 22 Ulicoten | 0.40 | 0.41 |

The relation of asexual frequency with regional climate, landuse and wetness of the habitat were allready recognized from the samples taken in 1978. At the new sites, sampled in 1979, predictions for the asexual frequency, based on regional climate and habitat features, were made before the population was actually sampled. In this way our ideas could be tested. As Table 7 shows, the predictions were pretty good. Therefore it seems justified for the moment to state that the *gynogenetic Muellerianella* forms occur more abundant in cool, wet and disturbed (mown) habitats, whereas the *sexual* species *M. fairmairei* is favoured at warmer, moist and less disturbed localities.


DISCUSSION

A fundamental question in the population ecology of gynogenetic forms and the sexual species on which they depend for sperm, is what allows the two forms to coexist (Moore 1976). When the twofold reproductive advantage of the asexuals is realized in the field, they will rapidly displace the sexual species on which they depend. The instability of such mixed populations may be reduced if the asexuals have less chance to be inseminated or if their primary fitness is much lower than that of the sexual species (Moore 1976). By studying the competitive relations between the asexual and

sexual forms we may find out what the use of sexuality is, or at least in what situations sexuality is favoured.

As was shown in this paper, the fecundity of gynogenetic *Muellerianella* females is at least as high as that of the sexual *M. fairmairei* females, with which they are associated. Moreover, *M. fairmairei* males probably do not discriminate against the asexual females and may inseminate several tens of females of both forms. In this respect the planthopper complex differs essentially from comparable complexes in fishes and salamanders, where mate-discrimination is an important mechanism to stabilize mixed asexual-sexual populations (Uzzel, 1964, Wilbur 1971, McKay 1971, Moore and McKay 1971, Moore 1975, 1976). Yet the asexual frequency in mixed *Muellerianella* populations appeared to be rather stable.

It was found that in the field the asexual females tend to be less frequently inseminated than the sexual females. We suggest that this tendency may be caused by the microdistribution of sexuals and asexuals in the field. Especially when males are scarce it is likely that many of the asexuals are not in close contact with the males, while most of the sexual females are. In other words, the insemination of asexuals is only effectively reduced at very high asexual frequencies. Since at many places the asexual frequency is balanced at a lower level, which means that sufficient males are present to inseminate all females, it is likely that at those places the primary fitness of the asexual females is lower than that of the sexual females.

Since the level at which the asexual frequency is balanced is a function of the fitness-differences between asexual and sexual females (Moore 1976), local differences in asexual frequencies indicate that environmental factors affect the fitness differences. The present paper has shown that the asexual frequency in mixed *Muellerianella* populations varies geographically and depends on ecological conditions. This suggest that the two forms are adapted to different environments and different geographic regions.

When the distribution of asexual organisms in general, is compared with that of the related sexual species, it appears that asexual forms tend to occur at higher altitudes and latitudes (Suomalainen 1950, Glesener and Tilman 1978 and others). As Glesener and Tilman have argued, these geographic trends are not merely geographic, but should be considered in relation to the underlying environmental factors which favour sexual or asexual reproduction respectively.

From a review on parthenogenetic *Cnemidophorus* lizards, Wright and Lowe

(1968) conclude that asexual forms are mostly restricted to so called "weed habitats". These habitats are generally characterized by terms as disturbed, disclimax, severe, ecotone, transient etc. This "weed" hypothesis is essentially supported by the studies on asexual fishes (Moore 1976) and by data given by Glesener and Tilman (1978) in their review on geographic parthenogenesis. Indeed, many asexual organisms have features which are typical for "r-strategists" such as high reproductive rates, phenotypic plasticity (due to heterosis and polyploidy), preservation of adaptive gene-complexes, and colonizing ability (in sperm-independent forms) (Moore 1976). In general, they are not able however, to displace the more "K-selected" sexual species from biologically complex (competitive) environments (Glesener and Tilman 1978). The latter kind of habitats can be described as abiotically more stable, climax, ecoclinal, undisturbed and mesic.

It might be argued, as was done by Cuellar (1977), that the ecogeographic trends outlined above do not hold for gynogenetic and hybridogenetic forms, because these can only occur together with the sexual species on which they depend for sperm.

From the present study on gynogenetic and sexual *Muellerianella* forms, however, several conclusions can be drawn which are in general agreement with the ecogeographic trends of parthenogenesis.

The first conclusion is that the gynogenetic *Muellerianella* forms are absent from the southernmost part of the range of the sexual species *M. fairmairei* and that the asexual frequencies increase when going from SW to NE Europe. Thus there is good evidence for a geographic replacement. The second remarkable trend is that the asexual forms occur without *M. fairmairei* in Denmark and at higher altitudes in Central Europe. In these regions the asexual populations maintain at low densities, by using males of another species, *M. brevipennis*, as sperm donor. At these places the asexual females live on their usual hostplant, *Holcus lanatus*, which grows there intermixed with the hostplant of *M. brevipennis*, *Deschampsia cespitosa*. Although *M. fairmairei* also lives on *Holcus lanatus*, this species is apparently unable to maintain itself at these sites. Its absence may be due to environmental conditions or to competition with the asexual forms. In the latter case the asexuals can, thanks to the presence of *M. brevipennis*, exclude *M. fairmairei* from part of its potential range.

It seems likely that the present distribution of the gynogenetic *Muellerianella* forms is correlated with their possible hybrid origin.

106

According to Drosopoulos (1977, 1978), the gynogenetic forms probably arose through hybridization between the originally south-european species *M. fairmairei* and the more northern species *M. brevipennis* (see also Booij 1981, 1982). The restriction of the gynogenetic forms to the area of sympatry between the parental species, and high asexual frequencies in regions where both species frequently occur syntopically, strongly support the hybrid origin hypothesis. This is also in agreement with the idea that asexual forms of hybrid origin are most successful in ecological conditions which are intermediate between those of the parental species (Uzzel and Darevsky 1975, Thibault 1978, Moore 1976).

Finally we like to discuss the ecological differentiation between the asexual *Muellerianella* forms and the sexual *M. fairmairei*. As was shown in this paper, the frequency of asexuals in mixed populations varies considerably between the 27 sample sites in the Netherlands, which is a relatively small area. Part of the variation between sites can be attributed to regional climatic differences. The correlations of the asexual frequencies with Dutch climatic data are consistent with the trends found in Europe, but they are weak. Apparently the success of the asexuals is highly affected by other factors like microclimate, vegetation structure and landuse. In the mixed Dutch populations high asexual frequencies were especially found in meadows, trenches and steep transitions between wet and dry. In extensively grazed pastures and unmanaged grassy places, however, the sexual species is usually dominating.

Abiotic and biotic features which characterize these two habitat categories may have significant effects on the reproduction and survival of the animals. Meadows are usually wet in spring which keeps temperature relatively low during the first months of the growing season. The meadows in which *Muellerianella* occurs, are mown only once in late summer. This causes a sudden change in the structure of the vegetation and consequently in the microclimate. As was shown by Morris (1981), cutting of grasslands has severe negative effects on many insect species. After cutting the insects are suddenly exposed to drought and higher temperatures. Compared with extensively grazed pastures and natural habitats, meadows are structurally rather homogeneous. Because regular mowing prevents tussock-information the microclimatic differentiation is rather poor (Larsson 1976). Also trenches and ditch-sides are usually mown once a year. In addition to this, ground-water-levels at these places often fluctuate throughout the year. The same can be

said about ecotones between dry and wet.

In extensively grazed pastures, the oviposition plant of both forms, *Juncus effusus*, forms tussocks which are little affected by cattle. In and around these tussocks, *Holcus lanatus* grows optimally. Such a vegetation offers a wide variety of microniches to the planthoppers. Moreover, the vegetation structure is relatively stable throughout the year. In spring the microclimate of pastures is usually less wet and warmer than that of meadows. Also in more natural habitats there is a wide variety of microniches and there are no sudden changes like in meadows or trenches.

All these considerations justify the conclusion that the asexuals are better adapted to environments which are wet and cold in spring, which are poorly structured, and where abiotic factors suddenly change during the season. The sexual species is more successful at places where abiotic factors are more stable, which are warmer and drier in spring, and where the vegetation structure offers a lot of variation.

These conclusions agree well with the idea of Glesener and Tilman (1978), who suggest that asexual organisms tend to occur in "physically controlled" environments which are poorly structured and where "biological uncertainty" is low. Sexuality, however, seems to be favoured in abiotically more stable environments which are complex and "biologically unpredictable".

SUMMARY

Triploid gynogenetic planthoppers of the genus *Muellerianella* depend on sperm supplied by males of related sexual species. This relationship creates a situation in which sexual and asexual females compete for copulations with males of the sexual species. The gynogenetic *Muellerianella* forms are usually associated with the sexual species *M. fairmairei* because they have similar life-histories.

The frequency of asexuals in such mixed asexual-sexual populations appeared to be rather stable from year to year, but the stabilizing factors could not be clarified yet.

By sampling asexual-sexual *Muellerianella* populations at 62 European sites, data were obtained which indicate that the asexual gynogenetic forms and the sexual species *M. fairmairei* are adapted to different climatic regions and ecological conditions.

The asexual forms are absent from the southernmost part of the range of *M. fairmairei*. In other parts of this range the frequency of asexual females in mixed populations tend to increase with altitude and latitude. In north-eastern borderline populations the gynogenetic forms occur, without *M. fairmairei*, in association with the more northern species *M. brevipennis*.

More detailed information about the occurrence of asexual forms was obtained from 27 Dutch populations. It was found that the asexuals are most frequent in cool, wet and disturbed habitats like wet meadows, trenches and sharp transitions between wet and dry. The sexual species *M. fairmairei* is favoured at warmer, moist and less disturbed habitats like extensively grazed pastures and grassy places in forests.

The results are discussed in relation to ecological and geographic trends for sexual vs. asexual reproduction in general, which have been discussed by Suomalainen (1950), Glesener and Tilman (1978) and others.

LITERATURE CITED

Booij, C.J.H. 1981. Biosystematics of the *Muellerianella* complex (Homoptera, Delphacidae), taxonomy, morphology and distribution. Neth.J.Zool.31: 572-595.
Booij, C.J.H. 1982. Biosystematics of the *Muellerianella* complex (Homoptera,

Delphacidae): hostplants, habitats and phenology. Ecol.Entomol. 7: 9-18

Cuellar, O. 1977. Animal parthenogenesis, a new evolutionary-ecological model is needed. Science 197: 837-843.

Drosopoulos, S. 1976. Triploid pseudogamous biotype of the leafhopper *Muellerianella fairmairei*. Nature 263: 499-500.

Drosopoulos, S. 1977. Biosystematic studies on the *Muellerianella* complex (Delphacidae, Homoptera, Auchenorrhyncha). Meded. Landbouwhogeschool, Wageningen 77: 1-133.

Drosopoulos, S. 1978. Laboratory synthesis of a pseudogamous triploid "species" of the genus *Muellerianella* (Homoptera, Delphacidae). Evolution 32: 916-920.

Glesener, R.R. and D. Tilman. 1978. Sexuality and the components of environmental uncertainty: Clues from geographic parthenogenesis in terrestrial animals. Amer. Natur. 112: 659-673.

K.N.M.I. 1972. Klimaatatlas van Nederland, K.N.M.I. Staatsuitgeverij, The Hague.

Larsson, A. 1976. Wet meadows in southern Sweden: vegetation, succession, and management. Meded. Avd. Ekol. Bot. Lunds Univ. 31: 1-107.

Maynard Smith, J. 1978. The Evolution of Sex. Cambridge University Press.

McKay, F.E. 1971. Behavioural aspects of population dynamics in unisexual-bisexual *Poeciliopsis* (Pisces: Poeciliidae). Ecology 52: 778-790.

Moore, W.S. 1975. Stability of small unisexual-bisexual populations of *Poeciliopsis* (Pisces, Poeciliidae). Ecology 56: 791-808.

Moore, W.S. 1976. Components of fitness in the unisexual fish *Poeciliopsis monacha-occidentalis*. Evolution 30: 564-578.

Moore, W.S. and F.E. McKay. 1971. Coexistence in unisexual-bisexual species complexes of *Poeciliopsis* (Pisces: Poeciliidae) Ecology 52: 791-799.

Moore, W.S., R.R. Miller, and R.J. Schultz. 1970. Distribution, adaptation and probable origin of an all-female form of *Poeciliopsis* (Pisces: Poeciliidae) in northwest Mexico. Evolution 24: 789-795.

Morris, M.G. 1981. Responses of grassland invertebrates to management by cutting. III Adverse effects on Auchenorrhyncha. J.Appl.Ecol. 18: 107-123.

Schultz, R.J. 1971. Special adaptive problems associated with unisexuality in fish. Amer. Zool. 11: 351-360.

Suomalainen, E. 1950. Parthenogenesis in animals. Adv. Genet. 3: 193-253.

Thibault, R.E. 1978. Ecological and evolutionary relationships among diploid and triploid unisexual fishes associated with the bisexual species *Poeciliopsis lucida*. Evolution 32: 613-623.

Uzzel, T.M. 1964. Relations of diploid and triploid species of the *Ambystoma jeffersonianum* complex (Amphibia, Caudata). Copeia 1964: 257-300.

Uzzel, T. and I.S. Darevsky. 1975. Biochemical evidence for the hybrid origin of the parthenogenetic species of the *Lacerta saxicola* complex (Suaria: Lacertidae) with a discussion on some ecological and evolutionary implications. Copeia 1975: 204-222.

Wilbur, H.M. 1971. The ecological relationship of the salamander *Ambystoma laterale* to its all-female gynogenetic associate. Evolution 25: 168-179.

Williams, G.C. 1975. Sex and Evolution. Princeton University Press.

Wright, J.W. and C.H. Lowe. 1968. Weeds, polyploids, parthenogenesis and the geographical and ecological distribution of all female species of *Cnemidophorus*. Copeia 1968: 128-138.

# An evolutionary model for the *Muellerianella* complex

C.J.H. BOOIJ

## ABSTRACT

Using all available information about the *Muellerianella* species, an attempt is made to reconstruct the phylogenetic relations between the species and to postulate a model of speciation for the genus. Although the absence of synapomorphies impede a cladistic analysis, phenetic similarities between the species and results of cross-experiments suggest that *M. extrusa* and *M. fairmairei* are phylogenetically very closely related whereas *M. brevipennis* is more differentiated from both other species. In the hypothetical speciation model, it is assumed that both *M. brevipennis* and *M. fairmairei* originated from a *M. extrusa*-like ancestor species and that the split-off of *M. fairmairei* has been the most recent. It is suggested that the development of man-made grasslands in Europe has influenced the evolutionary processes in the genus *Muellerianella*, in particular the origin of triploid gynogenetic forms.

## INTRODUCTION

When all results of the foregoing papers are brought together, the question arises which evolutionary processes have led to the present diversity patterns in the genus *Muellerianella*. Of course an evolutionary model, as it will be described in this paper, can only be preliminary because more, as yet undiscovered, species may be involved which may complicate the overall picture. Moreover, little is known about the *Muellerianella* species reported from the Eastern Palearctic.

For a reconstruction of the evolutionary processes which possibly have taken place, it is necessary to assess the phenetic and phylogenetic relationships between the species in order to establish the most likely splitting pattern. Based on this splitting pattern, a speciation model may be postulated which includes the possible speciation mechanisms and which relates evolutionary processes to their ecological and geographical context.

SPLITTING PATTERNS

Several problems arise when we try to trace the evolutionary pathways within groups of closely related species. The most serious one is the minor differentiation between the species which makes it hard to find sufficient derived characters which are necessary to clarify the phylogenetic relationships between the species. For closely related species a cladistic analysis is further impeded because parallelism and convergence are probably more common in such groups since the species have similar gene pools and often comparable ecological adaptations (Arnold 1981).

Another problem is that anagenetic differentiation in ancestor species may be negligible, whereas the species which split off may rapidly differentiate because a new niche is occupied (Gould and Eldredge, 1977). Because of this, synapomorphies for certain groups of closely related species may be absent or very difficult to detect. This problem is most explicit when several species in succession split off from a well established ancestor species. These considerations are relevant when discussing the evolutionary relationships between the *Muellerianella* species.

If we assume that *M. brevipennis*, *M. extrusa* and *M. fairmairei* form a monophyletic group, and that no other species are involved, we have three possible combinations of species which have differentiated from each other. Thus, three splitmodels (cladograms) can be imagined for the phylogenetic relationships (Fig. 1).

If any derived character would be known which is shared by two of the three species, this would be an argument to choose for one of the alternatives given in Fig. 1. Unfortunately such derived characters are not known for any combination of species (Table 1). Although several morphological
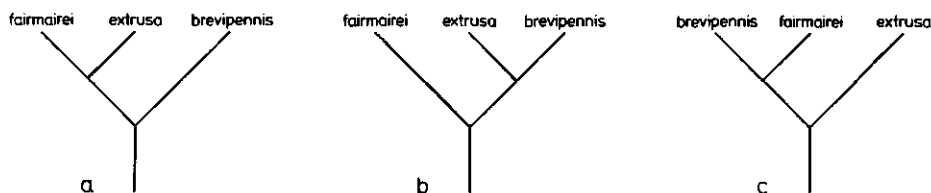


*Fig. 1* Theoretically possible cladograms for the three *Muellerianella* species.

Table 1. Polarity conditions of biotaxonomic characters among species of the genus *Muellerianella*.

| Characters | plesiomorphic in | apomorphic in |
|---|---|---|
| morphology | ? | ? |
| acoustic behaviour | *M. extrusa, M. fairmairei* | *M. brevipennis* |
| ecology | *M. extrusa, M. brevipennis* | *M. fairmairei* |
| distribution | *M. extrusa, M. brevipennis* | *M. fairmairei* |

characters are the same in *M. extrusa* and *M. fairmairei* and different in *M. brevipennis*, none of these can with certainty be regarded as derived. Also the acoustic behaviour patterns of *M. extrusa* and *M. fairmairei* are characterized by similar features, but unfortunately these should be considered as plesiomorphic. With regard to distribution and ecology, *M. brevipennis* and *M. extrusa* show the largest degree of similarity, but for both aspects the condition should be considered as plesiomorphic. For these reasons a cladistic analysis is not fruitful at present for the *Muellerianella* species.

Consequently the problem should be approached in another way. The only clues which we have are the differences and similarities between the species, their interfertility and their distributions. It should be realized that the morphological, ecological and behavioural similarities and also the results of cross-experiments, can only be an indirect measure of the genetic relations between the species. However, if there is coincidence of the various similarities, the evidence for a particular kinship-scheme becomes stronger.

As was shown in the first paper, *M. fairmairei* and *M. extrusa* can be distinguished only by a few morphological characters, whereas *M. brevipennis* differs in more respects from these two species. The hybridization experiments showed that *M. extrusa* and *M. fairmairei* can be crossed easily in the laboratory. Although the Fl hybrids are partly sterile, backcrossing products are fertile and genetic exchange between these species is feasible. Crosses of either of the two species with *M. brevipennis* are less successful. Furthermore there is a general similarity between *M. fairmairei* and *M. extrusa* with regard to acoustic behaviour, whereas the sound-production of *M. brevipennis* is more differentiated. The minor differentiation of *M. fairmairei* and *M. extrusa* regarding the foregoing aspects, justifies to my

113

opinion the conclusion that they are genetically more closely related to each other than either of them is to *M. brevipennis*.

When comparing *M. brevipennis* with *M. extrusa* and *M. fairmairei*, it appears that the phenetic similarity between *M. brevipennis* and *M. extrusa* is larger than that between *M. brevipennis* and *M. fairmairei*. This becomes clear when the ecology of the species is compared. Both *M. brevipennis* and *M. extrusa* are adapted to temperate and boreal climates and usually complete one generation per year. *M. fairmairei* has an atlantic mediterranean distribution and has two or more generations per year. The ecological resemblance between *M. brevipennis* and *M. extrusa* is correlated with the climatic preferences and distribution of their hostplants, which are similar.

Another similarity between *M. brevipennis* and *M. extrusa* is that both species are widespread in natural habitats, in contrast with *M. fairmairei* which lives mainly in man-made habitats. Moreover, the tussock-structure of their hostplants, *Deschampsia cespitosa* and *Molinia cearulea* is similar and both species lay their hibernating eggs in the stems of their hostplant, whereas *M. fairmairei* does not. Finally it should be mentioned that *M. extrusa* can be reared on the hostplant of *M. brevipennis*, *Deschampsia cespitosa*.

Regarding the phenetic similarities and differences between the three species, our tentative conclusion is that *M. fairmairei* and *M. extrusa* are genetically most closely related, whereas *M. fairmairei* and *M. brevipennis* show the largest amount of divergence.

A phenetic analysis of acoustic and morphological characters of the *Muellerianella* species would lead to a phenogram such as that given in Fig. 2a. Evolutionary diagrams which are theoretically plausible and which agree with this phenogram are given in Figs. 2b and 2c. These diagrams correspond with the cladograms of Figs. 1a and 1b respectively. Diagram 2c assumes that the divergence of *M. fairmairei* and *M. extrusa* has been much slower than the divergence of *M. brevipennis* from both other species. This seems unlikely because one would expect the reverse on account of the specialized ecology of *M. fairmairei*. It should be mentioned here that the differences between *M. fairmairei* and *M. extrusa* are extremely small compared with differences found between species within other delphacid genera. Also the crossability of the three species among each other does not support a splitting pattern as that in Fig. 2c, for one expects that cross barriers are least developed in species which diverged most recently.
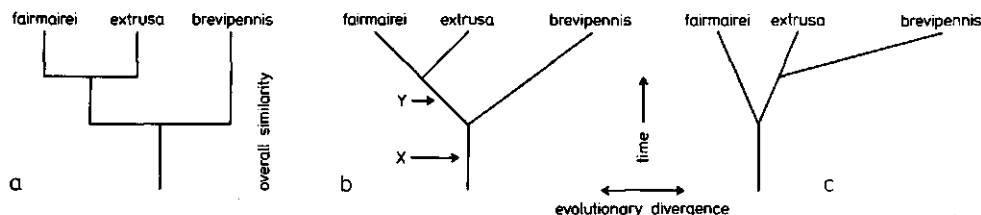
*Fig. 2* Phenogram for the *Muellerianella* species, based on morphological and behavioural similarity (a) and two theoretically corresponding evolutionary diagrams (b) and (c). Explanation, see text.

All these arguments lead to the conclusion that a split model as given in Fig. 2b is more plausible.

THE SPECIATION PROCESS

Assuming that the *Muellerianella* species split according to the pattern of Fig. 2b, one may wonder what the acestral species at points x and y looked like and how the speciation process took place.

For reasons given above it seems likely that the differentiation of *M. fairmairei* and *M. extrusa* is of rather recent origin. The following arguments suggest that the present species *M. fairmairei* originated from a *M. extrusa*-like ancestral species. First, *M. fairmairei* can be regarded as ecologically most specialized. Secondly it occurs mainly in man-made habitats. Finally it has probably been a very local species in the past with a small mediterreanean distribution. In contrast, *M. extrusa* has an extensive range and lives on hostplants which are widespread in natural habitats.

It is more difficult to argue what ancestral species x (Fig. 2b) looked like. Again it seems likely that it was similar to the present species *M. extrusa*. The fact that *M. extrusa* is more or less oligophagous, whereas *M. brevipennis* is strictly monophagous, suggests that *M. brevipennis* evolved from a *M. extrusa*-like ancestor species. The complicated acoustic behaviour of *M. brevipennis*, which should be considered as derived from the more simple pattern in *M. extrusa*, supports this hypothesis.

From the foregoing a final speciation model can be postulated for the *Muellerianella* species (Fig. 3). In this model a *M. extrusa*-like species, which lived primarily on *M. caerulea*, is supposed to be the original species.
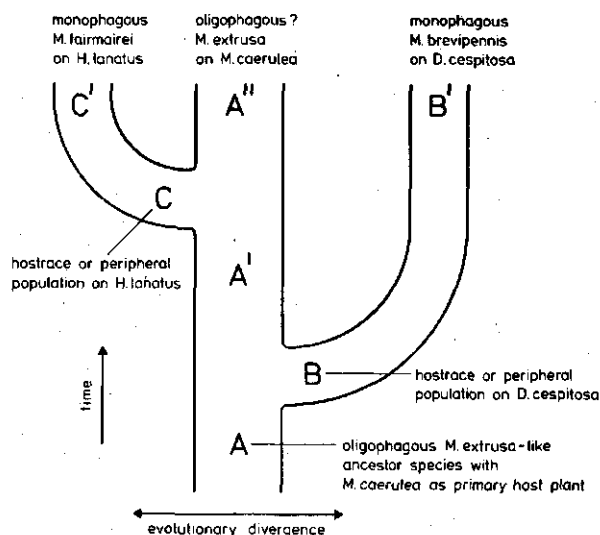
115

*Fig. 3* Speciation model for the *Muellerianella* species.

From this potentially oligophagous species a hostrace or peripheral
population, which was able to maintain itself on *Deschampsia cespitosa,*
became isolated and evolved into the present *M. brevipennis.* Since *M. extrusa*
and *M. brevipennis* are sympatric over large areas at present, we have no
obvious geographic arguments for an allopatric speciation. Regarding the
distribution and ecological preferences of the hostplants, it seems likely
also that the hostplants have had very similar ranges for a very long time.

Without discussing arguments given by several authors pro and contra
sympatric speciation (e.g. White 1978, Futuyma and Mayer, 1980, respectively),
the conditions necessary for such speciation seem to be favourable in the
genus *Muellerianella.* The species are predominantly monophagous and complete
their life-cycle on the hostplant. Although vagility is high due to
macropterous forms, which occur in small proportions (0-30%) in natural
populations, most adults are brachypterous and stay on the hostplants, where
also mating takes place. Therefore homogamy is favoured which is important
to reduce geneflow between incipient species. Finally, evidence from
hybridization studies and the existence of specialized hostplant-clones
among the asexual forms suggest that hostplant selection is under rather
simple genetic control. Although a hostplant shift from *M. caerulea* to *D.*

*cespitosa* under sympatric conditions seems ecologically feasible (similar hostplant structures, habitats and microclimates), no further convincing evidence can be given which supports a sympatric speciation model for *M. extrusa* and *M. brevipennis*.

The second step in the model is the differentiation of *M. fairmairei* from *M. extrusa*. This divergence may have occurred allopatrically, for example after the last glaciation. The withdrawal of *M. extrusa* with its hostplant *M. caerulea* to northern Europe, when the climate became warmer again, may have left isolated populations of *M. extrusa* in the mediterranean region, which could maintain themselves on *Holcus lanatus*. The present sympatry of *M. extrusa* and *M. fairmairei* can be explained by the expansion of *M. fairmairei* together with its hostplant *Holcus lanatus* due to the development of semi-natural grasslands.

The evolution of grasslands in temperate Europe may also have contributed directly to the divergence of *M. fairmairei* from *M. extrusa*. After the development of woodland pasture, where *M. caerulea* occurred frequently on poor acid soils, the gradual intensification and extension of agricultural practices favoured the expansion of other grasses like *H. lanatus*. A replacement of vegetation types with *M. caerulea* by those in which *H. lanatus* is a dominant species, is especially clear during the development of meadows. Early meadows were not fertilized and only mown once a year or less (Ellenberg 1963). In these meadows *M. extrusa* probably occurred frequently on *M. caerulea*. As soon as these are fertilized or mown somewhat more frequently and earlier in the season, *M. caerulea* is replaced by grasses such as *H. lanatus* or *Arrhenatherum elatius*. Under these circumstances subpopulations of *M. extrusa* which were able to live on these grasses, may have had great advantages and could have given rise to a new species which diverged from *M. extrusa* under sympatric or micro-allopatric conditions.

In this context the puzzling *Muellerianella* population from Castor Hanglands (Great Britain) should be mentioned. This population seems to be associated with *Arrhenatherum elatius*, a common grass species of many european grasslands. It is still not clear whether this population represents a local hostplant-race of *M. extrusa*, an incipient species or another sibling species.

Since the origin of meadows and large scale development of grasslands in general dates back not more than 1000-3000 years, the hypothesis that *M. fairmairei* evolved from *M. extrusa* due to this development, I agree, is

117

extreme and supposes a very high rate of evolutionary divergence. It should be realized however, that selection pressures during this development may have been very intense. Rapid evolution during the evolution of grasslands has also been suggested for a number of grass species (Scholz 1975).

The human impact on the origin of the triploid gynogenetic *Muellerianella* forms has allready been discussed in several of the foregoing papers. The distribution and ecology of the triploids strongly support the idea that they are of hybrid origin (see first, second and final paper). The distribution of the triploids coincides with the area in which *M. brevipennis* and *M. fairmairei* are sympatric. Mixed populations of the two sexual species are especially common in extensively used grasslands of NW Europe. Since these grasslands are probably completely man-made, the development of these grasslands by man has caused possibilities for hybridization on a large scale. The restriction of the triploids to such man-made habitats supports the idea that the triploids arose in and completely depend on such habitats.

LITERATURE CITED

Arnold, E.N. (1981) Estimating phylogenies at low taxonomic levels. Z. zool. Syst. Evolut.-forsch. 19: 1-35.

Ellenberg, H. (1963) Vegetation Mitteleuropas mit den Alpen (2nd edition 1974). Verlag Eugen Ulmer, Stuttgart.

Futuyma, D.J. and G.C. Mayer (1980) Non-allopatric speciation in animals. Syst. Zool. 29: 254-271.

Gould, S.J. and N. Eldredge (1977) Punctuated equilibria: the tempo and mode of evolution considered. Paleaobiology 3: 115-151.

Scholz, H. (1975) Grassland evolution of Europe. Taxon 24: 81-90.

White, M.J.D. (1978) Modes of speciation. Freeman, San Francisco.

# Samenvatting

Van 1977 tot 1980 werd een biosystematies onderzoek verricht aan cicaden-
soorten van het geslacht *Muellerianella* (Homoptera, Delphacidae). De nauw-
verwante soorten van dit geslacht werden vergeleken wat betreft de morfologie,
de relaties met waardplanten en habitats, de geografiese verspreiding, het
akoesties gedrag en de onderlinge kruisbaarheid.

Het doel van het onderzoek was inzicht te krijgen in de variabiliteit
binnen de soorten, de mate van isolatie tussen de soorten en de onderlinge
verwantschap. Daarnaast werd veel aandacht besteed aan de oecologie en de
geografiese verspreiding van parthenogenetiese vormen, hun relaties met de
sexuele soorten en hun mogelijke oorsprong.

In W. Europa kunnen momenteel drie *Muellerianella* soorten onderscheiden
worden: *M. brevipennis, M. extrusa* en *M. fairmairei*. Deze soorten zijn
diploid en hebben een normale sexuele voortplanting. Daarnaast komen
triploide, parthenogenetiese vormen voor die zich slechts kunnen voortplanten
na copulatie met ♂♂ van één van de drie bovengenoemde soorten. Er treedt
echter geen bevruchting op en alle nakomelingen zijn ♀♀. Dit verschijnsel
wordt *gynogenese* of pseudogamie genoemd. De triploide gynogenetiese ♀♀
komen morfologies overeen met *M. fairmairei* ♀♀.

De grote morfologiese overeenkomst tussen de *Muellerianella* soorten
heeft in het verleden tot veel verwarring geleid. Dit geldt met name voor de
zeer nauwverwante soorten *M. fairmairei* en *M. extrusa*, die voorheen ten on-
rechte als één soort werden beschouwd. Na gedetailleerd morfologies onderzoek
aan *Muellerianella* materiaal uit heel Europa, konden betrouwbare verschillen
worden aangetoond voor zowel de ♀♀ als de ♂♂ van de drie soorten. Bij deze
revisie werd tevens aandacht geschonken aan geografiese variatie in bepaalde
kenmerken. Er werden geen verschillen gevonden tussen triploide gynogenetiese
♀♀ en *M. fairmairei* ♀♀. Deze kunnen slechts cytologies onderscheiden worden
op basis van hun karyotype.

Op basis van gecontroleerde oude gegevens uit de literatuur en van musea,
en daarnaast van veel nieuw verzameld materiaal, kon een goed beeld gevormd
worden van de geografiese verspreiding van de *Muellerianella* soorten. Hierbij

bleek dat *M. fairmairei* beperkt is tot Zuid en West Europa. *M. brevipennis* en
*M. extrusa* hebben een veel groter verspreidingsgebied. Ze komen waarschijn-
lijk in grote delen van het gematigd klimaatsgebied van de Palearctis voor,
maar ontbreken in Zuid Europa. Het verspredingsgebied van de triploide
gynogenetiese vormen valt min of meer samen met het gebied waar alle drie
sexuele soorten sympatries voorkomen.

De *Muellerianella* soorten worden het best gekarakteriseerd door hun
waardplanten en het milieu waarin ze voorkomen. *M. brevipennis* leeft op
*Deschampsia cespitosa* (ruwe smele) in extensief gebruikte graslanden, in
vochtige bossen en in venige terreinen. *M. extrusa* leeft primair op *Molinia
caerulea* (pijpestrootje) maar er zijn ook enkele populaties op andere gras-
soorten aangetroffen, waarbij het niet geheel zeker is of deze populaties tot
*M. extrusa* behoren. *M. extrusa* wordt vooral gevonden in hoogvenen, in
vochtige heideterreinen en in vochtige bossen op voedselarme zure grond. Zo-
wel *M. extrusa* als *M. brevipennis* leggen de wintereieren in de voedselplant.
*M. fairmairei* en de meest voorkomende triploide vormen leven op *Holcus lanatus*
(echte witbol) en soms op *Holcus mollis* (zachte witbol). Er moet steeds
*Juncus effusus* (pitrus) in de buurt groeien waarin de overwinterende eieren
worden gelegd. *M. fairmairei* en de triploide gynogenetiese ♀♀ komen, meestal
samen, voor in extensief gebruikte hooi- en weilanden, langs greppels en
slootkanten en andere sterk door de mens beinvloede milieu's. Lokaal werden
in W. Europa triploide vormen op andere grassoorten aangetroffen.
Kweekproeven in de kas lieten zien dat de drie sexuele soorten zich
slecht of niet kunnen handhaven op elkaars voedselplanten. *M. extrusa* en *M.
fairmairei* kunnen wel redelijk gekweekt worden op een aantal andere gras-
soorten.
Zowel *M. extrusa* als *M. brevipennis* hebben gewoonlijk slechts één
generatie per jaar. Op gunstige plaatsen heeft *M. brevipennis* soms twee
generaties per jaar. *M. fairmairei* produceert meestal twee of, op plaatsen
met een lang groeiseizoen, drie of meer generaties per jaar. De triploide
gynogenetiese vormen hebben op plaatsen waar ze samen met *M. fairmairei* voor-
komen gewoonlijk twee generaties per jaar. In gebieden waar ze samen met
*M. brevipennis* voorkomen wordt slechts één generatie voltooid.

De geluiden die door de *Muellerianella* soorten worden geproduceerd zijn
opgenomen en geanalyseerd. Elke soort produceert karakteristieke geluiden die

120

waarschijnlijk een belangrijke rol spelen bij de reproduktieve isolatie. Met
name in de roepzang van de ♂♂ zijn duidelijke verschillen gevonden. Daarbij
is vooral de struktuur van de voor het geslacht *Muellerianella* kenmerkende
roffels karakteristiek. Naast de roepzang, die potentiële partners tot
elkaar brengt, hebben de soorten een uitgebreid baltsgedrag met daarbij
behorende geluiden. Vooral het baltsrepertoire van *M. brevipennis* is complex
en bevat een aantal signalen die bij beide andere soorten niet voorkomen.
Tenslotte worden bij alle drie soorten door de ♂♂ luide en langgerekte signa-
len geproduceerd bij onderlinge interakties (rivaliteitszang).

Bij de uitgebreid onderzochte soorten *M. brevipennis* en *M. fairmairei*
blijkt er geografiese variatie te zijn in de geproduceerde geluidspatronen.
De verschillen tussen de soorten zijn echter groter dan de verschillen tussen
ver uit elkaar gelegen populaties van één soort. Er zijn enige aanwijzingen
dat in het gebied waar beide soorten voorkomen, de verschillen meer uitge-
sproken zijn dan in gebieden waar slechts één van hen voorkomt. Dit zou
kunnen wijzen op een vorm van character displacement.

Ondanks de verschillen in gedrag blijkt het mogelijk te zijn de
*Muellerianella* soorten in het laboratorium met elkaar te kruisen. De meeste
hybriden worden verkregen uit kruisingen tussen *M. fairmairei* en *M. extrusa*.
Het merendeel van de hybride ♂♂ blijkt daarbij steriel te zijn. Vele van de
hybride ♀♀ zijn echter fertiel en kunnen met succes teruggekruist worden met
de oudersoorten. Kruisingen van *M. brevipennis* met óf *M. fairmairei* óf
*M. extrusa* leveren veel minder nakomelingen op, waarbij de hybride ♂♂ altijd
volledig steriel zijn. Sommige hybride ♀♀ kunnen met succes worden terugge-
kruist met één van de oudersoorten.

Hoewel er geen overtuigende bewijzen zijn voor hybridizatie onder
natuurlijke omstandigheden, worden de soorten regelmatig samen aangetroffen
en is het niet uitgesloten dat kruisingen tussen de soorten voorkomen.

De aanwijzingen van Drosopoulos (1978) dat door kruising en terugkruising
van *M. fairmairei* en *M. brevipennis* triploide gynogenetiese vormen kunnen
worden verkregen, konden niet bevestigd worden. Verscheidene pogingen om
via deze weg triploiden te synthetiseren mislukten. Er werden uitsluitend
diploide produkten verkregen.

Mede met het doel inzicht te krijgen in de ontstaanswijze en de variabi-
liteit van triploide gynogenetiese vormen, werd de variatie in chromosoom-

aantallen tussen en binnen *Muellerianella* populaties onderzocht. Door verbetering van de prepareertechniek kon dit, ook voor de triploide ♀♀, routinematig gedaan worden.

De sexuele *Muellerianella* soorten hebben alle 14 paar chromosomen (2n= 28) en bezitten een neo-XY sex-determinatie systeem. Er werden slechts enkele afwijkende individuen gevonden met extra chromosomen of fragmenten daarvan. Bij de triploide gynogenetiese vormen, die theoreties 3n=42 moeten zijn, werd een aanzienlijke variatie in chromosoomaantallen ontdekt. Het feit dat 600 van de 711 onderzochte ♀♀ 3n=41, 3n=42 of 3n=43 bleken te zijn, bevestigde het triploide karakter van deze vormen. De rest van de ♀♀ had een ander chromosoomaantal dat varieerde van 3n=32 of 3n=44. De meeste variatie bij deze triploide vormen werd gevonden in Ierse populaties, de minste variatie in centraal europese populaties. Tenslotte werden twee, waarschijnlijk gynogenetiese ♀♀ gevonden met een chromosoomaantal op diploid niveau (2n=29).

De triploide gynogenetiese vormen worden het meest frekwent aangetroffen in associatie met *M. fairmairei*. Dit ligt voor de hand omdat ze op dezelfde voedselplant leven als *M. fairmairei* en omdat ze eenzelfde fenologie hebben. Bovendien zijn *M. fairmairei* ♂♂ voor de triploide ♀♀ reproduktief gezien het meest geschikt om mee te paren. Bij paring met *M. extrusa* of *M. brevipennis* ♂♂ worden aanzienlijk minder nakomelingen geproduceerd.

In mengpopulaties concurreren de gynogenetiese ♀♀ met de *M. fairmairei* ♀♀ om copulaties met de aanwezige ♂♂ en mogelijk ook om ovipositieplaatsen, voedsel etc. Omdat bij ongeslachtelijke voortplanting alleen ♀♀ worden geproduceerd, zijn de gynogenetiese ♀♀ sterk in het voordeel en zijn potentieel in staat *M. fairmairei*, waarvan ze afhankelijk zijn, te verdringen. Toch blijkt het percentage gynogenetiese ♀♀ in gemengde populaties van jaar tot jaar weinig te veranderen. Er is geprobeerd inzicht te krijgen in de faktoren die van invloed zijn op het percentage gynogenetiese vrouwtjes in zulke mengpopulaties.

Hoewel de *M. fairmairei* ♂♂ geen neiging hebben om meer met hun eigen ♀♀ te paren dan met de gynogenetiese ♀♀, hebben de eerste waarschijnlijk toch een betere kans om bevrucht te worden. Dit hangt mogelijk samen met de ruimtelijke verdeling van sexuele en gynogenetiese dieren in het veld. Door het bepalen van percentages gynogenetiese ♀♀ in een groot aantal gemengde populaties in verschillende delen van Europa, werd duidelijk dat dit percentage gemiddeld van zuid naar noord toeneemt. Bovendien lijkt het percentage

negatief gecorreleerd te zijn met de lengte van het groeiseizoen en positief
met de vochtigheid van het klimaat. Hoge percentages triploiden worden aan-
getroffen op plaatsen waar slechts één generatie per jaar wordt geproduceerd
(submontane gebieden van C. Europa, Denemarken) of op plaatsen met een
extreem atlanties klimaat (Ierland).

Overeenkomstige trends worden ook binnen Nederland waargenomen in rela-
tie met het regionale klimaat. Lokaal kunnen echter sterke afwijkingen van
deze trends gevonden worden ten gevolge van microklimaatsfaktoren en andere
omgevingsfactoren. In Nederland blijkt het percentage gynogenetiese ♀♀
sterk samen te hangen met het terreinbeheer en de grondwaterstand. Op
plaatsen waar gemaaid wordt en waar de grondwaterstand hoog is of sterk wis-
selend (hooilanden,   slootkanten etc.), worden meestal veel gynogenetiese
♀♀ gevonden. Op relatief drogere plaatsen en in extensief begraasde gras-
landen is het percentage gynogenetiese ♀♀ in populaties met *M. fairmairei*
veel kleiner.

Op basis van alle verzamelde gegevens is geprobeerd om soortsvormings-
model te reconstrueren. Hoewel het niet mogelijk bleek een verwantschaps-
schema te maken op basis van afgeleide kenmerken, wijst alles erop dat *M.*
*fairmairei* en  *M. extrusa* fylogeneties het meest nauw verwant zijn. Verschil-
lende argumenten leiden tot een soortsvormingsmodel waarin zowel *M. brevipen-*
*nis* als *M. fairmairei* afgeleid zijn van een *M. extrusa*-achtige voorouder-
soort, waarbij *M. fairmairei* het meest recent is afgesplitst.

Het sympatries voorkomen van *M. fairmairei* met beide andere soorten moet
waarschijnlijk als secundair beschouwd worden. Vermoedelijk kwam *M. fairmai-*
*rei* oorspronkelijk alleen in het mediterrane gebied voor op *Holcus lanatus*.
De huidige verspreiding van *H. lanatus* in NW en C. Europa is grotendeels te
danken aan de ontwikkeling van cultuurgraslanden, die ongeveer 5000 jaar
geleden begon. Dankzij deze ontwikkeling heeft *M. fairmairei* zijn areaal
naar NW Europa kunnen uitbreiden. Hierdoor zijn de van oorsprong geografies
en oecologies geisoleerde soorten *M. fairmairei* en *M. brevipennis* veelvuldig
met elkaar in kontakt gekomen. Hybridizatie tussen deze soorten heeft
mogelijk geleid tot het ontstaan van de triploide gynogenetiese vormen.
Deze veronderstelling wordt sterk ondersteund door de oecologiese en geo-
grafiese verspreiding van de triploiden.

# Curriculum vitae

Cornelis Johan Hendrik Booij werd geboren op 6 april 1952 te Oss.
Hij bezocht het Stedelijk Lyceum te Zutphen waar hij in 1970 zijn
HBS-B diploma behaalde. In hetzelfde jaar begon hij zijn biologie-
studie aan de Landbouwhogeschool te Wageningen. Het doctoraalexamen
biologie (specialisatie populatie/oecosysteem) werd in januari 1977
afgelegd, met als hoofdvakken dieroecologie en entomologie en als
bijvak natuurbeheer.
In maart 1977 begon hij  met het promotieonderzoek bij de Afdeling
Entomologie van de Landbouwhogeschool onder dagelijkse begeleiding
van prof. dr. ir. R.H. Cobben. Dit onderzoek werd mogelijk gemaakt
door steun van de Stichting voor Biologies Onderzoek in Nederland
(BION) die wordt gesubsidieerd door de Nederlandse Organisatie voor
Zuiver Wetenschappelijk Onderzoek (ZWO). Het onderzoek werd in juni
1980 afgesloten. Momenteel is hij werkzaam bij het Instituut voor
Onderzoek van Bestrijdingsmiddelen (IOB) te Wageningen.