BIOSYSTEMATIC STUDIES ON THE *MUELLERIANELLA* COMPLEX (DELPHACIDAE, HOMOPTERA AUCHENORRHYNCHA)

Dit proefschrift met stellingen van

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De rector magnificus van de Landbouwhogeschool,

PROF. DR. H. C. VAN DER PLAS

Wageningen, 15 september 1977

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SAKIS DROSOPOULOS

BIOSYSTEMATIC STUDIES ON THE *MUELLERIANELLA* COMPLEX (DELPHACIDAE, HOMOPTERA AUCHENORRHYNCHA)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS,
DR. H. C. VAN DER PLAS,
HOOGLERAAR IN DE ORGANISCHE SCHEIKUNDE,
IN HET OPENBAAR TE VERDEDIGEN
OP WOENSDAG 9 NOVEMBER 1977
DES NAMIDDAGS TE VIER UUR IN DE AULA
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN

H. VEENMAN & ZONEN B.V. – WAGENINGEN – 1977

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STELLINGEN

I

The fact that pseudogamy is rare could be due to the necessity of a special degree of genetic differentiation and the availability of proper mechanisms.

This thesis

II

Since the sperm nucleus of the bisexual species can fuse with the egg nucleus of the F_1 hybrids, thus resulting in the triploid female, it is likely that there is some mechanism preventing a similar fusion in the pseudogamous triploid female.

DROSOPOULOS, S., In press.

III

White's statement: 'From an originally diploid continuum, triploid biotypes may have evolved on several occasions, in different geographic areas. To speak of *the* triploid biotype in such cases would be wrong.' exemplifies the confusion prevailing around the term 'biotype'.

WHITE, M. J. D., 1973.
Cambridge Univ. Press.

IV

The opinion of Muller (1925) that polyploidy is rarer in animals than in plants is still shared by many authors. At present, this opinion might be reconsidered.

MULLER, H. J., 1925, Amer. natur., **59**: 346–353.

V

It would seem that there is a balance between the bisexual and unisexual populations of *M. fairmairei* in Leersum, the Netherlands.

this thesis.

VI

The scale insect *Saissetia oleae* became a serious pest on the olive trees in Greece, after applying insecticides against *Dacus oleae*. This is one more example, which shows that more work should be done on the inplications of pest-management.

VII

Polluting industries can never be relieved of the responsibility to cease the deterioration of the environment by employing scientists, sponsoring programs and publishing papers in defence of nature conservation.

VIII

At the present time in many parts of Greece the sea has changed its nice blue colour. Unfortunately people experience this change mainly in summer time.

IX

Socrates' statement: 'One thing I know is that I know nothing' seems to imply that even *this* we cannot know.

S. DROSOPOULOS
Wageningen, 9 november 1977

CONTENTS

1. INTRODUCTION

Two years ago a brief report was made on some biological differences between *Muellerianella fairmairei* (PERRIS) and *M. brevipennis* (BOHEMAN) in which the interest in biosystematic studies of the two species was emphasized (DROSOPOULOS 1975). KONTKANEN (1953) defined them as 'sibling species' on morphological grounds. The present author was surprised to find that the Dutch populations of *M. fairmairei* contained more females than males in both the field and in the laboratory colonies, in contrast to *M. brevipennis* which had the normal sex ratio of 1 : 1. A year later (DROSOPOULOS 1976), an all-female pseudogamous triploid biotype of *M. fairmairei* was discovered for the first time in Hemiptera. This gave an insight into the population structure of *M. fairmairei* and into its abnormal sex-ratio. Since the origin of such a rare unisexual biotype among vertebrates and invertebrates favored allopolyploidy, its laboratory synthesis and its possible origin in the field was reported recently (DROSOPOULOS 1977).

This paper contains data from the field and laboratory experimentation and their analyses, accumulated since my preliminary report in 1975. It is considered that these biosystematic studies touch on the problems of speciation, parthenogenesis and polyploidy, which have been previously discussed by *e.g.* ASTAUROV (1969), BROWN (1959), DARLINGTON (1953), DOBZHANSKY (1951), MAYR (1963, 1971), ROSS (1973), SCHULTZ (1969), STEBBINS (1940), SUOMALAINEN (1969), UZZELL (1964), WHITE (1973). The present data combine studies on morphology, behaviour, ecology, physiology and cytogenetics which in the sense of MAYR (1971) form the study of 'isolating mechanisms' which has led to the development of a new branch of biology.

In addition, this study contributes to the knowledge on the biology of Auchenorrhyncha, some of which are harmful to crops. One of the species studied here, *M. fairmairei*, is a vector of the Northern Cereal Mosaic, a rice virus disease in Japan (KISIMOTO, 1973).

Data on mating behaviour, accoustic communication and some aspects of cytology are not included here, but will be presented in later publications by the author and by his colleagues.

2. GENERAL DESCRIPTIONS

2.1. TAXONOMY

The genus *Muellerianella* was erected by WAGNER (1963) in his revision of european Delphacidae, to include the only two species then known: *M. fairmairei* (PERRIS, 1857) and *M. brevipennis* (BOHEMAN, 1847).

M. fairmairei has previously been called *Delphax fairmairei* PERRIS, 1857, *Delphax neglecta* FLOR, 1861, partim, *Liburnia extrusa* SCOTT, 1871, *Delphax fairmairei signicollis* REY, 1894 (NAST, 1971).

M. brevipennis has previously been called *Fulgora flavescens* (FABRICIUS, 1794, primary homonym), *Delphax brevipennis* BOHEMAN, 1847, *Delphax bivittata* BOHEMAN, 1850, *Delphax hyalinipennis* STÅL, 1854, *Delphax neglecta* FLOR, 1861, partim, *Delphacodes brevipennis sordidella* (METCALF, 1943) (NAST, 1971).

Another species of this genus was described recently as *M. relicta* from U.S.S.R. (LOGVINENKO, 1976).

In addition to these three species a pseudogamous female biotype was found, which at present is called *M. fairmairei* (3n) (DROSOPOULOS, 1976).

2.2. MORPHOLOGY

Males

The male genital segment in side view provides a good morphological character (Fig. 1). In *M. fairmairei* (Fig. 1a) its caudal margin has a shallower concavity, terminating dorsally with a blunt projection not directed downwards, while in *M. brevipennis* a deeper concavity is present and the dorsal projection is sharp, slightly flexed downwards (Fig. 1c). In all hybrids (both ways crossings) this character is intermediate (Fig. 1b).

The phalli of *M. fairmairei* (F), *M. brevipennis* (B) and the hybrid (B × F) are shown in Fig. 2. To enable comparison of these figures with those of LOGVINENKO (1976) for *M. relicta* (R) (Fig. 2) the side views of the phallus of each species also are presented. It may be a coincidence, but it is very striking from these drawings that the phallus of *M. relicta* is very close to that of the hybrid (B × F). Unfortunately there are no drawings available of the male genital segment of *M. relicta*.

Females

No morphological differences between the females of the *Muellerianella* complex including the female hybrids have been found. It is likely that the size of the pseudogamous biotype of *M. fairmairei* (3n) is larger than that of the diploid and specially of *M. brevipennis*. Unfortunately, this character is very

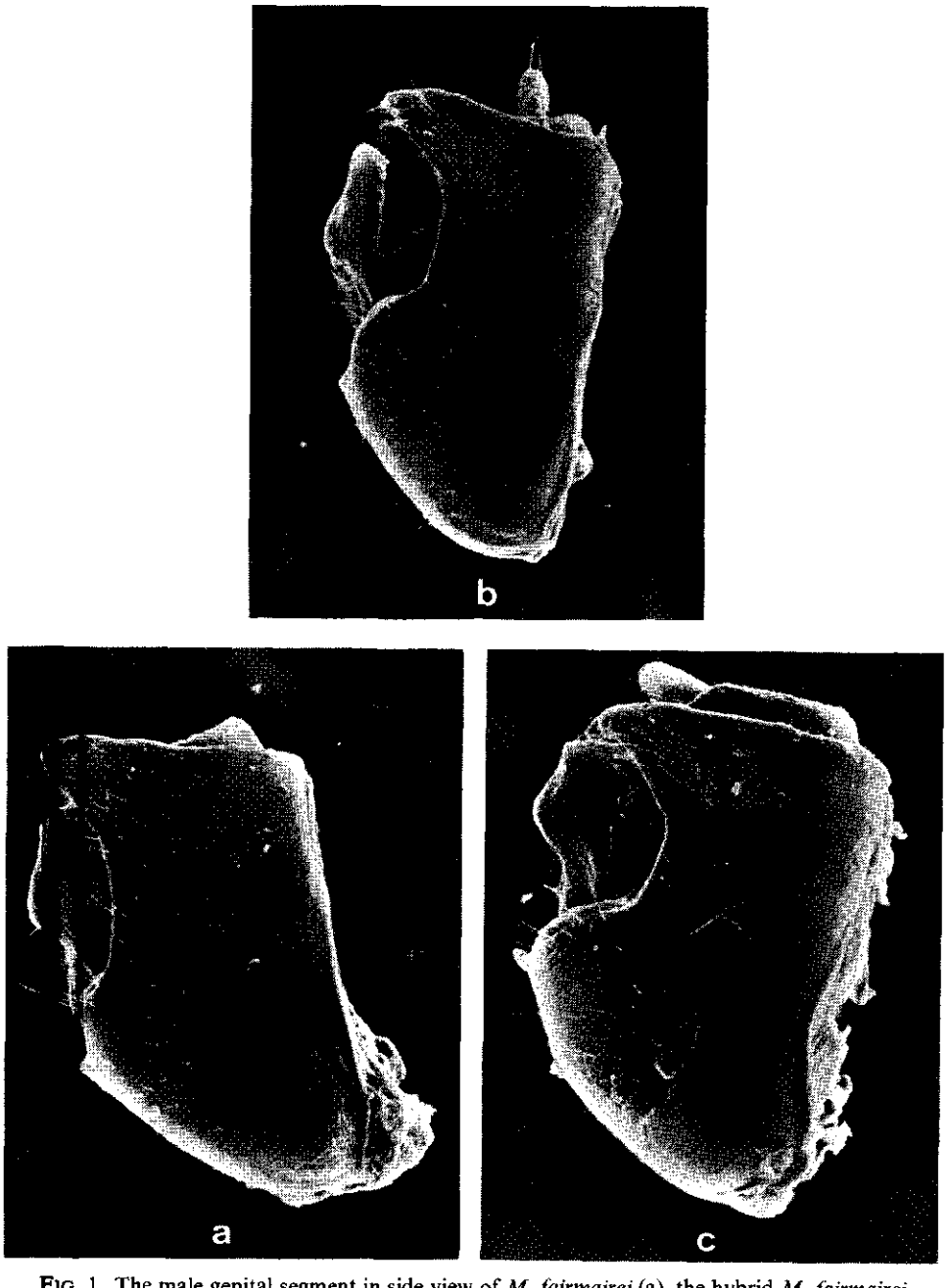


FIG. 1. The male genital segment in side view of *M. fairmairei* (a), the hybrid *M. fairmairei* ♀♀ × M. brevipennis ♂♂ (b), and *M. brevipennis* (c) (Scanning photomicrographs made by F. Tiel of the Electron Microscopy Section of the Service Institute for Technical Physics in Agriculture, Wageningen).

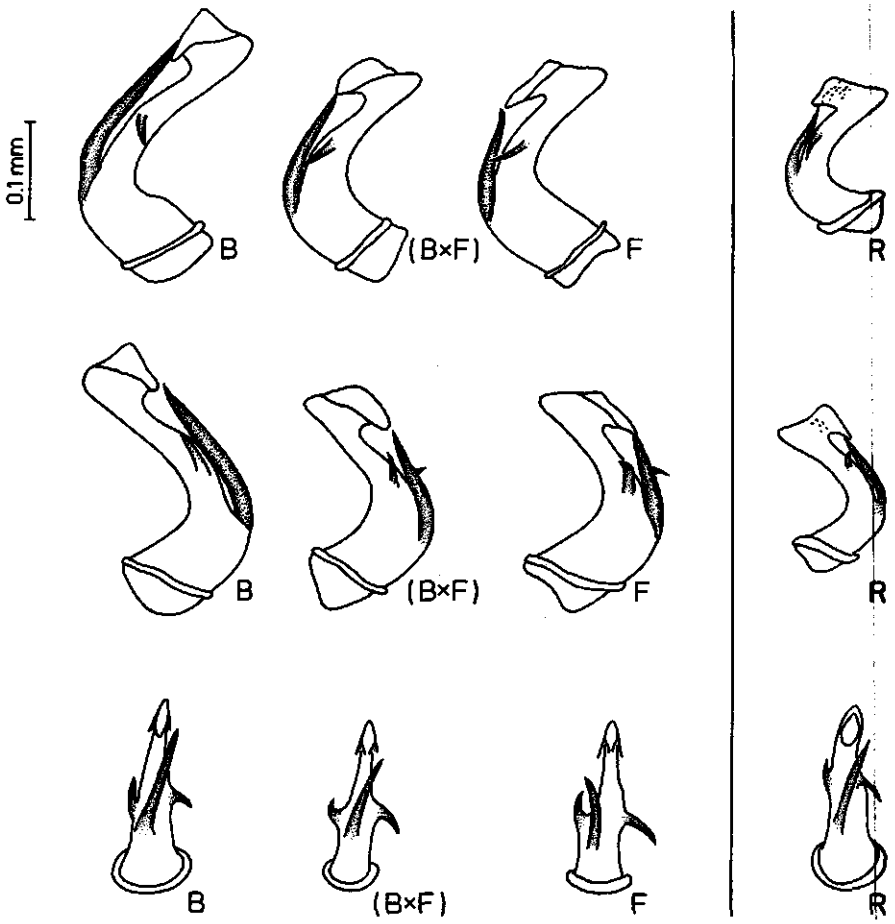


FIG. 2. Various views of the phallus of *M. brevipennis* (B), the hybrid *M. fairmairei* ♀ × *M. brevipennis* ♂ (B × F), *M. fairmairei* (F) and *M. relicta* (R; redrawn from Logvinenko, 1976).

variable, depending upon density of the colony, temperature and nutrition during the larval stages. However, there is some evidence that the length of the hind tibia of the pseudogamous biotype is longer than that of *M. fairmairei* (2n) and of *M. brevipennis*.

Larvae

The larvae of the *Muellerianella* complex are easily distinguisable from other delphacid species which I have studied. They are white in colour with a dark longitudinal stripe on each side of the body from the apex of the head to the caudal end of the body (HASSAN, 1939). In my experience this stripe tended to be dark in *M. brevipennis*, while in *M. fairmairei* it was frequently brownish.

TABLE 1. The number of sense organs counted on left side (L) and right side (R) of the body of 5th instar larvae.

Species \ No. larva / side	1		2		3		4		5		6		7		8		9		10		Mean	
	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
M. fairmairei																						
Head	13	13	13	13	13	13	13	13	13	13	8	13	13	13	13	13	13	13	13	13	12.5	13.0
Pronotum									No variation												7.0	7.0
Mesonotum									No variation												5.0	5.0
Metanotum									No variation												1.0	1.0
Abdomen																						
5th segment									No variation												1.0	1.0
6th segment	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3.0	2.9
7th segment									No variation												3.0	3.0
8th segment	3	3	3	3	3	2	3	3	3	2	3	3	3	3	3	2	2	3	3	3	2.9	2.7
9th segment									No variation												3.0	3.0
Total																					38.4	38.6
M. brevipennis																						
Head	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	14	13	13.1	13.0
Pronotum									No variation												7.0	7.0
Mesonotum									No variation												5.0	5.0
Metanotum									No variation												1.0	1.0
Abdomen																						
5th segment									No variation												1.0	1.0
6th segment									No variation												3.0	3.0
7th segment									No variation												3.0	3.0
8th segment									No variation												3.0	3.0
9th segment	3	3	3	3	3	3	4	3	3	3	3	3	3	3	3	3	3	.3	3	3	3.1	3.0
Total																					39.1	39.0

The number of sense organs in fifth instar larvae (see VILBASTE, 1968) revealed no distinct differences between *M. fairmairei* and *M. brevipennis* (Table 1). Both species had approximately the same number of organs symmetrically distributed.

Eggs

There were no morphological differences observed in the eggs of both *Muellerianella* species. The egg-nucleus is located approximately in the central longitudinal axis of the yolk column at about the level of the micropyle.

2.3. CHROMOSOME NUMBERS

Methods and material

Caryological studies of the males of *Muellerianella* have been based mainly on the squashing method described by GUT (1976). Other squashing methods (*e.g.* HALKKA, 1959; JOHN and CLARIDGE, 1974) were also suitable for such studies. Testes of last instar larvae provided the best results.

Studies by the squashing method in females are generally more tedious to work than in males. However, such preparations in the present study have given satisfactory results by squashing semi-mature eggs from 3–5 day old females.

Ovaries with semi-mature eggs were placed in propionic acid 50% for 2–6 minutes. During this period these eggs swell considerably and become free of their follicle cells and chorion. Before these eggs are diluted in propionic acid, they have to be transferred to a slide with a capillary glass-tube. When most of the propionic acid on the slide is removed the egg may be squashed under a cover-slip, applying constant pressure for 4–6 minutes. Additional fixation was made by submerging the slide with the coverslip in a petri-dish containing CARNOY (6 : 3 : 1) for 2–5 minutes. In this fluid, when the coverslip was not removed, one of its sides was fixed properly while under the other a fine needle was added. Both the coverslip and the slide were transferred to absolute alcohol for more than 10 minutes. Staining of the dried preparation was made in aqueous crystal violet 0.1%. Permanent preparations were mounted in Gurr's Neutral Medium.

For gross chromosome countings mature ovarian eggs were fixed directly in CARNOY. Thereafter their chorion was removed and the egg was squashed in lacto-acetic orcein after 3–5 hours.

The material of *Muellerianella* studied is shown in Table 2.

Males

Somatic metaphases of *M. fairmairei* and *M. brevipennis* (Fig. 3a–b) revealed consistently 28 chromosomes, while meiotic metaphases 14 bivalents (DROSOPOULOS and SYBENGA, 1977). Hybrid males had somatic metaphases also with

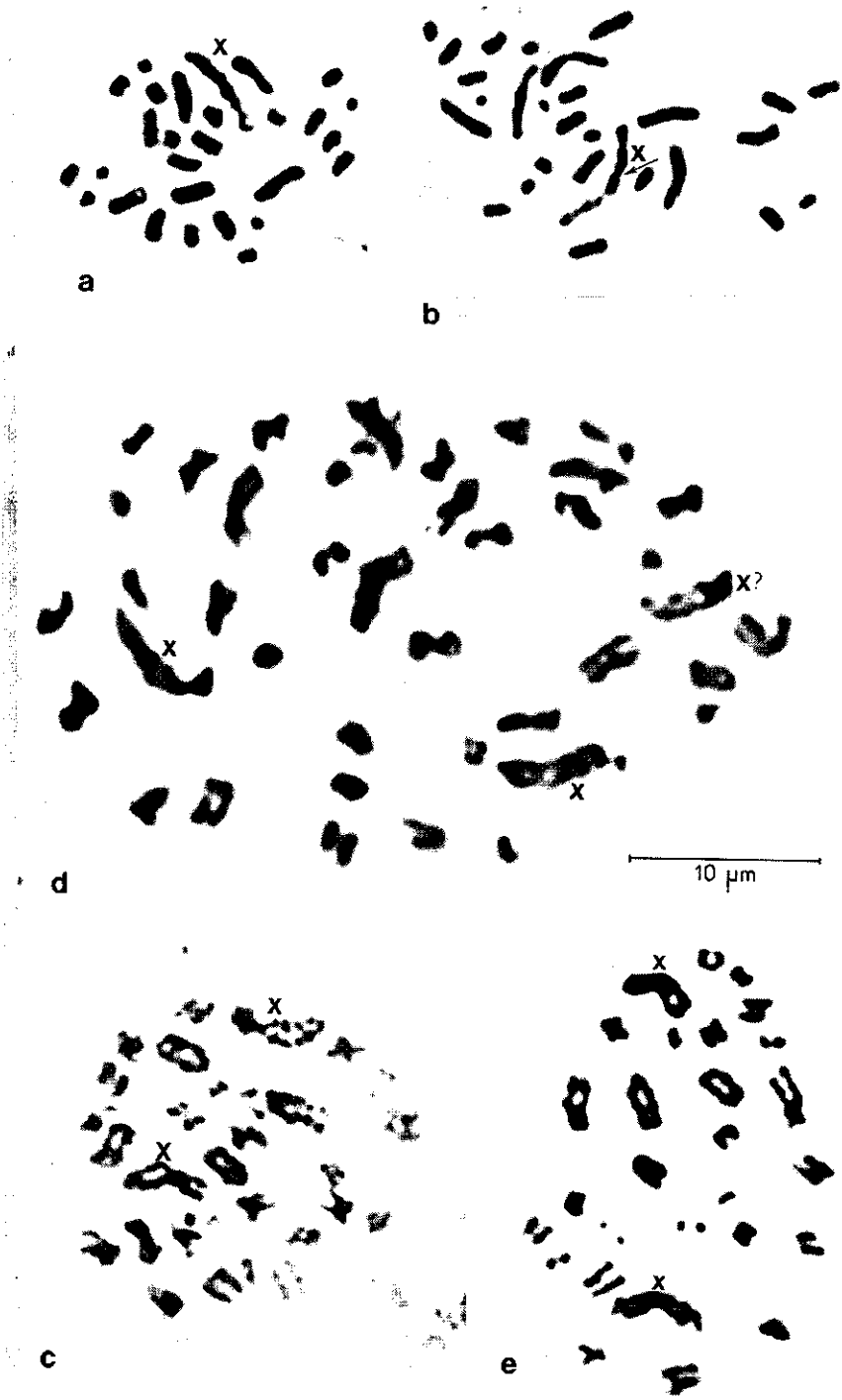


FIG. 3. Somatic metaphases of *M. fairmairei* males (a), females (2n) (c) and females (3n) (d), and *M. brevipennis* males (b), and females (e). The bar in (d) represents 10 μm. All photomicrographs are reproduced at the same magnification.

28 chromosomes, while meiotic plates consistently had 28 pseudobivalents (DROSOPOULOS, 1977).

Females

M. brevipennis possessed 28 chromosomes at somatic metaphase (Fig. 3e) and 14 bivalents at meiotic metaphase. The same was found for *M. fairmairei* (2n) (Fig. 3c). In the pseudogamous biotype somatic metaphase had 41 chromosomes (Fig. 3d) (3 plates counted), while meiotic plates comprised 41 pseudobivalents (4 plates) (DROSOPOULOS, 1976). Female hybrids of the F1 had 28 chromosomes, while in meiotic plates either a mixture of pseudobivalents and closely apposed bivalents or only pseudobivalents were observed (DROSOPOULOS, 1977).

Several populations of *M. fairmairei* studied cytologically revealed no variation in the chromosome number both in males and females (Table 2).

TABLE 2. Chromosome numbers of *M. fairmairei* and *M. brevipennis*.

Species	number of individuals tested	Chromosome number	
		somatic metaphases	meiotic metaphases
M. fairmairei (Dutch colony)			
Males (diploid)	27 (*5)	2n = 28 (2)	n = 13 II + XY (25)
Females (diploid)	10 (*1)	2n = 28 (4)	n = 13 II + XX (6)
Females (triploid)	23 (*3)	3n = 41 (15)	n = 41 II/2 (XXX) (8)
M. fairmairei (Greek colony)			
Males (diploid)	25	2n = 28 (2)	n = 13 II + XY (23)
Females (diploid)	16	2n = 28 (6)	n = 13 II + XX (10)
M. fairmairei (S. French colony)			
Males (diploid)	10	2n = 28 (2)	n = 13 II + XY (10)
Females (diploid)	6 (*6)		n = 13 II + XX (6)
M. fairmairei (C. French colony)			
Males (diploid)	1		n = 13 II + XY (1)
Females (diploid)	–		–
Females (triploid)	3		n = 41 II/2 (XXX) (3)
M. fairmairei (English colony)			
Males (diploid)	3	2n = 28 (1)	n = 13 II + XX (3)
Females (diploid)	–	–	–
Females (triploid)	4	3n = 41 (2)	n = 41 II/2 (XXX) (2)
M. brevipennis (Dutch colony)			
Males (diploid)	35(*3)	2n = 28 (3)	n = 13 II + XY (35)
Females (diploid)	17	2n = 28 (8)	n = 13 II + XX (9)

(*n), number of individuals collected directly from the field.

Sex chromosomes

At somatic metaphase of males of both species the large X chromosome is clear (Fig. 3a–b), which probably has resulted from a fusion of one autosome and the original X chromosome. The Y chromosome, which is also large, is likely to be confused with two pairs of autosomes having approximately the same size (of this sex-chromosome). It is remarkable that during meiosis this XY system of sex chromosomes forms always a sort of trivalent in *M. fairmairei* (similar to that described for *Oncopsis* by JOHN and CLARIDGE, 1974) but a heteromorphic bivalent in *M. brevipennis* (DROSOPOULOS and SYBENGA, 1977).

In *M. fairmairei* (2n) and *M. brevipennis* females, two distinct X chromosomes were always present (Fig. 3c–e) which during meiosis form the XX bivalent. In the pseudogamous biotype of *M. fairmairei* probably three X chromosomes are present (Fig. 3d).

2.4. HOST PLANTS

M. fairmairei

This species has been sampled by the author only in localities where either *Holcus lanatus* or *H. mollis* (food plants) and *Juncus effusus* were growing in close proximity. The latter is utilised only as an oviposition plant for overwintering eggs (HASSAN, 1939, WHALLEY, 1955 and DROSOPOULOS, 1975). The same food plant *H. lanatus* is reported for this species in England (MORCOS, 1953 and WALOFF, personal communication). According to MORRIS (1974), *M. fairmairei* from Ireland is probably associated with *Sesleria albicans* in particular.

Quite another list of host plants is recorded from Japan (MOCHIDA and OKADA, 1971): *Arthraxon hispidus* (Thunb.), *Digitaria adscendens* (H.B.K.), *Isachne globosa* (Thunb.), *Oryza sativa* (L.), *Phalaris arundinacea* (L.) and *Setaria viridis*.

The material for the present study from various european sources, was successfully reared in the laboratory on *H. lanatus*.

M. brevipennis

Records from England (WALOFF and SOLOMON, 1973), East Germany (WITSACK, 1971), Finland (TÖRMÄLÄ and RAATIKAINEN, 1976) and Holland (DROSOPOULOS, 1975) suggested that this species feeds and oviposits exclusively on *Deschampsia caespitosa*.

In the laboratory it was maintained also on this grass-species.

M. relicta

LOGVINENKO (1976) reported that this species was collected from *Luzula* sp. in May 1967 and 7 July 1970 at three localities in the Caucasus.

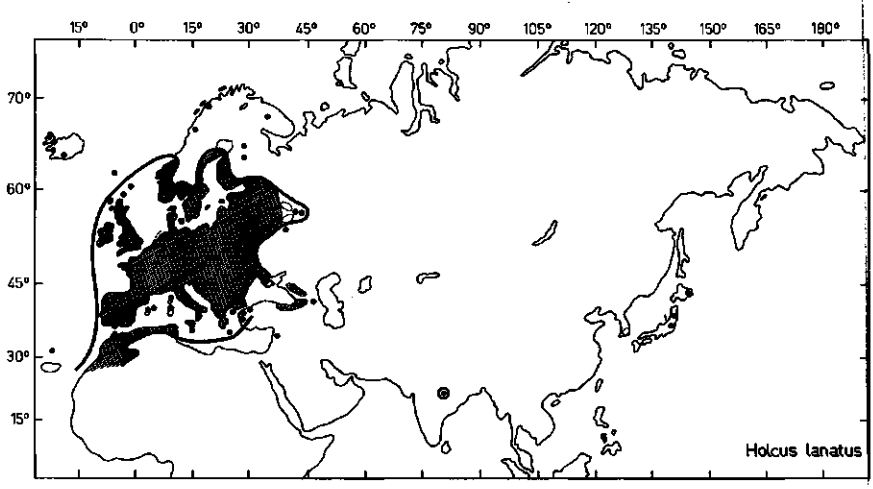


FIG. 4. Geographic distribution of *Holcus lanatus*.

2.5. GEOGRAPHIC DISTRIBUTION

a. Host plants

H. lanatus

According to BÖCHER and LARSEN (1958) and from the map Fig. 4 (after HULTEN, 1950) this grass-species is hardly native in the north of Europe, but it has its main distribution in Western, Central and Southern Europe. Its palaearctic distribution seems to run from southern Iceland north of the Faeroes to West Norway and further on in the U.S.S.R. towards the south-east of the Caucasus, thus approaching the limits of a Mediterranean-Atlantic species. In Japan and China this grass-species is also present, while in the Neartic region (North America) it has a wide range. *H. lanatus* is a fairly variable species especially in the southern part of its range (HULTEN, 1958). LÖVE and LÖVE (1956) suggest that *H. lanatus* was introduced into most localities in Iceland, but think that it is native in a few of them south of Eyjatjöll, and that it probably survived the last glaciation there. *H. mollis* and *J. effusus* have an almost similar distribution as *H. lanatus* (HULTEN, 1958).

D. caespitosa.

The palaearctic distribution of this grass-species is shown in Fig. 5 (after HULTEN, 1958). Obviously, its distribution extends more to the north and less to the south, compared to that of *H. lanatus*. On the other hand, this grass has a wide range in Asia and North-America. HULTEN mentioned that *D. caespitosa* is extremely variable, at least 40 varieties having been described from most parts of the northern hemisphere.

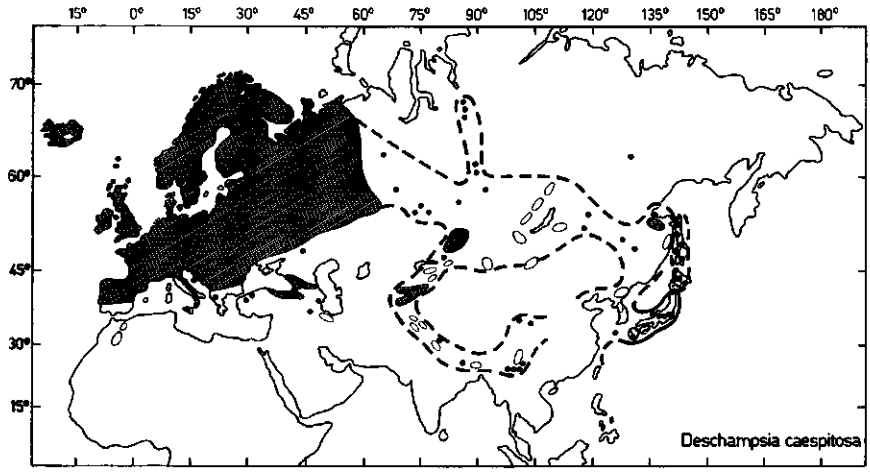


FIG. 5. Geographic distribution of *Deschampsia caespitosa.*

TABLE 3. Distribution of *Muellerianella* species.

Country	*M. fairmairei*	*M. brevipennis*
JAPAN (Hokkaido, Honshu)	+	
CHINA (Kansu)	+	
U.S.S.R. (1. Estonia 2. Latvia 3. Maritime Territory 4. N. and 5. M. Russia 6. Ukraine)	+(1–6)	+ (1, 2, 4)
AUSTRIA	+	+
BELGIUM	+	
CZECHOSLOVAKIA (1. Bohemia 2. Moravia 3. Slovakia)	+ (1–2)	+ (1–3)
DENMARK	+	+
FINLAND	+	+
FRANCE	+	+
GERMANY (East)	+	+
GERMANY (West)	+	+
GREECE	+	
GREAT BRITAIN (1. England 2. Scotland 3. Wales)	+ (1–3)	+ (1–3)
HUNGARY		+
IRELAND	+	
ITALY	+	+
NETHERLANDS	+	+
NORWAY	+	+
POLAND	+	+
ROMANIA	+	+
SWEDEN	+	+
SWITZERLAND	+	+
YUGOSLAVIA		+
AZORES	+	
MADEIRA	+	

b. *Muellerianella* species

Table 3 shows the presently known distribution of *Muellerianella* species. The Azores has been recorded as a local place also for *M. brevipennis* (NAST, 1972), but personal examination of this material (1♂) (leg. LINDBERG) revealed that it was closely similar to *M. fairmairei*. In addition, *D. caespitosa* does not occur in that region. As can be seen from Table 3, the range of *M. fairmairei* exceeds far that of *M. brevipennis* from far East to West and its range is pronounced more to the south following more or less the distribution of *H. lanatus* (Fig. 4).

In contrast to *M. fairmairei*, *M. brevipennis* is distributed more to the north, occupying a smaller range than that of its host plant (Fig. 5).

M. relicta seems to be an isolated species, although *Luzula* is a cosmopolitan genus.

The populations of *M. fairmairei* from the Azores, Japan and China need to be reexamined. There is a recent example of another delphacid: *Javesella pellucida* from the Azores, which proved to be a distinct new species (*J. azorica*, STRÜBING and HASSE, 1975).

3. THE STUDY AREA IN HOLLAND

3.1. THE GENERAL AREA

The area of study is situated between the villages of Leersum and Langbroek, both belonging to the province of Utrecht.

Samples of *M. fairmairei* were taken at Broekhuizen (Fig. 6), a site which belongs to the Research Institute for Nature Management (R.I.N.). There, three grassy fields occur which are maintained under different treatments, either by grazing with sheep or cattle, or by seasonal mowing. Control plots are used for botanical investigations by staff members of R.I.N. The soil, which abuts on standing water at its lowest level, varies from a fine sand to clay. The altitude of this area is 1–5 m above sea level.

Samples of *M. brevipennis* were taken near Overlangbroek, which is situated 1.5 km west of Broekhuizen. This area consists of *Fraxinus excelsior* coppice, 2–12 years old. Its soil type is heavy clay.

The area between these two localities consists of more or less open cultivated land with pastures. Observations on the occurrence of the two species and their host plants were made also in this area.

3.2. THE BIOTOPES STUDIED

Samples of *M. fairmairei* were taken from the three open biotopes in Broekhuizen mentioned above. Those of *M. brevipennis* were from one biotope in Overlangbroek.

Biotope K (Fig. 6) is a pasture meadow. Since 1969 it has been divided into two parts. One (biotope K+) was grazed by 4–6 cows and the other remained ungrazed, but is mown during July (biotope K—). The vegetation of the K biotope is close to Arrhenatherion elatioris, Agropyro-Rumicion crispi and Violion caninae alliances (JONGELEEN, MENKHORST and DROSOPOULOS 1972). The vegetation type from which samples were taken is very similar to the Lolio-Cynosuretum association. The most common plants are *Holcus lanatus*, *Agrostis tenuis*, *Juncus effusus*, *J. acutiflorus*, *Festuca rubra*, *Rumex acetosa*, *Stellaria graminea*, *Ranunculus repens*, *Potentilla reptans*, *Trifolium pratense*, *Cirsium palustre*, *Achillea millefolium*, *Carex disticha*, *Cynosurus cristatus*, *Anthoxanthum odoratum*, *Luzula campestris*, *Bellis perennis*, *Cardamine pratensis*.

Biotope G (Fig. 6) lies 300–400 m. north of the biotope K, from which it is separated by a forest (mostly tall beeches). Formerly it was used as a gazon terrain. Since 1969 it is mown once or twice in a year (middle of July and at the end of August). Within this biotope a small part is left unmown (biotope G_A). The vegetation type of the G-area is that of Agropyro-Rumicion crispi with

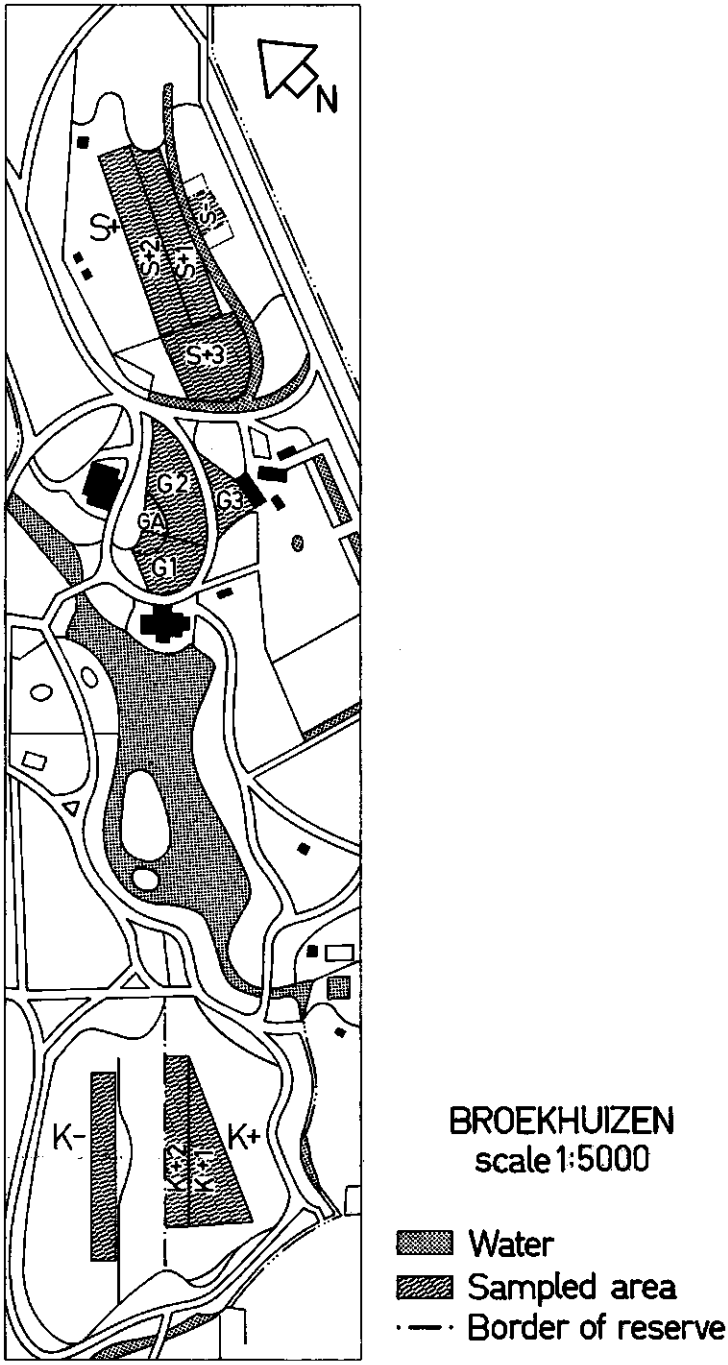


FIG. 6. The area of Broekhuizen (Leersum) including the biotopes where samples of *M. fairmairei* were taken.

tendencies to Arrhenatherion elatioris and Violion caninae. *Juncus* species were not growing in this biotope, while *Holcus mollis*, *H. lanatus*, *Agrostis tenuis*, *Festuca rubra*, *F. pratensis*, *Anthoxanthum odoratum*, *Rumex acetosa*, *Stellaria graminea*, *Ranunculus repens*, *Veronica chamaedrys* are the most common plants.

Biotope S (Fig. 6) is near biotope G, separated from it by a road and ditch. In contrast to the other biotopes it is dry and sandy. It has been grazed for some years by domesticated deers and since 1972 by 5–12 sheep (biotope S+). Within the biotope a small part is kept ungrazed (biotope S–). The vegetation type of the major part of biotope S is close to Violion caninae with *Equisetum arvense*, *Urtica dioica*, *Rumex acetosella*, *Cirsium palustre*, *Achillea millefolium*, *Galium verum*, *Plantago lanceolata*, *Hieracium pilosella*, *Juncus subuliflorus*, *J. effusus*, *Festuca rubra*, *F. pratensis*, *F. ovina*, *Agrostis tenuis*, *Deschampsia flexuosa* as most common plants (biotopes S+2 and S–). In its lower part near the ditch the most common plants are: *Trifolium pratense*, *Hydrocotyle vulgaris*, *Juncus acutiflorus*, *J. bulbosus*, *Festuca pratensis*, *F. rubra*, *Holcus lanatus*, *Agrostis tenuis* etc. (biotope S+1).

Biotope L is a 2–3 years old copse of *Fraxinus excelsior*. Samples of *M. brevipennis* were taken from clearings (4 × 250 m.) bordering a trail. Among the first 110 m. *Deschampsia caespitosa* covers 80% of the study area with *Phalaris arundinacea*, *Rubus sp.*, *Juncus effusus*, *Holcus lanatus* occurring at the edges (biotope L1). The remaining 150 m. of the trail margins consists predominantly of *D. caespitosa* intermixed with *Alopecurus pratensis*, *Phalaris arundinacea*, *Phragmites australis*, *Calamagrostis canescens*, *Cirsium palustre*, *Carex acuta*, *J. effusus*, *Urtica dioica*, *Valeriana officinalis*, *Heracleum sphondylium*, *Angelica sylvestris* etc. (biotope L2).

3.3. WEATHER AND CLIMATE

There is no meteorological station in the area of study. Reference is made here to such data as monthly temperature, precipitation and humidity (Table 4), recorded by the meteorological Station at the Bilt, which is situated about 19 km. western of the study area. Temperature was measured at 1.5 m. above the soil level and humidity at 0.4 m. Daily records of these meteorological data are to be found in 'Maandelijks Overzicht Der Weersgesteldheid'.

Since photoperiod was important in the experiments of the present study the mean length of day in each month at latitude 52° N is also given in Table 4.

There were some remarkable aspects of the weather during the four years of study. 1974 was characterized by an early spring. Temperatures of about 20 °C occurred as early as the second half of March, whereas these temperatures in 1973 and 1975 did not occur until the first half of May. The year of 1975 had a dry and warm summer during which the ditches at Broekhuizen dried out. The summers of 1972 and 1974 were wet and the late summer and autumn of 1974 were wet and cold. High humidities occurred during the summer of 1972 and 1974.

TABLE 4. Monthly means of temperature, precipitation, relative humidity and day length (52°N) in the years 1972–1975 at the 'De Bilt Meteorological Station'.

	Temperature (°C)					Precipitation (m.m.)					Relative humidity (%)					
Months	Mean 1941–1970	1972	1973	1974	1975	Mean 1941–1970	1972	1973	1974	1975	Mean 1941–1970	1972	1973	1974	1975	Day-length (L.D)
J	1.7	0.5	2.9	5.2	6.2	68.0	40.6	25.9	60.8	87.8	87	88	93	91	89	8.4 : 15.6
F	2.0	3.6	2.9	4.6	3.1	54.1	31.9	77.7	30.1	17.8	84	87	90	85	81	10.0 : 14.0
M	5.0	6.2	5.3	5.6	4.7	44.6	34.5	21.1	62.5	72.7	79	74	81	84	83	11.9 : 12.1
A	8.5	7.7	6.1	9.1	7.4	48.8	61.9	71.1	10.0	61.4	75	80	78	73	82	13.9 : 10.1
M	12.4	11.8	12.0	11.6	11.1	51.5	81.0	110.0	54.2	34.3	74	77	76	76	76	15.7 : 8.3
J	15.5	13.4	16.0	14.8	15.1	58.0	68.1	26.1	87.7	85.5	74	77	72	80	70	16.6 : 7.4
J	17.0	17.2	17.0	15.4	17.8	76.8	100.3	65.1	81.6	24.8	78	82	76	82	76	16.2 : 7.8
A	16.8	15.5	17.7	16.4	19.9	88.0	58.8	64.7	79.4	41.6	80	80	73	81	71	14.6 : 9.4
S	14.3	11.9	14.9	12.7	15.2	71.2	39.0	80.8	141.6	65.0	82	82	83	87	81	12.7 : 11.3
O	10.0	9.0	8.7	7.0	8.5	72.2	34.1	96.8	139.9	12.7	86	81	88	90	87	10.6 : 13.4
N	5.9	6.0	5.1	6.5	5.2	70.0	85.2	76.3	121.2	97.2	89	89	88	90	90	8.8 : 15.2
D	3.0	3.3	2.7	7.3	3.5	63.4	21.0	63.4	123.7	34.2	90	87	90	89	90	7.9 : 16.1

4. GENERAL MATERIAL AND METHODS

Insect samples

The sweep-netting method was used during 1972 for investigating the composition and seasonal fluctuations of Auchenorrhyncha and Heteroptera in the open biotopes at Leersum. This investigation was conducted by Dr. R. H. Cobben in cooperation with the State Institute for Nature Conservation (R.I.N.) in Broekhuizen – Leersum.

The samples, taken at weekly intervals from April until October, resulted in a detailed analysis of the composition of the hemipterous fauna composition and the phenology of some abundant species (JONGELEEN, MENKHORST and DROSOPOULOS, 1972).

In order to obtain a reliable estimation of the population densities of *M. fairmairei* and *M. brevipennis* in subsequent years (1973–1975), the 'D-Vac' suction apparatus (DIETRICK, 1969) was also used.

The advantages and disadvantages of sweep-netting and suction methods have been widely considered and discussed previously (*e.g.* PHILLIPS 1931, DE LONG 1932, GRAY and TRELOAR 1933, BEALL 1935, ROMNEY 1945, KONTKANEN 1950, JOHNSON and SOUTHWOOD 1957, HEIKINHEIMO and RAATIKAINEN 1962, ANDRZEJEWSKA 1965, JURISSO 1970 and MORRIS 1974).

Samples using both methods were taken between 13–16 hours on each sampling date, which was preferred to be a sunny and dry one. When samples using both methods were taken on the same date from the same biotope, the suction method was preceding the netting in order to avoid disturbance of the fauna by sweeping. During 1973–1975 sweep-net and suction samples were taken from the same site in each biotope. Sweep-net samples were taken by walking across the sampling site of each biotope as is shown in Fig. 7. Special care was taken to ensure standardization of the speed and length of the sweeping stroke. The back stroke quickly followed the forward stroke and resulted in two strokes being made over the same vegetation. Suction samples were taken by moving the collection cone of the apparatus rapidly over the sites as shown in Fig. 7. The time required to take one sample unit (0.08 m^2) was approximately one minute. Coarse material was removed from the collection net, while the engine of the apparatus was still operating. The collection net was replaced by an other after 1–3 sample units had been taken.

In comparing the two methods 7 suction sample units were taken to equal 100 sweeps. This proportion is approximately the same to that taken by HEIKINHEIMO and RAATIKAINEN (1962) who studied an other delphacid, *Javesella pellucida*. The size of samples taken with the two sampling methods in each biotope during 1972–1975 is recorded in Table 5.

Sweep-net and suction samples were taken particularly in biotopes K+ and L. The samples collected by each method were kept in a glass jar with a filter paper soaked in ethyl acetate. In the laboratory the numbers, sex and wing

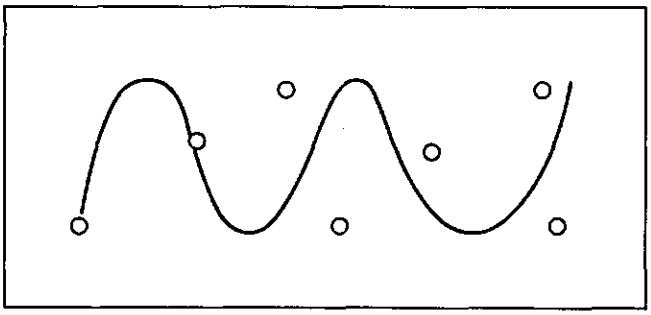


FIG. 7. Diagrammatical representation of the sampling procedure in each biotope (square frame) with the netting method (100 sweeps along the curved line) and suction method (7 partial samples indicated by circles).

form of each species of Auchenorrhyncha and Heteroptera were recorded under a binocular microscope.

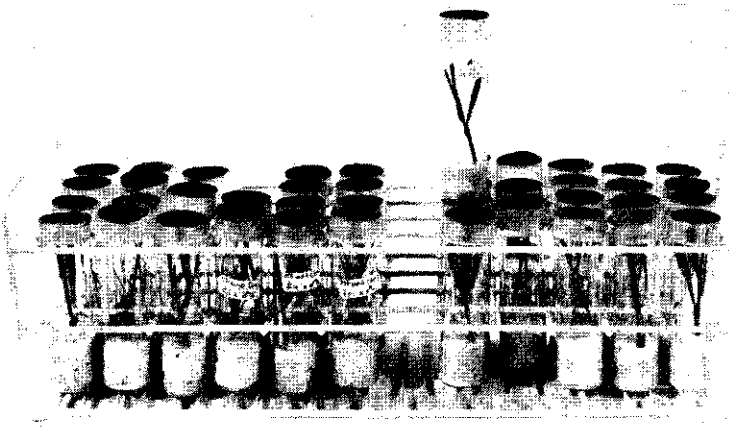
A comparison of the frequency of all species sampled with the sweep-net and suction method has not yet been reported. However, such a comparison for the two sibling species of *Muellerianella*, and their associated species, is given in the samples taken by each method from the K+ and L biotopes during 1974 (Tables 7–10). It can be seen that the sweep-net method was unsuitable for sampling of *M. brevipennis*, due to the structure of its host plant.

Plant samples

In order to investigate the oviposition sites of *M. fairmairei* and *M. brevipennis*, plant samples were taken in the field. They were taken approximately at

TABLE 5. Size of sweep-net samples (number of sweeps) and suction samples (number of sample units) taken in each biotope during the years of 1972–1975

Biotope	Sampling method	Sweep-net				Suction		
		1972	1973	1974	1975	1973	1974	1975
	K–	200	200	100	100	15	7	7
(K)	K+1	100	100	100	100	7	7	7
	K+2	100	100	100	100	7	7	7
	G_1	200	100	100	100	–	–	–
(G)	G_2	100	100	100	100	–	–	–
	G_3	100	100	100	100	–	–	–
	G_A	100	100	100	100	–	–	–
	S+1	250	100	100	100	–	–	–
(S)	S+2	–	100	100	100	–	–	–
	S+3	–	–	100	100	–	–	–
	S–	100	100	50	50	–	–	–
(L)	L_1	–	–	100	–	–	7	–
	L_2	–	–	150	–	–	10	–



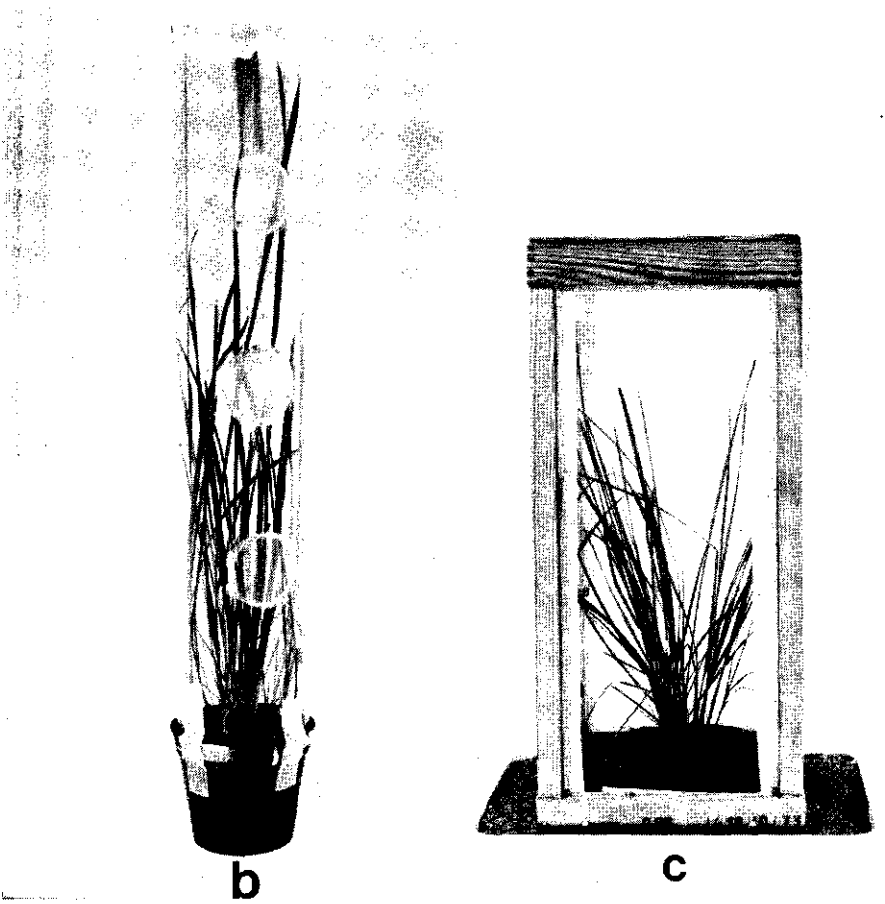


FIG. 8. Material for rearing the *Muellerianella* species; test-tubes (a), plastic cylinder (b) and cage (c).

the end of each of the two adult generations. Plants with their roots were collected at random from the sampled biotope and in the laboratory all plants were examined under the binocular microscope. Eggs which had been laid were recorded according to their egg-group size. In all the biotopes studied there was no other delphacid species hibernating in the egg stage except *Conomelus anceps*, whose eggs are morphologically different from those of the two *Muellerianella* species. Thus, eggs laid by *M. fairmairei* and *M. brevipennis* were recorded with certainty, especially hibernating eggs.

Treatment of eggs

Eggs were extracted from the plant under the binocular microscope. Newly deposited eggs were detached with some plant tissue attached. In order to follow gross embryogenesis the eggs were stored in an embryoblock with a substrate of sterilized filter paper soaked in distilled water. When some eggs were dead, remaining healthy eggs were transferred to another embryo-block. Bouin's fluid was used as a fixative for the eggs (15 minutes). After puncturing the chorion embryos were stained with borax carmin solution (COBBEN, 1968).

Rearings

a. Test-tubes. Larvae and adults of Delphacidae were reared in the laboratory in test-tubes (Fig. 8a). They were made of a glass-tube (2 cm. diameter and 7 cm. long) covered by a plastic stopper the top of which was cut out and replaced by a metallic sheath fixed to the plastic stopper. The test-tube litter was made of cotton wool, which was rolled in tissue paper and pressed to the bottom of the glass-tube. After cut stems of the food plants were planted in this litter a quantity of white fine sand was placed on top of the litter. The litter of the test-tube was kept watered, in order to avoid accidental mortality, especially in rearing first instar larva. Care was taken that the litter was not more than 0.25 of the length of the tube.

This method of rearing has given more satisfactory results than any other method described previously, because the tubes are easily handled and the cut plant is suitable for feeding 1–3 larvae for a period of 5–15 days. Moreover, larval mortality in such test-tubes was much less than in other methods tried previously.

b. Plastic cylinders. Cellulose nitrate cylinders (12 cm. diameter and 60 cm. high) were fixed to plastic pots as shown in Fig. 8b. Six openings covered by a fine nylon mesh were made in the side of each cylinder. A stopper was fixed to the top of the cylinder in a similar way as described for the test-tubes (a). These cylinders were used for rearing colonies in the laboratory.

c. Cages. Cages ($18 \times 18 \times 48$ cm.) were made of a wooden frame (Fig. 8c). The two side openings of the frame were covered by glass, while its top and two other sides were covered by nylon gause. The bottom of the cage, which was in contact with the soil, was made of a galvanized frame, which was attached to the wooden frame. Cages were used both for indoor and outdoor rearing.

Statistical analysis

In the present study the mean value and standard deviation are given for each set of observations or samples. Analysis of variance is based on the F-test. Comparison of regression lines (in fact between two regression coefficients) was made using the 'formulae' reported by SOKAL and ROHLF (1969 p. 455). The levels of significance of differences are indicated with asterisks as follows:

* $0.05 > P > 0.01$
** $0.01 > P > 0.001$
*** $P \leqslant 0.001$

5. HABITATS

Several records have been given of the habitats of Auchenorrhyncha and Heteroptera communities, which include *M. fairmairei* and *M. brevipennis*, from Europe. In those reported by KONTKANEN (1950) from South Finland, *M. brevipennis* is defined as a stenotopic species typical of 'fresh' biotopes. The community it belongs to is that of *Philaenus spumarius*, *Arthaldeus pascuellus* and *Elymana sulphurella*. There, *M. fairmairei* is recorded as occurring in only one rather moist biotope. LINNAVUORI (1952) listed low frequencies of *M. brevipennis* in moist sloping meadows also in South Finland. VILBASTE (1974) reported that *M. fairmairei* usually occurred in forests and rarely in open meadows at 17 localities in the baltic countries (Latvia and Lithuania), while *M. brevipennis* was common in forests but also in meadows at 25 localities in this region. LE QUESNE (1960) described *M. brevipennis* as a local species of damp places and ditches in England, Wales and Scotland, and *M. fairmairei* as a widely distributed species of damp places in England, Wales, Scotland and Ireland. MORCOS (1953) referred to the collection site of *M. fairmairei* on wet ground adjacent to a stream near Silwood Park – England. In Germany, KUNTZE (1937) reported that the densities of *M. fairmairei* were higher in intermediate types of peat-bogs and wet meadows than in peat-bogs proper or in transient habitats between peat-bogs and dryer banks, being totally absent from dry banks. In this area *M. brevipennis* was present also, occurring in wet and peat-bog biotopes. In addition KUNTZE referred to the presence of both species in forest meadows, being less common in forest tracks and totally absent in forest marshes. He concluded that open localities in woods and open meadows are favoured habitats of both species. REMANE (1958) reported that *M. fairmairei* occurred only in original, natural biotopes in Germany, being absent from cultivated areas.

At three localities in Holland the two sibling species were observed to occur together. In the sampled biotopes of Leersum only *M. fairmairei* occurred. However, a few individuals of *M. fairmairei* were found in the biotopes of Langbroek (at a distance of 1.5 km. of Leersum), where *M. brevipennis* was frequent. In the area between Leersum and Langbroek both species appeared to exist in each other's company.

In sweep-net samples from all biotopes at Leersum (Fig. 6) taken during April-October 1972, *M. fairmairei* was dominant (> 15%, after KONTKANEN 1950) in the K+ biotope, which is grazed by cattle (Table 6). In the adjacent K– biotope, which is not grazed but mown, the frequency of this species was lower. The frequency of *M. fairmairei* in the mown G biotope was lower than in the unmown GA one. In the S+ biotope grazed by sheep, *M. fairmairei* was also present, while the ungrazed S– biotope was unsuitable for *M. fairmairei*.

Deltocephalus pulicaris and *Florodelphax leptosoma* are species closely associated with *M. fairmairei*, while the eurytopic species *Philaenus spumarius*,

Arthaldeus pascuellus, *Javesella pellucida* and *Macrosteles laevis* (a species which rapidly recolonizes disturbed biotopes, ANDRZEJEWSKA, 1962) are also associated with it. *Jassargus pseudocellaris*, *Acanthodelphax spinosus* and *Conomelus anceps* are evidently uncommon where *M. fairmairei* is frequent (Table 6). More detailed data on the precise association of *M. fairmairei* with Auchenorrhyncha and Heteroptera are given from samples taken by the suction and sweep-net method during 1974 (Tables 7 and 8). In this year the population density of *M. fairmairei* appeared to be higher than any of the other species occurring in the K+ biotopes. Particularly, in suction samples the frequency of *M. fairmairei* (including the number of larvae) was 86.4% and 74.2% in the K+1 and K+2 biotopes respectively, which shows that the mean number of individuals of this species in a single sample unit (0.08 m^2) was 77.6 in the K+1 biotope and 57.2 in the K+2 biotope. Among the Heteroptera, *Stenotus binotatus* was the most common species in sweep-net samples and *Pachytomella parallela* in suction samples.

In its main features the association of *M. fairmairei* with Auchenorrhyncha from the K+ biotope coincides with that reported by KONTKANEN (1950) from South Finland, and KUNTZE (1937) from Germany. In particular, there is high coincidence of both floristic and faunistic associations (Auchenorrhyncha – Heteroptera) of *M. fairmairei* with similar records given by REMANE (1958) from Germany.

The association of *M. brevipennis* with Auchenorrhyncha and Heteroptera is shown in Tables 9 and 10. Evidently, this species is less frequent in sweep-net than in suction samples. In suction samples *M. brevipennis* is also more

TABLE 6. Dominant and influent (>6.0%) species of Auchenorrhyncha in sweep-net samples taken from the biotopes in Leersum during April-October 1972.

Species \ Biotope	K+	K–	G_A	G	S+	S–
Conomelus anceps	0.1	0.1	0.0	0.0	2.1	14.6
Muellerianella fairmairei	20.2	6.6	9.2	2.9	7.8	0.0
Florodelphax leptosoma	7.5	2.2	0.0	0.0	5.7	4.6
Acanthodelphax spinosus	0.0	0.0	1.0	1.5	7.2	6.1
Javesella pellucida	5.4	6.3	5.6	20.2	7.1	4.8
J. dubia	3.8	2.9	0.8	11.3	1.2	0.5
Philaenus spumarius	19.2	3.2	7.4	2.3	3.1	0.0
Macrosteles laevis and *sexnotatus*	15.8	43.7	22.8	23.9	2.0	1.5
Arthaldeus pascuellus	14.4	26.7	17.8	16.1	10.6	1.7
Jassargus pseudocellaris	0.0	0.0	23.1	14.5	37.8	40.1
Deltocephalus pulicaris	7.5	1.0	0.0	0.8	1.8	0.0
Eupteryx vittata	0.1	0.0	8.1	1.3	0.4	0.0
Total (%)	94.0	92.7	95.8	94.8	86.8	73.9
Total number of individuals	3314	2667	2108	5444	1788	603
Total number of species	24	26	26	35	43	30

TABLE. 7. The numbers of larval and adult Auchenorrhyncha taken in sweep-net samples and suction samples (in brackets) from the K+ biotopes in Leersum, on 10 and 30 July, 20 August and 19 September 1974.

Biotope	K + 1					K + 2				
Species	No. ♂	No. ♀	No. larvae	No. ♂♀	% ♂♀	No. ♂	No. ♀	No. larvae	No. ♂♀	% ♂♀
Muellerianella fairmairei	22	93	5	115	23.8	77	285	30	362	39.8
	(258)	(531)	(1384)	(789)	(78.4)	(266)	(417)	(919)	(683)	(63.4)
Conomelus anceps	0	1	0	1	0.2	5	10	0	15	1.6
	(2)	(1)	(0)	(3)	(0.3)	(9)	(2)	(0)	(11)	(1.0)
Florodelphax leptosoma	18	12	0	30	6.2	51	45	0	96	10.5
	(7)	(4)	(0)	(11)	(1.1)	(6)	(8)	(0)	(14)	(1.3)
Xanthodelphax straminea	0	1	0	1	0.2	0	0	0	0	0.0
	(2)	(1)	(0)	(3)	(0.3)	(0)	(1)	(0)	(1)	(0.1)
Javesella pellucida	14	11	0	25	5.2	11	8	0	19	2.1
	(10)	(10)	(0)	(20)	(2.0)	(11)	(9)	(0)	(20)	(1.9)
J. dubia	2	6	0	8	1.7	3	7	0	10	0.9
	(10)	(10)	(0)	(20)	(2.0)	(8)	(5)	(0)	(13)	(1.2)
Delphacidae larvae			2					30		
			(68)					(67)		
Philaenus spumarius	26	21	0	47	9.7	20	22	0	42	4.6
	(2)	(0)	(0)	(2)	(0.2)	(5)	(6)	(0)	(11)	(1.0)
Aphrodes flavostriatus	0	0	0	0	0.0	0	0	0	0	0.0
	(2)	(4)	(14)	(6)	(0.6)	(14)	(12)	(12)	(26)	(2.4)
A. albifrons	0	0	0	0	0.0	0	0	0	0	0.0
	(12)	(12)	(0)	(24)	(2.4)	(16)	(23)	(0)	(39)	(3.6)
Cicadella viridis	8	1	0	9	1.9	8	10	4	18	2.0
	(1)	(0)	(0)	(1)	(0.1)	(3)	(1)	(2)	(4)	(0.4)
Macrosteles spp.	9	29	0	38	7.8	18	36	0	54	6.0
	(0)	(0)	(0)	(0)	(0.0)	(0)	(8)	(0)	(8)	(0.7)
Deltocephalus pulicaris	4	5	0	9	1.9	8	8	0	16	1.8
	(0)	(1)	(0)	(1)	(0.1)	(0)	(0)	(0)	(0)	(0.0)
Recilia coronifera	0	0	0	0	0.0	0	0	0	0	0.0
	(0)	(1)	(0)	(1)	(0.1)	(0)	(0)	(0)	(0)	(0.0)
Elymana sulphurella	3	1	0	4	0.8	5	11	0	16	1.8
	(18)	(16)	(0)	(34)	(3.4)	(3)	(14)	(0)	(17)	(1.6)
Conosanus obsoletus	1	0	0	1	0.2	0	0	0	0	0.0
	(1)	(0)	(0)	(1)	(0.1)	(0)	(0)	(0)	(0)	(0.0)
Streptanus aemulans	2	7	0	9	1.9	4	4	0	8	0.9
	(16)	(16)	(0)	(32)	(3.2)	(22)	(22)	(0)	(44)	(4.1)
S. sordidus	2	0	0	2	0.4	2	3	0	5	0.5
	(7)	(0)	(0)	(7)	(0.7)	(3)	(3)	(0)	(6)	(0.6)
Arthaldeus pascuellus	84	101	0	185	38.2	98	151	0	249	27.3
	(28)	(24)	(0)	(52)	(5.2)	(59)	(122)	(0)	(181)	(16.8)
Notus flavipennis	0	0	0	0	0.0	1	0	0	1	0.1
	(0)	(0)	(0)	(0)	(0.0)	(0)	(0)	(0)	(0)	(0.0)
Cicadellidae larva			29					65		
			(41)					(82)		
Total	195	289	36	484	100.0	311	600	129	911	99.9
	(376)	(631)	(1507)	(1007)	(99.9)	(425)	(653)	(1082)	(1078)	100.1)
No. species				15					15	
				(17)					(16)	

TABLE 8. The numbers of larval and adult Heteroptera taken in sweep-net and suction samples (in brackets) from the K+ biotopes in Leersum, on 10 and 30 July, 20 August and 19 September 1974.

Species \ Biotope	K+1 No. ♂	K+1 No. ♀	K+1 No. larvae	K+1 No. ♂♀	K+1 % ♂♀	K+2 No. ♂	K+2 No. ♀	K+2 No. larvae	K+2 No. ♂♀	K+2 % ♂♀
Myrmus miriformis	0	0	0	0	0.0	0	0	0	0	0.0
	(0)	(1)	(0)	(1)	(0.7)	(0)	(1)	(0)	(1)	(0.7)
Peritrechus geniculatus	0	0	0	0	0.0	0	0	0	0	0.0
	(1)	(0)	(0)	(1)	(0.7)	(0)	(0)	(0)	(0)	(0.0)
Stygnocoris pedestris	0	0		0	0.0	0	0	0	0	0.0
	(1)	(0)	(0)	(1)	(0.7)	(0)	(0)	(0)	(0)	(0.0)
Nabis spp. (ferus group)	1	1	0	2	1.0	0	1	0	1	0.3
	(1)	(3)	(0)	(4)	(3.0)	(3)	(2)	(0)	(5)	(3.3)
Dolichonabis limbatus	0	0	0	0	0.0	1	0	0	1	0.3
	(2)	(4)	(1)	(6)	(4.5)	(2)	(1)	(0)	(3)	(2.0)
Myrmedobia coleoptrata	0	0	0	0	0.0	0	0	0	0	0.0
	(0)	(1)	(0)	(1)	(0.7)	(0)	(0)	(0)	(0)	(0.0)
Capsus ater	1	0	0	1	0.5	0	0	0	0	0.0
	(0)	(1)	(0)	(1)	(0.7)	(0)	(0)	(0)	(0)	(0.0)
Cyrtorhinus pygmaeus	0	0	0	0	0.0	0	0	0	0	0.0
	(1)	(0)	(0)	(1)	(0.7)	(0)	(0)	(0)	(0)	(0.0)
Harpocera thoracica	1	0	0	1	0.5	0	0	0	0	0.0
	(0)	(0)	(0)	(0)	(0.0)	(0)	(0)	(0)	(0)	(0.0)
Leptopterna dolobrata	1	3	0	4	2.0	9	22	0	31	8.5
	(3)	(2)	(0)	(5)	(3.7)	(3)	(2)	(0)	(5)	(3.3)
Lopus decolor	0	0	0	0	0.0	1	2	0	3	0.8
	(0)	(0)	(0)	(0)	(0.0)	(0)	(1)	(0)	(1)	(0.7)
Notostira elongata	14	12	0	26	12.8	12	19	0	31	8.5
	(11)	(3)	(0)	(14)	(10.4)	(11)	(11)	(0)	(22)	(14.5)
Pachytomella parallela	15	13	0	28	13.8	35	24	0	59	16.2
	(27)	(45)	(6)	(72)	(53.7)	(22)	(50)	(1)	(72)	(47.4)
Pithanus maerkeli	0	0	0	0	0.0	1	1	0	2	0.5
	(4)	(2)	(0)	(6)	(4.5)	(0)	(1)	(0)	(1)	(0.7)
Plagiognathus chrysanthemi	0	0	0	0	0.0	0	0	0	0	0.0
	(0)	(2)	(0)	(2)	(1.5)	(1)	(0)	(0)	(1)	(0.7)
Stenodema laevigatum	0	3	0	3	1.5	2	2	0	4	1.1
	(1)	(1)	(0)	(2)	(1.5)	(0)	(4)	(0)	(4)	(2.6)
S. calcaratum	2	2	0	4	2.0	1	2	0	3	0.8
	(2)	(2)	(0)	(4)	(3.0)	(1)	(2)	(0)	(3)	(2.0)
Stenotus binotatus	31	103	0	134	66.0	51	172	0	223	61.3
	(8)	(5)	(0)	(13)	(9.7)	(20)	(14)	(0)	(34)	(22.4)
Trigonotylus ruficornis	0	0	0	0	0.0	2	4	0	6	1.6
	(0)	(0)	(0)	(0)	(0.0)	(0)	(0)	(0)	(0)	(0.0)
Miridae larvae			233					221		
			(125)					(105)		
Total	66	137	233	203	100.1	115	249	221	364	99.9
	(62)	(72)	(132)	(134)	(99.7)	(63)	(89)	(106)	(152)	(100.3)
No. species				9					11	
				(16)					(11)	

TABLE 9. The numbers of larval and adult Auchenorrhyncha taken in sweep-net and suction samples (in brackets) from Langbroek on 10 and 30 July, 20 August and 19 September 1974.

Species / Biotope	L_1 No. ♂	L_1 No. ♀	L_1 No. larvae	L_1 No. ♂♀	L_1 % ♂♀	L_2 No. ♂	L_2 No. ♀	L_2 No. larvae	L_2 No. ♂♀	L_2 % ♂♀
Muellerianella brevipennis	7 (146)	9 (176)	0 (241)	16 (322)	7.7 (39.7)	3 (107)	11 (83)	0 (161)	14 (190)	4.2 (29.9)
Cixius sp.	1 (0)	0 (0)	0 (0)	1 (0)	0.5 (0.0)	0 (0)	0 (0)	0 (0)	0 (0)	0.0 (0.0)
Stenocranus major	36 (15)	27 (10)	1 (7)	63 (25)	30.4 (3.1)	106 (50)	59 (43)	4 (81)	165 (93)	50.0 (14.6)
Conomelus anceps	12 (51)	32 (73)	0 (0)	44 (124)	21.3 (15.3)	15 (27)	33 (19)	0 (0)	48 (46)	14.5 (7.2)
Euides speciosa	0 (0)	0 (0)	0 (0)	0 (0)	0.0 (0.0)	0 (0)	0 (2)	0 (0)	0 (2)	0.0 (0.3)
Chloriona sp.	1 (0)	1 (0)	0 (0)	2 (0)	1.0 (0.0)	0 (0)	0 (0)	0 (0)	0 (0)	0.0 (0.0)
Paraliburnia adela	0 (1)	0 (3)	0 (0)	0 (4)	0.0 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0.0 (0.0)
Acanthodelphax denticauda	2 (36)	1 (31)	0 (0)	3 (67)	1.4 (8.3)	5 (46)	10 (28)	0 (0)	15 (74)	4.5 (11.6)
Javesella pellucida	4 (5)	4 (3)	0 (0)	8 (8)	3.9 (1.0)	8 (4)	10 (8)	0 (0)	18 (12)	5.5 (1.9)
J. dubia	0 (0)	0 (0)	0 (0)	0 (0)	0.0 (0.0)	0 (0)	0 (1)	0 (0)	0 (1)	0.0 (0.2)
Delphacidae larvae			1 (152)					0 (222)		
Philaenus spumarius	5 (0)	4 (0)	0 (0)	9 (0)	4.3 (0.0)	6 (0)	9 (1)	0 (0)	15 (1)	4.5 (0.2)
Megophthalmus scanicus	0 (0)	0 (0)	0 (0)	0 (0)	0.0 (0.0)	0 (1)	0 (0)	0 (0)	0 (1)	0.0 (0.2)
Aphrodes bicinctus	1 (1)	1 (0)	0 (0)	2 (1)	1.0 (0.1)	0 (1)	0 (0)	0 (0)	0 (1)	0.0 (0.2)
A. flavostriatus	0 (5)	0 (13)	0 (10)	0 (18)	0.0 (2.2)	0 (13)	0 (10)	0 (28)	0 (23)	0.0 (3.6)
Balclutha punctata	0 (0)	0 (0)	0 (0)	0 (0)	0.0 (0.0)	1 (0)	2 (0)	0 (0)	3 (0)	0.9 (0.0)
Macrosteles sp.	0 (0)	0 (0)	0 (0)	0 (0)	0.0 (0.0)	0 (0)	0 (1)	0 (0)	0 (1)	0.0 (0.2)
Doratura stylata	0 (0)	0 (0)	0 (0)	0 (0)	0.0 (0.0)	0 (7)	0 (0)	0 (0)	0 (7)	0.0 (1.1)
Streptanus sp.	2 (42)	1 (57)	0 (2)	3 (99)	1.4 (12.2)	1 (24)	11 (32)	0 (0)	12 (56)	3.6 (8.8)
Arthaldeus pascuellus	21 (49)	34 (91)	6 (20)	55 (140)	26.6 (17.2)	19 (34)	17 (55)	2 (5)	36 (89)	10.9 (14.0)
Mocuellus sp.	0 (0)	0 (0)	0 (0)	0 (0)	0.0 (0.0)	1 (10)	3 (26)	0 (0)	4 (36)	1.2 (5.7)
Eupteryx sp.	1 (2)	0 (0)	0 (0)	1 (2)	0.5 (0.2)	0 (0)	0 (1)	0 (0)	0 (1)	0.0 (0.2)
Erythroneura scutellaris	0 (2)	0 (0)	0 (0)	0 (2)	0.0 (0.2)	0 (0)	0 (2)	0 (0)	0 (2)	0.0 (0.3)
Cicadellidae larvae			0 (13)					9 (40)		
Total	93 (355)	114 (457)	8 (445)	207 (812)	100.0 (100.0)	165 (324)	165 (312)	15 (537)	330 (636)	99.8 (100.2)
No. Species				12 (12)					10 (18)	

TABLE 10. The numbers of larval and adult Heteroptera taken in sweep-net and suction samples (in brackets) from Langbroek on 10 and 30 July. 20 August and 19 September 1974.

Species / Biotope	No. ♂	No. ♀	L_1 No. larvae	No. ♂♀	% ♂♀	No. ♂	No. ♀	L_2 No. larvae	No. ♂♀	% ♂♀
1. *Ischnodemus sabuleti*	804	551	1868	1355	91.9	1410	1017	81	2427	92.0
	(3541)	(2131)	(2899)	(5672)	(98.8)	(3444)	(2141)	(2554)	(5585)	(97.6)
2. *Picromerus bidens*	1	1	0	2		0	0	0	0	
	(0)	(0)	(0)	(0)		(0)	(0)	(0)	(0)	
3. *Peritrechus geniculatus*	0	0	0	0		0	0	0	0	
	(0)	(1)	(0)	(1)		(0)	(1)	(0)	(1)	
4. *Scolopostethus sp.*	0	0	0	0		0	0	0	0	
	(0)	(0)	(0)	(0)		(1)	(0)	(0)	(1)	
5. *Stygnocoris pedestris*	0	0	0	0		0	0	0	0	
	(0)	(0)	(0)	(0)		(16)	(5)	(0)	(21)	
6. *Piesma maculatum*	0	0	0	0		0	0	0	0	
	(0)	(0)	(0)	(0)		(0)	(0)	(5)	(0)	
7. *Nabis spp. (ferus group)*	1	1	0	2		1	2	0	3	
	(2)	(2)	(2)	(4)		(0)	(0)	(2)	(0)	
8. *Dolichonabis limbatus*	0	2	0	2		2	6	0	8	
	(3)	(3)	(5)	(6)		(7)	(4)	(1)	(11)	
9. *Myrmedobia spec.*	0	0	0	0		0	0	0	0	
	(0)	(0)	(0)	(0)		(0)	(2)	(0)	(2)	
10. *M. tenella*	0	0	0	0		0	0	0	0	
	(1)	(1)	(0)	(2)		(0)	(0)	(0)	(0)	
11. *Adelphocoris quadripunctatus*	0	0	0	0		4	0	0	4	
	(0)	(0)	(0)	(0)		(0)	(0)	(0)	(0)	
12. *Blepharidopterus angulatus*	0	0	0	0		0	1	0	1	
	(0)	(0)	(0)	(0)		(0)	(0)	(0)	(0)	
13. *Capsus ater*	0	0	0	0		1	0	0	1	
	(0)	(0)	(0)	(0)		(1)	(0)	(0)	(1)	
14. *Cyrtorhinus pygmaeus*	0	0	0	0		1	0	0	1	
	(5)	(12)	(0)	(17)		(3)	(8)	(0)	(11)	
15. *Leptopterna dolobrata*	0	1	0	1		1	1	0	2	
	(1)	(0)	(0)	(1)		(0)	(0)	(0)	(0)	
16. *Notostira elongata*	6	3	2	9		5	2	2	7	
	(2)	(0)	(1)	(2)		(3)	(2)	(2)	(5)	
17. *Orthops campestris*	0	0	0	0		0	1	0	1	
	(0)	(0)	(0)	(0)		(0)	(0)	(0)	(0)	
18. *Pachytomella parallela*	0	1	0	1		0	0	0	0	
	(0)	(0)	(0)	(0)		(1)	(0)	(0)	(1)	
19. *Plagiognathus arbustorum*	14	8	0	22		2	3	0	5	
	(0)	(1)	(0)	(1)		(0)	(0)	(0)	(0)	
20. *Stenodema laevigatum*	1	1	0	2		0	1	0	1	
	(0)	(1)	(0)	(1)		(0)	(0)	(0)	(0)	
21. *S. calcaratum*	8	17	26	25		36	43	147	79	
	(9)	(6)	(13)	(15)		(14)	(17)	(26)	(31)	
22. *Stenotus bionatus*	22	31	0	53		44	55	0	99	
	(8)	(8)	(0)	(16)		(33)	(21)	(0)	(54)	
23. Miridae larvae			248					325		
			(47)					(146)		
Total (2–23)	53	66	276	119	8.1	97	115	474	212	8.0
	(31)	(35)	(68)	(66)	(1.2)	(79)	(60)	(182)	(139)	(2.4)
No. species				11					13	
				(12)					(13)	

numerous than other Auchenorrhyncha species. The mean number of larvae and adults of this species in a sample unit was 20.1 and 12.5 in the L1 and L2 biotopes respectively. Among the Heteroptera species occurring in the L biotopes *Ischnodemus sabuleti* was more abundant than any of the other species. This species, which was abundant on *Glyceria maxima* in Leersum, appeared at high frequencies both in sweep-net and suction samples in Langbroek. Observations in the latter area revealed that *I. sabuleti* occurred in high densities on both *P. arundinacea* and *D. caespitosa*. The extremely high density of this lygaeid (mean number of larvae and adults in a sample unit 306.1 and 203.5 in the L1 and L2 biotopes respectively) might have had consequences for *M. brevipennis*, which inhabits the same food plant.

The general species-associations of *M. brevipennis* in this biotope contrasts with those of the K+ biotope in Leersum. Since the frequency of *C. anceps*, being restricted to *J. effusus* which in this biotope is less common than in the K+ one, was higher, there is evidence that this biotope is less wet than the K+ one. *Acanthodelphax denticauda* and *Streptanus sp.* (which were reared in the laboratory on *D. caespitosa*) are species closely associated with *M. brevipennis*. *Javesella discolor*, another delphacid species observed there and reared in the laboratory on *D. caespitosa*, belongs also to the community of *M. brevipennis*. The eurytopic Auchenorrhyncha species *P. spumarius*, *A. pascuellus*, *J. pellucida* and most of the Heteroptera species (excluding *I. sabuleti*) were also common in the L– biotope. *Stenocranus major*, more common in sweep-net samples in this biotope, particularly colonized *Phalaris arundinacea*. In its main features, this association of *M. brevipennis* with other Auchenorrhyncha agrees with that reported by KONTKANEN (1950) from South Finland.

Our conclusion may be that high frequencies of each of the *Muellerianella* species occur in different habitats, while low ones (especially of *M. fairmairei*) may be expected at localities where the other species is common. In general *M. fairmairei*, occurring in higher densities than *M. brevipennis*, is a typical species of wet natural meadows, although it may be found in forests as well. *M. brevipennis* could be characterized as a stenotopic species of 'fresh' biotopes mainly in wooded areas.

The data reported by KONTKANEN, LINNAVUORI and VILBASTE, indicate that *M. brevipennis* was sampled more frequently than *M. fairmairei* in the Northern part of Europe. In contrast to this, *M. fairmairei* was more frequently sampled than *M. brevipennis* from Central Europe (KUNTZE, REMANE, MORRIS, LE QUESNE). Our experience from Southern Europe suggests that *M. brevipennis* may be rare, while *M. fairmairei* has been more often collected. So far these data support the hypothesis that *M. brevipennis* is a cold-adapted species, while *M. fairmairei* is more adapted to moderate climates.

6. PHENOLOGY

Records from Europe

Faunistic records on the phenology and ecology of Auchenorrhyncha from Southern Finland (62°N) (KONTKANEN 1947, 1950a, 1954 and LINNAVUORI 1952) have shown that *M. fairmairei* and *M. brevipennis* are univoltine. Their adult period extends from half July untill half of September.

Records from Germany (52°N) (KUNTZE 1937, MÜLLER 1972, REMANE 1958, SCHIEMENZ 1972, and WITSACK 1971), England (52°N) (HASSAN 1939, WALOFF and SOLOMON 1973) and Ireland (52°N) (MORRIS 1974) have demonstrated that each of the two species is bivoltine. The two adult periods extend in succession from June into Ocotber.

In South Europe the phenology of the two species is not known, but there are some records, which suggest that *M. fairmairei* might be polyvoltine at lower latitudes. Samples taken by the author in South Greece (38°N) on 5th of May 1975 comprised two females and 18 third to fifth instar larvae. Material of what is supposed to be the same species from Azores (38°N) and Madeira (32°N) was borrowed from the museum of Helsinki (leg. H. Lindberg). This material collected at four localities in the Azores in the period between 13–30 of May comprised 8 females and one male. In ten localities of Madeira 22 females and one male were collected in the period between 20–24 of April 1959, and 3 females and 2 males in the period between 8–15 of May. Other material of *M. fairmairei* collected in South-East France (43°N) at the end of June 1975 and 1976 (leg. Cobben and De Vrijer) comprised mainly females with swollen abdomen apparently from the end of their generation.

Records from Holland

Netting samples of *M. fairmairei* taken from all biotypes at Leersum-Holland (52°N) during four successive years of 1972–1975 resulted in two distinct generations in each year (Fig. 9). The first adult generation extended from middle of June till the end of July, with a maximum number of collected individuals occurring at about the first third of July. The adult period of the second generation started at about 10th of August and terminated at the end of October. The maximum number of collected individuals of this generation occurred in the middle of September. Quantitatively, the second generation is several times larger than the first. The occurrence of each of the two generations was rather constant over the years, and corresponded with the data given by KONTKANEN (1954) from Germany.

Netting samples of *M. brevipennis* taken at Langbroek during the summer of 1974 have resulted in a low number of individuals, which are not suitable for comparison with those of *M. fairmairei*. Therefore, comparison of the phenology of the two species is based on samples taken with the suction method on the same dates during 1974. The number of larvae and adults of *M. fair-*

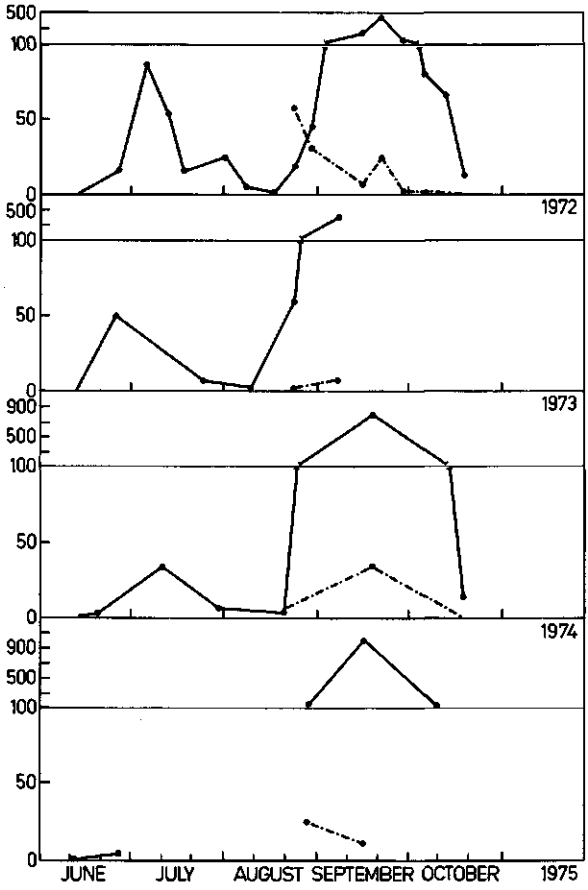


FIG. 9. Numbers of larvae (dashed line) and adults (solid line) of *M. fairmairei* in netting samples taken from all biotopes at Leersum during 1972–1975.

mairei obtained from the K+ biotope in Leersum (14 × 0.08 m²) and those of *M. brevipennis* from the L1 and L2 biotopes at Langbroek (17 × 0.08 m²) are drawn in Fig. 10. Thus, occurrence and quantity of each generation of *M. fairmairei* are closely similar to those obtained by the netting method (Fig. 9). It is clear, however, that the phenology of *M. brevipennis* does not match that of *M. fairmairei*. The beginning of the first generation counted from the adults of *M. brevipennis* occurred at about the first-third of July, probably 3–4 weeks later than that of *M. fairmairei*. This generation is extended partly in the second generation of *M. fairmairei*. Four larvae of *M. brevipennis* collected on 30th of June were in the fourth and fifth instar. On the same date observations at Leersum revealed that the larvae of the second generation of *M. fairmairei* were in the second and third instar. The second adult generation of *M. brevipennis* probably starts at the end of August extending till the end of October as

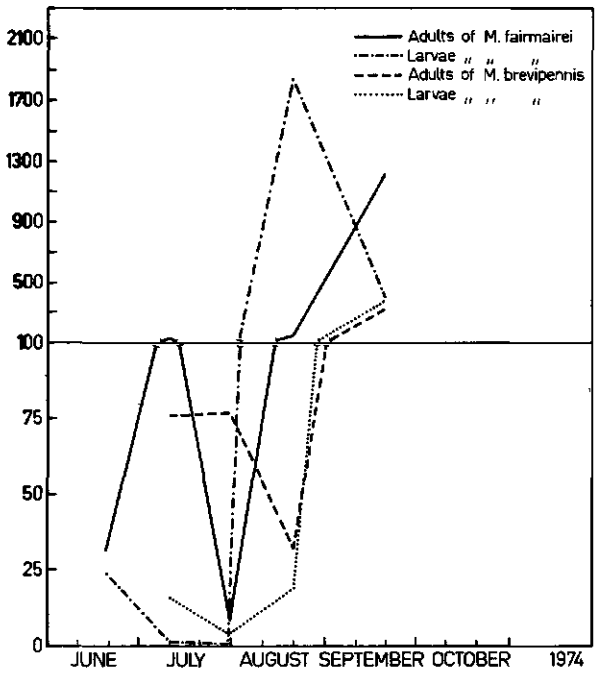


FIG. 10. Numbers of larvae and adults of *M. fairmairei* and *M. brevipennis* in suction samples taken from the K+1 and K+2 biotopes at Leersum, and the L1 and L2 biotopes at Langbroek. The samples of the two species were taken on the same dates in 1974.

well. Thus, there is no distinct isolation between the two generations of *M. brevipennis*. From this, it is strongly suggested that part of the first generation does not result in a second one, due to the fact that the stragglers of the first generation deposit eggs in August which go straightly in diapause (see chapter 12).

The second generation of *M. brevipennis* is quantitatively larger than the first as in *M. fairmairei*. The population densities of both generations of *M. brevipennis* are smaller than the respective ones of *M. fairmairei* (Fig. 10), although the sample size was somewhat larger in the former species. Similar records on the occurrence and abundance of the two siblings are given by KUNTZE (1937). Thus at several localities in Germany, where the two species occurred together, *M. fairmairei* was sampled from June till October, while *M. brevipennis* was collected from July till September. *M. fairmairei* is recorded as a more abundant species than *M. brevipennis*.

Conclusively, the phenological data presented demonstrate that although there are certain differences between the occurrences and quantities of the two generations of each species, seasonal isolation between the two sibling species is not apparent.

7. POPULATION BALANCE

It has been reported (DROSOPOULOS, 1975) that the percentage of *M. fairmairei* males in field samples varied from 0–25, while *M. brevipennis* had a 1 : 1 sex-ratio, both in field samples and laboratory colonies. Now, it is known (DROSOPOULOUS 1976, 1977) that this peculiar sex-ratio of *M. fairmairei*, which is very exceptional for invertebrates, is due to the coexistence of two female biotypes. One of the two biotypes is diploid and bisexual. The other is triploid, most probably of hybrid origin, reproducing gynogenetically, but requiring to be mated with males of the bisexual species in order to give progeny (pseudogamy).

Relative low numbers of males have been reported by KONTKANEN (1952) also for *M. brevipennis* from South Finland. This author concluded that those few males apparently had the same longevity as the females. In 1953 the same author reported that *M. brevipennis* was never found to occur at the same localities in South and Central Finland together with *M. fairmairei*, although the two species occurred very often together in the same regions in Germany (KUNTZE, 1937). If the data reported by KONTKANEN from South Finland indeed represent a consistent low percentage of males of *M. brevipennis* it is probable that the *Muellerianella* complex comprises one more unisexual biotype. Two triploid unisexual 'species' are also known from members of the salamander *Ambystoma jeffersonianum* complex (UZZELL, 1964) and in the teleost *Poeciliopsis* (SCHULTZ, 1969). To the opinion of the writer it is also possible that the species recorded by KONTKANEN as *M. brevipennis* could be either a 'taxonomic error' (in place of *M. fairmairei*) or that the samples were containing males and females of *M. brevipennis* and mainly females of the triploid biotype of *M. fairmairei*. A similar association of the *Muellerianella* complex has been observed in Goor and Langbroek (Holland), where *H. mollis*, *D. caespitosa* and *J. effusus* are closely growing.

VILBASTE (1974) referred also to the absence of males in *M. fairmairei* (26 ♀♀ from 17 localities), but to the presence of males in *M. brevipennis* (42 ♂♂ and 78 ♀♀ from 25 localities) in samples taken between 7 July and 28 August from Latvia and Lithuania.

7.1. THE POPULATION STRUCTURE OF M. FAIRMAIREI

In Table 11, material of *M. fairmairei* is recorded, obtained at some localities in Europe. This material comprises either populations reared in the laboratory and tested cytogenetically for the presence or absence of the unisexual biotype, or preserved material loaned by the museum of Helsinki (Azores-Madeira), LE QUESNE'S personal collection (Jersey) and sampling material taken by MORRIS (Ireland and West England).

TABLE 11. *M. fairmairei:* Biotype, relative number of males and dates of samples taken at some localities in Europe. (Net-samples; *, suction samples).

No. sample	Locality	Latitude (°N)	Date	No. ♂, ♀	♂♂ No.	(%)	X^2	Biotype
1.	Burren, (Ireland)	53.00	23–25.7.71	52	23	(44.2)	0.69	
2.	Burren, (Ireland)	53.00	29.8–5.9.71	185	63	(34.1)	18.82***	
3.	Burren, (Ireland)	53.00	5.9.71	134*	61	(45.5)	1.07	
4.	Castor Hanglands, (W. England)	52.30	25.7.68	86	39	(45.3)	0.74	
5.	Portreath Cornwall, (W. England)	53.17	21.7.70	258	7	(2.7)	230.76***	
6.	Portreath Cornwall, (W. England)	53.17	3.9.70	–	–	–		
7.	Ascot-Silwood Park, (S. England)	51.45	28.9.75	31	3	(9.7)		2n + 3n
8.	Quaine, (Jersey)	49.25	5.10.66	1	1			
9.	Quaine, (Jersey)	49.25	5.10.68	8	4			
10.	Quaine, (Jersey)	49.25	26.8–3.9.70	6	0			
11.	La Saline, (Jersey)	49.25	30.8.54	4	3			
12.	Val des Vaux, (Jersey)	49.25	21.9.65	2	0			
13.	Goor, (Holland)	52.45	4.7.76	41	3	(7.3)		2n + 3n
14.	Perpignan, (S. France)	43.00	23.6.75	14	3			2n?
15.	Perpignan, (S. France)	43.00	25.6.76	5	–			2n?
16.	Nevers, (C. France)	47.00	31.7.76	68	3			2n + 3n
17.	Skaloula-Doris (S. Greece)	38.40	5.5.75	20	10	(50.0)		2n
18.	Azores	38.00	13–30.5.59	9	1			
19.	Madeira	32.45	20–24.4.59	23	1			
20.	Madeira	32.45	8–15.5.59	5	2			

Two samples (No. 1, 3) taken in Burren (Ireland) had a ratio of males to females not significantly differing from the theoretical 1 : 1 (Table 11). The third sample (No. 2) from Ireland taken on 29.8–5.9.71 from several biotopes at Burren cannot indicate whether it includes the triploid biotype of *M. fairmairei*, because its relative number of males was high. Conclusively, it is likely that the triploid biotype of *M. fairmairei* is absent from that locality in Ireland. In addition, *M. brevipennis* was not sampled at those localities (MORRIS, 1974), so that in Ireland there may be no possibility of hybridization between the two diploid species, eventually resulting in a triploid biotype of *Muellerianella*. LE QUESNE (1960) reported also absence of *M. brevipennis* in Ireland, although *D. caespitosa* is common in this region.

The sex-ratio of the sample No. 4 (Table 11), taken at Castor Hanglands in South-West England, suggests that it consists only of the diploid biotype of *M. fairmairei*. However, it is evident that the other sample (No. 5) taken in Portreath, Cornwall includes mainly the triploid biotype of *M. fairmairei*. Similar samples taken at the latter locality of West England on 3.9.70 contained no *M. fairmairei* surprisingly enough (MORRIS, personal communication).

Absence of *M. fairmairei* at a given moment was noted as well in a locality of France (Nevers). There the author had observed abundance of *M. fairmairei* in October 1974. Two years later *M. fairmairei* was found in July only in another locality 200 m. separated from the former one. Local extinction of *M. fairmairei* caused by the 'reproductive parasite' (the triploid form) could be given as a reasonable answer to the mentioned phenomenon.

The number of specimens collected in Jersey, the Azores and Madeira are too small to allow suggestions to be made on the occurrence of the triploid biotype of *M. fairmairei.*

In conformity with all data of Table 11, in which also material from other localities of Europe has been analysed cytogenetically, it can be suggested that the triploid biotype of *M. fairmairei* occurs in the overlapping region of the two bisexual sibling species. Pure diploid populations of *M. fairmairei* should be found, either exclusively outside or in some isolated localities within the region of *M. brevipennis*. However, future work can elucidate better the geographic distribution of the *Muellerianella* complex.

7.2. Crossings

Raatikainen (1967) reported that it was very difficult to determine the actual sex-ratio of the delphacid *Javesella pellucida* in material collected in the field, because the method, frequency and period of sampling during the generation of the insect influence the sex-ratio found. In order to give insight into the peculiar case of sex-ratio of *Muellerianella* from field samples controlled crossings within and between populations of each species were made. The progeny obtained from each crossed female (Tables 12–16) did not correspond always with its maximum fecundity, because some females were isolated a long time before being mated. The total resulted number of offsprings of each cross is given. The X^2-test indicates whether the resulted number of sexes differs from the theoretical 1 : 1 ratio.

Table 12 shows pairwise crossings between females collected in the K+ biotope in Leersum and males of a Greek population reared in the laboratory for 1–5 months. One of the three females crossed with different males (A, B, C) produced a large progeny comprising only females. Therefore, two groups, consisting of three females each, were crossed with the same male. Two females of each group resulted in all-female progeny, while the remaining two females produced both males and females. Undoubtedly the male played no role in the sex determination of the five females (No. 2, 4, 5, 7, 8), which produced an all-female progeny. Back-crossings of some of these females resulted in all-female progeny as well (Table 12, III). However, the other four females (No. 1, 3, 6, 9) gave a bisexual progeny. There were no significant differences between the resulted male-progeny of the four crossed males (A, C, 1, 2) ($X^2 = 1.42$, $P > 0.05$). The F_2 had a similar sex-ratio as the bisexual F_1 (Table 12, II). The resulted total number of males in F_1 was not significant-

TABLE 12. Crossings between females of *M. fairmairei* from a Dutch population (F^H) and males of this species from a Greek population (F^G).

No. cross. ♀ × ♂	No. Pairs.	No. larvae	No. ♂,♀	No. ♂	No. ♀	Sex-ratio (♂♂:♀♀)	X^2
I. Parent crosses							
1. $F^H♀_A \times F^G♂_A$	(1)	147	114	65	49	57.0 : 43.0	2.25
2. $F^H♀_B \times F^G♂_B$	(1)	–	514	–	514	0 : 100	
3. $F^H♀_C \times F^G♂_C$	(1)	–	27	12	15	44.4 : 55.6	0.33
4. $F^H♀_1 \times F^G♂_1$	(1)	–	283	–	283	0 : 100	
5. $F^H♀_2 \times F^G♂_1$	(1)	–	291	–	291	0 : 100	
6. $F^H♀_3 \times F^G♂_1$	(1)	–	45	23	22	51.1 : 48.9	0.02
7. $F^H♀_4 \times F^G♂_2$	(1)	–	160	–	160	0 : 100	
8. $F^H♀_5 \times F^G♂_2$	(1)	–	283	–	283	0 : 100	
9. $F^H♀_6 \times F^G♂_2$	(1)	–	396	236	160	59.6 : 40.4	14.59***
Σ(1–9) (F_1)	(9)	147	2113	336	1777	15.9 : 84.1	
Σ(1, 3, 6, 9) (F_1)	(4)	147	582	336	246	57.7 : 42.3	13.92***
Σ(2, 4, 5, 7, 8) (F_1)	(5)	–	1531	–	1531	0 : 100	
II. Crosses between ♂♂ × ♀♀ of the bisexual F_1 (1, 3, 6, 9).							
Σ (F_2)	(5)	–	273	155	120	56.0 : 44.0	3.99*
III. Back crosses of the all-female F_1 (2,8).							
1. $(F^H♀_B \times F^G♂_B)♀ \times F^G♂_2$	(1)	–	99	–	99	0 : 100	
2. $(F^H♀_B \times F^G♂_B)♀ \times F^G♂_3$	(1)	–	92	–	92	0 : 100	
3. $(F^H♀_B \times F^G♀_B)♀ \times F^G♂_4$	(1)	–	21	–	21	0 : 100	
4. $(F^H♀_B \times F^G♂_B)♀ \times F^G♂_{1,2}$	(1)	–	8	–	8	0 : 100	
5. $(F^H♀_5 \times F^G♂_2)♀ \times F^G♂_2$	(1)	–	105	–	105	0 : 100	
6. $(F^H♀_5 \times F^G♂_2)♀ \times F^G♂_2$	(1)	–	73	–	73	0 : 100	
7. $(F^H♀_5 \times F^G♂_2)♀ \times F^G♂_5$	(1)	–	24	–	24	0 : 100	
8. $(F^H♀_5 \times F^G♂_2)♀ \times F^G♂_5$	(1)	–	105	–	105	0 : 100	
9. $(F^H♀_5 \times F^G♂_2)♀ \times F^G♂_{7,9}$	(2)	–	245	–	245	0 : 100	
10. $(F^H♀_5 \times F^G♂_2)♀ \times F^G♂_8$	(1)	–	137	–	137	0 : 100	
11. $(F^H♀_5 \times F^G♂_2)♀ \times F^G♂_{4,5,10,11}$	(4)	–	424	–	424	0 : 100	
Σ(1–11) (B_1)	(15)	–	1333	–	1333	0 : 100	

ly different from the F_2 ($X^2 = 0.15$, $P > 0.05$), while the ratio of males-females both in F_1 and F_2 was significantly different from the theoretical 1 : 1. Thus, in F_1 and F_2 the males were more numerous than the females.

All pairwise crossings of the reciprocal cross (females of the Greek colony and males from Holland) resulted in bisexual progeny (Table 13). There were no significant differences between the resulted male-progeny of the five parent males (A, B, C, 1, 2) ($X^2 = 0.77$, $P > 0.05$). However, the females produced by the individual nine parent crosses were more numerous than the males. There was a significant difference between the ratio of males to females of this cross and its reciprocal bisexual cross (Table 12 and 13, indicated by squares). The ratio of males to females of each of the two crosses was not significantly dif-

TABLE 13. Crossings between females of *M. fairmairei* from a Greek population (F^G) and males of this species from a Dutch population (F^H).

No. cross.	♀ × ♂	No. Pairs	No. larvae	No. ♂,♀	No. ♂	No. ♀	Sex-ratio (♂♂ : ♀♀)	X^2
1.	$F^G♀_A \times F^H♂_A$	(1)	–	231	99	132	42.9 : 57.1	4.71*
2.	$F^G♀_B \times F^H♂_B$	(1)	–	286	117	169	40.9 : 59.1	9.45**
3.	$F^G♀_C \times F^H♂_C$	(1)	–	11	5	6	45.5 : 54.5	0.09
4.	$F^G♀_1 \times F^H♂_1$	(1)	–	5	3	2	60.0 : 40.0	0.20
5.	$F^G♀_2 \times F^H♂_1$	(1)	41	231	105	126	45.5 : 54.5	1.91
6.	$F^G♀_3 \times F^H♂_1$	(1)	–	157	59	98	37.6 : 62.4	9.69**
7.	$F^G♀_4 \times F^H♂_2$	(1)	80	289	133	156	46.0 : 54.0	1.83
8.	$F^G♀_5 \times F^H♂_2$	(1)	75	218	95	123	43.6 : 56.4	3.60
9.	$F^G♀_6 \times F^H♂_2$	(1)	96	214	94	120	43.9 : 56.1	3.16
Σ	(1–9) (F_1)	(9)	292	1642	710	932	43.2 : 56.8	30.01***

ferent from the ratio females to males of the other ($X^2 = 0.17, P > 0.05$).

Two crossings, each of five pairs of *M. fairmairei* (Dutch bisexual population isolated previously in the laboratory), produced a progeny with ratio of males to females not different from the theoretical 1 : 1 (Table 14). However, significantly different ($X^2 = 8.68$**) was its ratio of males to females from the same Dutch population crossed with males of the Greek population (Tables 12,I and 14).

The results of the three mentioned combinations of crossings between the Dutch and Greek population of *M. fairmairei* (Tables 12, 13 and 14) were crucial for the present study. They revealed that the Dutch population

TABLE 14. Crossings between females and males of *M. fairmairei* (Dutch diploid population isolated in the laboratory).

No. cross	No. pairs	No. larvae	No. ♂,♀	No. ♂	No. ♀	Sex-ratio (♂♂ : ♀♀)	X^2
1.	(5)	98	241	116	125	48.1 : 51.9	0.34
2.	(5)	24	181	88	93	48.6 : 51.4	0.14
Σ (1–2) (F_1)	(10)	122	422	204	218	48.3 : 51.7	0.46

TABLE 15. Crossings (in pairs) between 8 females and 8 males of *M. fairmairei* (French population from Nevers).

No. cross	No. Pairs	No. larvae	No. ♂,♀	No. ♂	No. ♀	Sex-ratio (♂♂ : ♀♀)	X^2
1.	(1)	–	234	130	104	55.6 : 44.4	2.89
2.	(1)	–	121	61	60	50.4 : 49.6	0.08
Σ (1–2) (F_1)	(2)	–	355	191	164	53.8 : 46.2	2.05
Σ (3–8) (F_1)	(6)	–	434	–	434	0 : 100	

TABLE 16. Crossings between females and males of *M. brevipennis* (Dutch population).

No. cross.	♀ × ♂	No. pair	No. larva	No. ♂,♀	No. ♂	No. ♀	Sex-ratio (♂♂ : ♀♀)	X^2
1.	$♀_1 \times ♂_1$	(1)	–	41	16	25	39.0 : 61.0	1.98
2.	$♀_2 \times ♂_1$	(1)	–	50	23	27	46.0 : 54.0	0.32
3.	$♀_3 \times ♂_1$	(1)	–	83	42	41	50.6 : 49.4	0.01
4.	$♀_4 \times ♂_2$	(1)	–	23	16	7	69.6 : 30.4	3.52
5.	$♀_5 \times ♂_2$	(1)	–	41	24	17	58.5 : 41.5	1.20
6.	$♀_6 \times ♂_2$	(1)	–	86	46	40	53.5 : 46.5	0.42
Σ(1–6) (F_1)		(6)		324	167	157	51.5 : 48.5	0.31

comprised one bisexually and another gynogenetically reproducing female biotype, while the Greek population consisted only of the diploid females. A French population (Nevers) had a similar composition as the Dutch population, however, within a proportion of unisexual to bisexual females higher than that of the Dutch one (Table 15).

Six pairs of *M. brevipennis* (Dutch population reared in the laboratory) resulted in bisexual progenies with a total ratio of males to females not significantly different from the theoretical 1 : 1 (Table 16). There were no significant differences between the resulted male-progeny of the two parent males ($X^2 = 1.82$, $P > 0.05$).

The conclusions from the crossings recorded in Tables 12–16 are: 1. The triploid pseudogamous biotype of *M. fairmairei* is reproducing exclusively gynogenetically. 2. The sex-ratio in the progeny of crosses between males-females (2n) of the same population is closer to the theoretical 1 : 1, than the sex-ratio in the progeny of crosses between different diploid populations of the same species. The latter is more pronounced in crosses between females of *M. brevipennis* and males of *M. fairmairei* (see chapter 15). So far it can be assumed that the produced sex-ratio of the two bisexual sibling species remained approximately close to 1 : 1.

7.3. SEX-RATIO IN FIELD SAMPLES

7.3.1. *M. fairmairei in Holland*

During the summer of 1972 the sampling frequency in all biotopes at Leersum was with about one week intervals. This sampling-rate clearly showed that *M. fairmairei* passed through two generations in all biotopes investigated (Table 17). There was no difference between the occurrence of the two generations in each biotope because the highest density occurred on the same day in all biotopes (Table 17). According to the total relative numbers of males collected in each biotope, the percentages of ♂♂ in the K biotope appeared to be higher than in the G and S biotopes (for the situation of these localities, see chapter 3 and Fig. 6). There were no significant differences between the total

The second explanation could be that the males of *M. fairmairei* have longer longevity than the females (see Chapter 10), due to selection in maintaining the two populations of this species. If this is the case, the sex-ratio at about the end of the generation should be approximately 1:1 (Fig. 12b).

The third explanation could be that the phenology of the triploid biotype occurs somehow earlier than that of the diploid one, due to its possible higher rate of development and reproduction (DROSOPOULOS, 1977). Consequently a normal sex-ratio of 1:1 would appear at the end of the generation of the triploid biotype (Fig. 12c).

Moreover, a combination of the above mentioned explanations cannot be excluded.

7.3.2. *Ecological niche of the two biotypes*

Netting and suction samples were taken from the K biotopes on the same dates during the two generations of *M. fairmairei* (Table 20). It appeared, that the relative number of males in sweep-net samples of the first generation did not differ significantly from the second generation ($X^2 = 0.78$, $P > 0.05$). However, while the relative number of males sampled during the first generation did not differ between the two sampling methods ($X^2 = 0.001$, $P > 0.05$), males in suction samples were evidently more numerous than in sweep-net samples of the second generation. These data indicate that the sex-ratio of males to females had increased in the second generation as evidenced by the suction sampling method. The fact that this sex-ratio remained about the same in both sampling methods during the first generation could be due to the vegetation structure, which might be sampled with equal success by both methods. However, the fact that the total number of females of both generations was more numerous in sweep-net than in suction samples, suggests that the triploid females could be more abundant in the upper layers of the vegetation. The diploid females and males might be more readily extracted with the suction method from a lower vegetation layer, which is less suitable for the sweep-net method. This vertical zonation in the substrate presumes that the diploid males and females should occur in the suction and sweep-net samples in about equal proportion, as for example is found in the bisexual *M. brevipennis* (see section 7.3.3.), and in another delphacid, *J. pellucida* (HEIKINHEIMO and RAATIKAINEN 1962).

From the reasoning given above, one may deduce that either the females ($2n + 3n$) are vertically distributed within the stratum otherwise than the males, or that each of the two biotypes of *M. fairmairei* occupies a slightly different ecological niche, which for the bisexual population is the lower vegetation layer and for the unisexual the upper one. Unfortunately, this hypothesis could not be proven directly since the two female types are not morphologically different. However, the possibility that both female types occur on the upper layer of the vegetation and the males more down is less likely.

The total ratio of males to females obtained in the K biotopes by the suction

TABLE 20. Relative numbers of males of *M. fairmairei* in sweep-net samples and suction samples (in brackets) taken from Leersum.

Biotopes	K + 1			K + 2			K–			K+(1+2) and K–		
Year	Total ♂,♀	♂♂ No.	♂♂ %	Total ♂,♀	♂♂ No.	♂♂ %	Total ♂,♀	♂♂ No.	♂♂ %	Total ♂,♀	♂♂ No.	♂♂ %
1st. generation	1	0	0.0	3	2	66.7	29	6	20.7	33	8	24.2
1973	(39)	(8)	(20.5)	(53)	(19)	(35.8)	(119)	(24)	(20.2)	(211)	(51)	(24.2)
1st. generation	–	–	–	2	0	–	7	2	28.6	9	2	22.9
1974	(81)	(27)	(33.3)	(75)	(17)	(22.7)	(185)	(30)	(16.2)	(341)	(74)	(21.7)
2nd. generation	115	22	19.1	361	77	21.3	21	0	0.0	497	99	19.9
1974	(722)	(234)	(32.4)	(625)	(250)	(40.0)	(10)	(7)	(70.0)	(1357)	(491)	(36.2)
2nd. generation	332	61	18.4	318	67	21.1	355	27	7.6	1005	155	15.4
1975	(2752)	(1055)	(38.3)	(1462)	(496)	(33.9)	(695)	(230)	(33.1)	(4909)	(1781)	(36.3)
1st. generations	1	0	0.0	5	2	40.0	36	8	22.2	42	10	23.8
1973–1974	(120)	(35)	(29.2)	(128)	(36)	(28.1)	(304)	(54)	(17.8)	(552)	(125)	(22.6)
2nd. generations	447	83	18.6	679	144	21.2	376	27	7.2	1502	254	16.9
1974–1975	(3474)	(1289)	(37.1)	(2087)	(746)	(35.7)	(705)	(237)	(33.6)	(6266)	(2272)	(36.3)
Both generations	448	83	18.5	684	146	21.3	412	35	8.5	1544	264	17.1
1973–1975	(3594)	(1324)	(36.8)	(2215)	(782)	(35.3)	(1009)	(291)	(28.8)	(6818)	(2397)	(35.2)

method was 35.2 : 64.8 (Table 20). This proportion is not significantly different from the 1 : 2 one ($X^2 = 0.16$, $P > 0.05$). Since the sex-ratio of the diploid Dutch population was approximately 1 : 1 (Table 14) it is evident that the proportion of the two female biotypes of *M. fairmairei* in the K biotopes is about 1 : 1. In addition, this ratio (males to females of about 35 : 65) is frequently obtained with the suction method (Table 20).

Apparently the two biotypes of *M. fairmairei* maintain an equilibrium of equal densities in that biotope. This equilibrium could not be maintained so far in the laboratory, when mixed populations of the two biotypes from several localities in Europe were reared in the same cage. After 1–2 generations both populations became extinct due to the disappearance of males (DROSOPOULOS, 1975). Therefore, some mechanism is operating for the maintainance of the two female populations in the field. It might be either mate selection, as has been reported for the coexistence in unisexual-bisexual species complexes of *Poeciliopsis* fishes (MOORE and MCKAY, 1971), selective pressure of predators and egg-parasitism or other unknown factors.

7.3.3. *Samples of M. brevipennis at Langbroek*

The ratio of males to females of this species was not significantly different from the theoretical 1 : 1, both in netting samples ($X^2 = 3.33$, $P > 0.05$) and in suction samples ($X^2 = 0.07$, $P > 0.05$) (Table 21). Also there were no

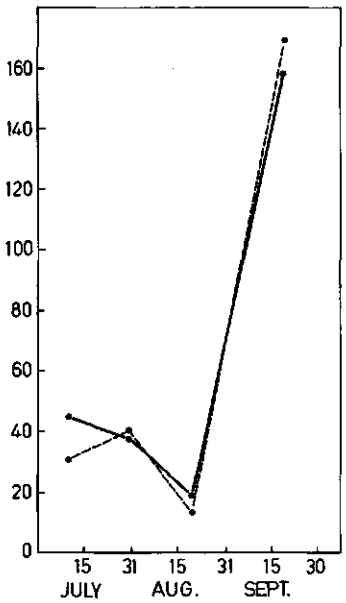


FIG. 13. *M. brevipennis:* Number of males (dashed line) and females (solid line) in suction samples taken from all biotopes at Langbroek during the summer of 1974.

TABLE 21. Relative numbers of males of *M. brevipennis* in sweep-net samples and suction samples (in brackets) taken from Langbroek.

Biotopes	L_1			L_2			$L_1 + L_2$		
Year	Total ♂,♀	♂♂ No	%	Total ♂,♀	♂♂ No.	%	Total ♂,♀	♂♂ No.	%
1st. generation 1974	2 (117)	1 (46)	50.0 (39,3)	10 (68)	1 (38)	10.0 (55.9)	12 (185)	2 (84)	16.7 (45.4)
2nd. generation 1974	14 (205)	6 (100)	42.9 (48.8)	4 (122)	2 (69)	50.0 (56.6)	18 (327)	8 (169)	44.4 (51.7)
Both generations 1974	16 (322)	7 (146)	43.8 (45.3)	14 (190)	3 (107)	21.4 (56.3)	30 (512)	10 (253)	33.3 (49.4)

significant differences between the numbers of males sampled with the two methods ($X^2 = 0.76, P > 0.05$). The appearance of males during the two generations of 1975 was closely matching that of the females (Fig. 13). Conclusively, the sex-ratio of *M. brevipennis* in this locality in the Netherlands corresponded to that of a normal bisexual species.

TABLE 23. Frequency of macropterous (M) males and females of *M. fairmairei* in sweep-net samples taken during the first and second generations (1972–1975) at Leersum.

Biotope	Generation	No. ♂ : ♂M	No. ♀ : ♀M	No. ♂♀ : ♂♀M	X^2
K	First	19 : 11	106 : 30	125 : 41	2.84
K	Second	470 : 20	2412 : 66	2882 : 86	2.92
(K + G)	First	21 : 12	235 : 59	256 : 71	4.62*
(K + G)	Second	490 : 23	2949 : 78	3439 : 101	5.75*

each macropterous sex were not significantly different from the other when the samples were taken in the K biotopes during both generations, but significantly different when the samples were taken from both biotopes together during the two generations. Proportionally, the macropterous males were more frequent than the macropterous females in samples taken from both biotopes together. This difference indicates that the triploid biotype, which occurs more frequently in biotope G than in biotope K, produced fewer macropterous females than the diploid biotype.

Comparison of the macropterous *M. fairmairei* sampled in the K biotope with the sweep-net and suction method (Table 24) revealed that the macropterous form was significantly more numerous in sweep-net than in suction samples both during the first generation ($X^2 = 25.29$***) and the second one

TABLE 24. Macropterous (M) adults of *M. fairmairei* in samples taken on the same dates with sweep-net and suction method (in brackets) in the K biotope at Leersum. Same material as in Table 20.

Biotopes	K+ (1+2) + K–						
	total	♂♂ M		♀♀ M		♂♀ M	
Years	♂♀	No.	%	No.	%	No.	%
1st generation	33	7	21.2	13	39.4	20	60.6
1973	(211)	(16)	(7.6)	(47)	(22.3)	(63)	(29.9)
1st generation	9	1	11.1	2	22.2	3	33.3
1974	(341)	(3)	(0.9)	(34)	(10.0)	(37)	(10.9)
2nd generation	497	1	0.2	2	0.4	3	0.6
1974	(1357)	(5)	(0.4)	(11)	(0.8)	(16)	(1.2)
2nd generation	1005	7	0.7	33	3.3	40	4.0
1975	(4909)	(33)	(0.7)	(62)	(1.3)	(95)	(1.9)
1st generation	42	8	19.0	15	35.7	23	54.7
1973–1974	(552)	(19)	(3.4)	(81)	(14.7)	(100)	(18.1)
2nd generation	1502	8	0.5	35	2.3	43	2.8
1974–1975	(6266)	(38)	(0.6)	(73)	(1.2)	(111)	(1.8)
both generations	1544	16	1.0	50	3.2	66	4.3
1973–1975	(6818)	(57)	(0.8)	(154)	(2.3)	(211)	(3.1)

TABLE 25. Frequency of macropterous (M) males and females of *M. fairmairei* in suction samples taken from the K biotope at Leersum during the first generations of 1973–1974 and the second ones of 1974–1975.

Generation	No. ♂ : ♂ M	No. ♀ : ♀ M	No. ♂♀ : ♂♀ M	X^2
First	125 : 19	427 : 81	552 : 100	0.65
Second	2272 : 38	3994 : 73	6266 : 111	0.19

($X^2 = 7.28^{**}$). Also, it appeared that the macropterous form in suction samples of the first generation was significantly more numerous than in those of the second generation ($X^2 = 439.86^{***}$). Moreover, there were no significant differences between the proportion of macropterous males to the number of males and the proportion of macropterous females to the total number of females in the suction samples in each of the two generations (Table 25). There was no evidence that either of the two sampling methods was more suitable in collecting macropterous males on the one hand or macropterous females on the other, either during the first generation ($X^2 = 2.73$, $P > 0.05$) or the second one ($X^2 = 3.61$. $P > 0.05$) (Table 24).

b. *M. brevipennis:* Table 26 shows that in this species also the brachypterous form was more numerous than the macropterous one. The macropterous form was more numerous in sweep-net than in suction samples during the first generation and almost absent in samples taken with both sampling methods during the second generation. Although suction samples taken on the same dates of the first generation revealed a higher frequency of macropters of *M. fairmairei* than of *M. brevipennis* ($X^2 = 6.74^{**}$), the low number of macropterous specimens in the sweep-net samples did not differ between the two

TABLE 26. Macropterous (M) adults of *M. brevipennis* in sweep-net and suction samples (in brackets) taken at Langbroek during 1974. Same material as in Table 21.

Biotopes	$L_1 + L_2$						
	total ♂♀	♂♂ M		♀♀ M		♂♀ M	
		No.	%	No.	%	No.	%
1st generation 1974	12 (185)	1 (0)	8.3 (0.0)	3 (7)	25.0 (3.8)	4 (7)	33.3 (3.8)
2nd generation 1974	18 (327)	0 (1)	0.0 (0.3)	0 (0)	0.0 (0.0)	0 (1)	0.0 (0.3)
both generations 1974	30 (512)	1 (1)	3.3 (0.2)	3 (7)	10.0 (1.4)	4 (8)	13.3 (1.6)

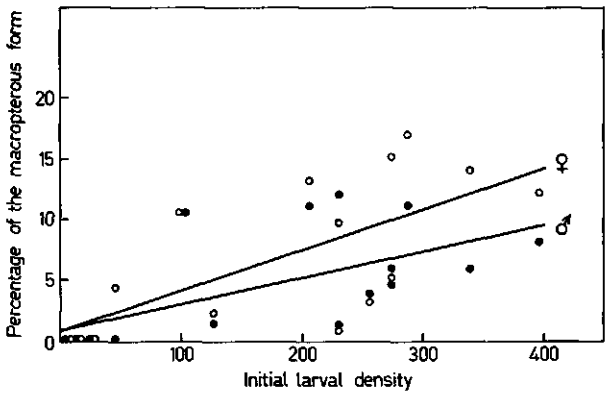


FIG. 15. *M. fairmairei* (2n): Correlation between initial larval density and the resulted percentage of macropterous males (♂, ●) and females (♀, ○).

8.3. DISCUSSION AND CONCLUSIONS

Field and laboratory data have demonstrated that short photoperiod is the major factor favoring production of the brachypterous form in both species. The suppression of macropterous adults is more pronounced under short day rearings in the laboratory than under the regime of short day during the second generation in the field. This is probably due to the relatively longer short photoperiod, under which adult development occurs during the second generation in the field, compared with the constant short photoperiod (L.D = 10 :14), under which the adults develop in laboratory colonies.

Under long photoperiod, field and laboratory data have shown that macropterous adults are produced. However, the larval density is another factor determining the proportion of the two wing forms under long photoperiod. Thus, at low larval densities (1–3 specimens per test-tube) only brachypterous adults of both species were produced. At higher densities of larvae, the subsequent proportion of macropterous *M. brevipennis* fluctuated around 60% (Fig. 14). In contrast to *M. brevipennis*, *M. fairmairei* revealed an increasing proportion of the less numerous macropterous adults as the larval density increased. However, this was more pronounced in the diploid biotype than in the triploid one (Fig. 14). This is in accordance with the significantly higher frequency of macropterous males than macropterous females in the G + K biotopes, where the population density of the triploid unisexual biotype of *M. fairmairei* was higher than that of the bisexual diploid biotype (Table 23). The same result was obtained in the laboratory, where the frequency of the macropterous females of both biotypes of *M. fairmairei* could be compared with the frequency of the macropterous males (Table 28).

That heredity (by means of genome dosage effect) is a factor determining wing dimorphism in the *Muellerianella* complex, is unlikely. Thus, because of

the hybrid origin of *M. fairmairei* (3n), this biotype should produce more macropterous adults than *M. fairmairei* (2n) and fewer than *M. brevipennis*, under long photoperiod. However, this did not occur and macropterous adults of the triploid were even less frequent than those of the diploid biotype.

It is obvious that wing dimorphism in the *Muellerianella* complex is photoperiodically influenced and in this respect it is closely linked with the phenomena of diapause and reproduction.

9. PARASITISM

9.1. Parasitoids of larvae and adults

Pipunculidae (Diptera), Dryinidae (Hymenoptera) and Elenchidae (Strepsiptera) are known as common parasitoids of larvae and adults of leafhoppers (*e.g.* Hassan 1939, Lindberg 1950, Kontkanen 1950, Cobben 1956, Raatikainen 1966, 1967 and Waloff 1975). Although other species of delphacids have been frequently reported to be highly parasitized, *M. fairmairei* and *M. brevipennis* were seldom infested by these parasitoids.

At Leersum and Langbroek, parasitism in Delphacidae was frequent. However, neither *Muellerianella* species was ever found to be parasitized by any of the parasitoids mentioned above. In other localities in Europe (Silwood Park – England and Nevers – France) two larvae of *M. fairmairei* were found which were parasitized by Dryinidae. In Holland (Wageningen) one female larva of *M. brevipennis* was found to be parasitized by a strepsipteron. The emerged female leafhopper, which had been reared in a test-tube behaved differently from other females. First, it refused to mate with any of the males which were offered in its test-tube for a period of 15 days. Secondly, it failed to oviposit although eggs were found in its abdomen.

9.2. Egg-parasites

In contrast to the rarity of larval and adult parasitism, eggs of both delphacids were found to be frequently parasitized by *Anagrus sp.* (Mymaridae)[1].

Witsack (1973) reported that up to 60% of the winter eggs of *M. brevipennis* were parasitized in East Germany. Morcos (1953) and Whalley (1956) referred to egg parasitism of *M. fairmairei* in England.

At Leersum and Langbroek first and second generation eggs of *M. fairmairei* and *M. brevipennis* were found to be highly parasitized (Table 29). Although summer eggs of *M. fairmairei* laid in *H. lanatus* were less frequently parasitized than winter eggs oviposited in *J. effusus*, the same difference in parasitism occurred between summer and winter eggs laid by *M. brevipennis* in *D. caespitosa*. Thus, egg parasitism was more frequent in hibernating eggs. It is possible that eggs with a continuous embryonic development during the first generation are exposed to mymarids for a relatively shorter period than diapausing eggs of the second generation.

One sample of eggs was taken at Leersum on 19th July. Externally, parasitized eggs could not be distinguished from unparasitized eggs, in which embryo-

[1] The material is in the hands of Dr. J. Walker (Imperial College of Science and Technology, London), who is presently engaged with the revision of the difficult *Anagrus*-complex.

TABLE 29. Parasitism of eggs by *Anagrus sp* in the field.

Species and locality	Sampling date (generation)	Plant with deposited eggs	No of eggs sampled	No.	Parasitized eggs %
M. fairmairei					
Leersum	12.12.73 (2nd)	*J. effusus*	370	105	28.4
Leersum	24.01.74 (2nd)	*J. effusus*	82	31	37.8
Leersum	13.02.74 (2nd)	*J. effusus*	162	42	25.9
Leersum	10.07.74 (1st)	*H. lanatus*	171	17	9.9
Langbroek	24.11.74 (2nd)	*J. effusus*	43	10	23.3
M. brevipennis					
Langbroek	20.08.74 (1st)	*D. caespitosa*	86	11	12.8
Langbroek	22.11.74 (2nd)	*D. caespitosa*	154	56	36.4

genesis was approximately at the embryo-rotation stage. Parasitism became apparent 3–5 days later, when infested eggs turned orange, and the parasites emerged between 23–26 July under room conditions.

The time at which parasites emerged from winter eggs laid by each of the two sibling species is shown in Fig. 16. The longer period required for the emergence of parasites from eggs of *M. brevipennis* than of *M. fairmairei* is remarkable. Whether this difference is due to the fact that the egg-diapause is stronger in *M. brevipennis* than in *M. fairmairei* (Chapter 12), cannot be explained so far; unparasitized winter eggs of both species hatched at 20°C in a period of 10–15 days (Fig. 32).

However, this important subject of egg parasitism has not been worked out in detail. For example, it is not known, whether the same parasite species infests eggs of both delphacid species. Also, it is not known if eggs laid by each biotype of *M. fairmairei* suffer the same rate of parasitism.

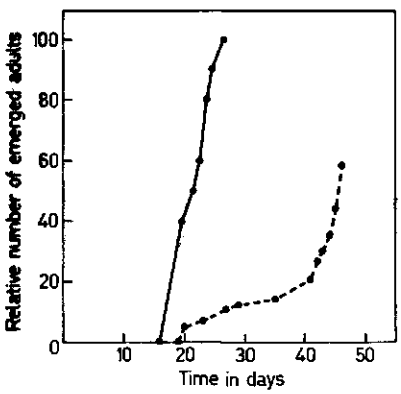


FIG. 16. Adults of *Anagrus sp.* emerged in the laboratory at 20°C from field eggs of *M. fairmairei* (solid line) and of *M. brevipennis* (dashed line) sampled in the same locality at Langbroek on 26.11.74.

10. REPRODUCTION

The population density of *M. fairmairei* in the field is distinctly higher than that of *M. brevipennis* (See chapters 5 and 6). Moreover it has been suggested that males of *M. fairmairei* might live longer than females (See chapter 7). In view of this, a detailed laboratory study on reproduction and adult longevity was undertaken in order to interpret the field data more accurately.

10.1. Material and methods

Last instar larvae of both species were collected in the field during their first generation, and they were reared individually in test-tubes. Each test-tube contained two stems of either *H. lanatus* or *D. caespitosa*. For each test-tube the time of adult moult, sex and wing form of the insect reared were noted at intervals of 12 hours.

The time of first mating was observed as follows: Every day after the adult moult a male was transferred into one tube of a female and observed for a period of four hours.

The pre-oviposition period was measured daily. The cut stems in each test-tube were replaced by new ones, and the egg-production was measured by dissecting the grass stems at intervals of two days throughout the female life span. In this way the number of eggs deposited by each female during the course of its oviposition period was established.

The egg-fertility was tested by replanting the marked stems in a pot of sandy soil. There the cut-stems were kept for a period of 7–10 days. After this period the appearance of a yellow mycetome or rotated embryos in the eggs indicated egg-fertility.

These experiments were carried out under temperatures of 20–25° and long photoperiod (L.D = 18 :6). The relative humidity in the test-tubes was 80–90%.

It should be mentioned here that the female material of *M. fairmairei* studied included four brachypterous specimens from a female colony.

10.2. Sexual maturity

Motile sperm is already present in the testis of the last larval instar of both species. Males of *M. fairmairei* may start mating during the first day following emergence, while those of *M. brevipennis* do so during the second day. In both species mature eggs were never observed before the fourth day of adulthood.

Brachypterous females of *M. fairmairei* seemed to mate somewhat earlier than macropterous females, but the difference is not significant ($t = 0.80$, $P > 0.05$) (Table 30). However, the difference in the time of sexual receptivity

between *M. fairmairei* and *M. brevipennis* is significant both in the brachypterous ($t = 5.88^{***}$) and the macropterous forms ($t = 2.37^{*}$).

10.3. Preoviposition period

It has been reported that in other delphacids (*e.g. Javesella pellucida*, Mochida, 1973. *Nilaparvata lugens*, Kisimoto, 1965, and *Struebingianella lugubrina*, De Vrijer, personal communication) preoviposition period in the macropterous form is longer than in the brachypterous form. The same difference was found between each wing form of *M. fairmairei* ($t = 3.49^{**}$) and *M. brevipennis* ($t = 4.59^{**}$) (Table 30). In addition the mean preoviposition period in the brachypterous form of *M. brevipennis* was significantly longer than in the corresponding form of *M. fairmairei* ($t = 2.57^{**}$). However, the mean preoviposition periods of the macropterous forms of the two species did not differ significantly ($t = 0.50$, $P > 0.05$).

This might be due to the wider range of the preoviposition period in *M. fairmairei* than in *M. brevipennis* (Figs. 17 and 18). In these figures is shown the correlation between the preoviposition period and the time of first mating of each wing form of the two species. The regression analysis has given:

M. fairmairei, brachypterous $Y = 1.42 + 0.280X$ ($r^2 = 0.10$)
M. fairmairei, macropterous $Y = 3.09 + 0.044X$ ($r^2 = 0.01$)
M. brevipennis, brachypterous $Y = 4.44 + 0.035X$ ($r^2 = 0.01$)
M. brevipennis, macropterous $Y = -0.59 + 0.643X$ ($r^2 = 0.37$)

where X is the preoviposition period in days, Y is the time required by each female for its first mating and r^2 the coefficient of determination. Obviously, there is no correlation between the two variables X and Y.

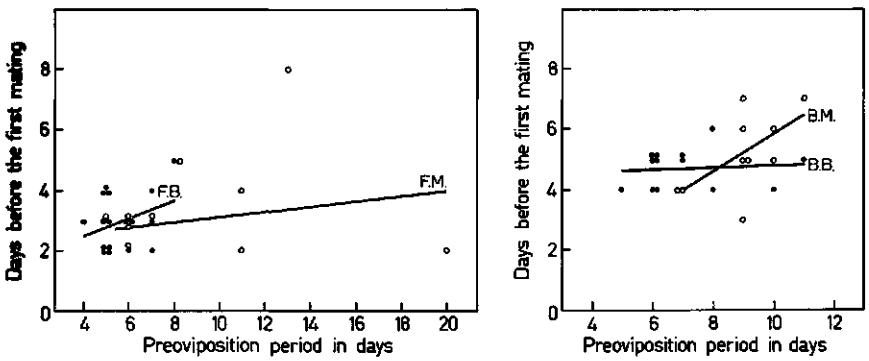


Fig. 17. *M. fairmairei:* correlation between preoviposition period and first mating in the brachypterous (FB, ●), and the macropterous form (FM, ○).
Fig. 18. *M. brevipennis:* correlation between preoviposition period, and first mating in the brachypterous (BB, ●) and the macropterous form (BM, ○).

10.4. Oviposition and post-oviposition periods

The analysis of variance of the mean oviposition period did not reveal significant differences between both wing forms of the two species ($F = 1.60$, d.f. 3 and 47, $P > 0.05$). That of the period after the termination of the oviposition period had similar result ($F = 0.49$, d.f. 3 and 47, $P > 0.05$), (Table 30).

Table 30. Reproduction and adult longevity in brachypterous and macropterous *M. fairmairei* and *M. brevipennis*.

	No ♂, ♀ observed	Mean ± S.D.	Range
M. fairmairei (brachypterous)			
Days before first mating of ♀	17	3.00 ± 0.94	2– 5
Preoviposition period (days)	17	5.65 ± 1.06	4– 8
Oviposition period (days)	14	38.29 ± 9.69	25– 55
Postoviposition period (days)	14	1.14 ± 1.46	0– 5
Egg production/♀	14	357.50 ± 128.92	124–525
Eggs/day/♀	14	9.16 ± 2.20	4.96– 12.84
♂ longevity (days)	16	60.50 ± 20.33	27– 92
♀ longevity (days)	14	45.07 ± 9.72	33– 64
M. fairmairei (macropterous)			
Days before first mating of ♀	10	3.50 ± 1.84	2– 8
Preoviposition period (days)	14	9.43 ± 3.94	5– 20
Oviposition period (days)	9	35.11 ± 11.44	21– 55
Postoviposition period (days)	9	1.56 ± 1.81	0– 5
Egg production/♀	9	331.78 ± 172.26	83–564
Eggs/day/♀	9	9.20 ± 2.93	2.59–12.00
♂ longevity (days)	1	64.00	
♀ longevity (days)	9	46.33 ± 11.57	30–67
M. brevipennis (brachypterous)			
Days before mating of ♀	13	4.69 ± 0.63	4– 6
Preoviposition period (days)	14	7.00 ± 1.71	5– 10
Oviposition period (days)	13	43.77 ± 12.94	21– 62
Postoviposition period (days)	13	0.77 ± 1.30	0– 4
Egg production/♀	13	160.77 ± 43.75	101–251
Eggs/day/♀	13	3.80 ± 1.25	2.16–6.09
♂ longevity (days)	10	57.10 ± 23.37	30– 82
♀ longevity (days)	13	51.62 ± 13.63	27– 75
M. brevipennis (macropterous)			
Days before first mating of ♀	10	5.20 ± 1.32	3– 7
Preoviposition period (days)	16	10.00 ± 1.79	7– 13
Oviposition period (days)	15	33.93 ± 14.98	16– 58
Postoviposition period (days)	15	1.00 ± 1.51	0– 4
Egg production/♀	15	154.87 ± 70.79	37–266
Eggs/day/♀	15	4.64 ± 1.57	2.31–8.22
♂ longevity (days)	9	53.22 ± 21.19	20– 77
♀ longevity (days)	15	44.47 ± 15.49	26– 70

10.5. EGG-PRODUCTION

Table 30 shows that the mean egg-production of the brachypterous form did not differ significantly from the macropterous one, either in *M. fairmairei* ($t = 0.38$, $P > 0.05$) or in *M. brevipennis* ($t = 0.27$, $P > 0.05$).

As was expected from the field data, the mean fecundity in *M. fairmairei* proved to be significantly larger than in *M. brevipennis* both in the brachypterous forms ($t = 5.39^{***}$) and the macropterous ones ($t = 2.94^{**}$). The fecundity of *M. fairmairei* was approximately twice that of *M. brevipennis*.

10.5.1. *Differences in the reproduction between the two biotypes of M. fairmairei*

Since there were no differences found between the mean fecundity of brachypterous and macropterous forms within each species, the fecundity of all females of each species were pooled as shown in the histograms of Fig. 19. It appeared that our samples unraveled two distinct populations of *M. faimairei* and one of *M. brevipennis*. Most probably, the population in *M. fairmairei* with the higher fecundity is that of the triploid pseudogamous biotype and the other that of the bisexual biotype. This is deduced from the fact that the mean fecundity of the four triploids was 438.75 ± 59.16 eggs and that very often large a progeny was obtained from such female colonies.

10.5.2. *The unisexual and bisexual species*

It may be seen in the data in Table 31 that:

a. The number of days before first mating does not differ significantly between the two biotypes of *M. faimairei* ($t = 0.16$, $P > 0.05$), but *M. brevipennis* is significantly different both from the triploid biotype ($t = 5.08^{***}$) and the diploid biotype of *M. fairmairei* ($t = 2.75^{**}$).

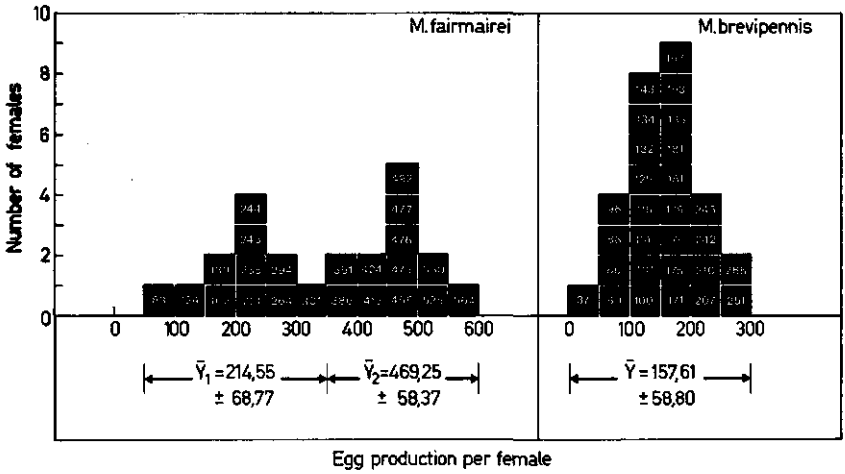


FIG. 19. Pooled fecundities of brachypterous and macropterous forms of *Muellerianella* species.

TABLE 31. Relationship of reproduction and longevity in females of the two biotypes of *M. fairmairei* and *M. brevipennis*. Same material as in Table 30.

	No. ♀ observed	Mean ± S.D.	Range
M. fairmairei (2n)			
Days before first mating	11	3.27 ± 1.85	2– 8
Oviposition period (days)	11	27.91 ± 3.62	21– 32
Postoviposition period (days)	11	1.45 ± 1.81	0– 5
Egg production/♀	11	214.55 ± 68.77	83–301
Eggs/day/♀	11	7.76 ± 2.47	2.59–11.62
M. fairmairei (3n)			
Days before first mating	12	3.17 ± 0.94	2– 5
Oviposition period (days)	12	45.42 ± 6.27	37– 55
Postoviposition period (days)	12	1.17 ± 1.40	0– 5
Egg production/♀	12	469.25 ± 58.37	386–564
Eggs/day/♀	12	10.47 ± 1.62	7.71–12.84
M. brevipennis			
Days before first mating	23	4.91 ± 1.00	3– 7
Oviposition period (days)	28	38.50 ± 14.69	16– 62
Postoviposition period (days)	28	0.89 ± 1.40	0– 4
Egg production/♀	28	157.61 ± 58.80	37–266
Eggs/day/♀	28	4.25 ± 1.47	2.31–8.22

b. The mean oviposition period of the triploid biotype differs significantly from the diploid one ($t = 8.28^{***}$) but not from that of *M. brevipennis* ($t = 2.09$, $P \simeq 0.05$). However, the mean ovipositon period is not significantly different between the two bisexual species, ($t = 3.55^{**}$).

c. The mean postoviposition periods in the two biotypes of *M. fairmairei* and *M. brevipennis* do not differ significantly ($F = 0.74$, d.f. 2 and 48, $P > 0.05$).

d. The mean fecundity of the triploid biotype is significantly higher than that of the diploid one of *M. fairmairei* ($t = 9.53^{***}$). In addition the mean fecundity of the diploid *M. fairmairei* was likewise higher than that of *M. brevipennis* ($t = 2.42^{*}$).

10.5.3. *Fecundity and oviposition period*

The correlation between fecundity and oviposition period is shown in Fig. 20. The regression analysis has given:

M. fairmairei $Y = -84.79 + 11.668X$ ($r = 0.832^{***}$)

M. brevipennis $Y = 58.43 + 2.575X$ ($r = 0.643^{***}$)

where X and Y are the oviposition period and total egg production of each species and r the correlation coefficient. There is a significant difference between the two regression lines ($F = 350.95^{***}$). This difference might be explained by the regression lines of the two biotypes of *M. fairmairei*, which are represented in the same figure. Thus the same analysis gives:

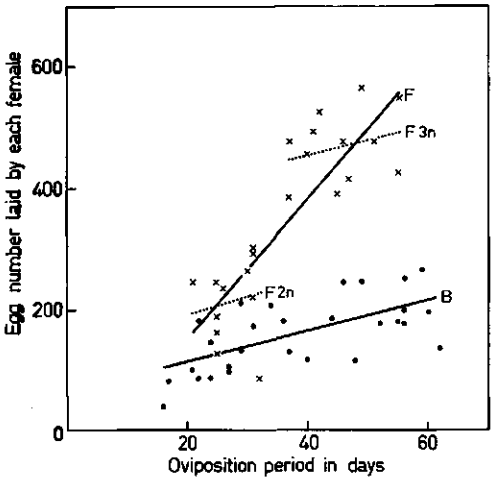


FIG. 20. Correlation between the duration of the oviposition period and the number of eggs produced per female of *M. brevipennis* (●) and *M. fairmairei* (x).

M. fairmairei (2n) $Y = 128.09 + 3.098X$ ($r = 0.163$)
M. fairmairei (3n) $Y = 353.14 + 2.556X$ ($r = 0.275$)

Obviously, there is no correlation between the two variables X and Y in this case. However, there is no significant difference both between the regression lines of the two females biotypes of *M. fairmairei* ($F = 0.215$, $P > 0.05$), and between the regression lines of the two bisexual species ($F = 0.012$, $P > 0.05$).

10.5.4. *Rate of reproduction*

There was not found significant difference between the rate of reproduction of each wing form both in *M. fairmairei* ($t = 0.035$, $P > 0.05$) and in *M. brevipennis* ($t = 1.57$, $P > 0.05$) (Table 30). However, the rate of egg production of *M. fairmairei* was significantly higher than that of *M. brevipennis* both in brachypterous females ($t = 7.85$***) and in macropterous ones ($t = 4.31$***).

The triploid biotype of *M. fairmairei* might have a higher rate of egg production than the diploid one ($t = 3.08$***), and the latter form might have a higher rate than *M. brevipennis* ($t = 4.41$***) (Table 31).

10.5.5. *Rate of reproduction during oviposition period*

The daily egg production in each wing form of *M. fairmairei* and *M. brevipennis* throughout the oviposition period is graphically presented in Figs. 21 and 22. In this case the regression analysis gives:

M. fairmairei brachypterous $Y = 9.10 + 0.154X - 0.005X^2$ ($r = 0.665$***)
M. fairmairei macropterous $Y = 8.44 + 0.165X - 0.005X^2$ ($r = 0.440$*)
M. brevipennis brachypterous $Y = 6.41 - 0.156X + 0.002X^2$ ($r = 0.770$***)
M. brevipennis macropterous $Y = 8.45 - 0.286X + 0.004X^2$ ($r = 0.652$**)

where X is the female longevity in days, Y the mean daily rate of deposited

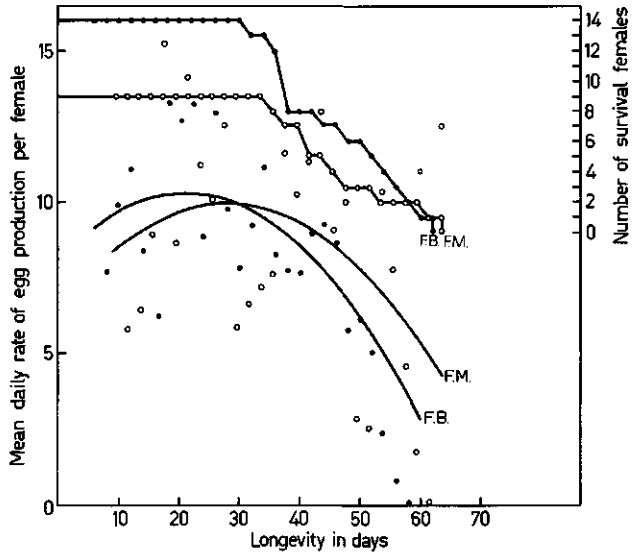


FIG. 21. *M. fairmairei:* relationship between the 'mean daily rate of egg production' (the mean number of eggs per female per day) and the course of the oviposition period. FB, ●, brachypterous; FM, ○, macropterous.

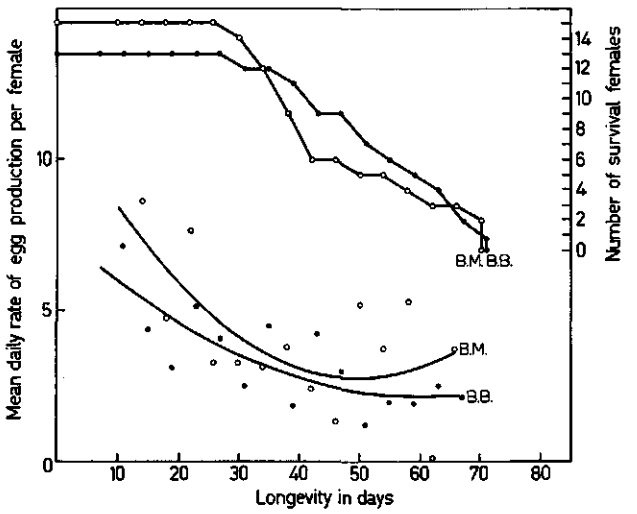


FIG. 22. *M. brevipennis*: relationship between 'mean daily rate of egg production' and the course of the oviposition period. B.B, ●, brachypterous; BM, ○, macropterous.

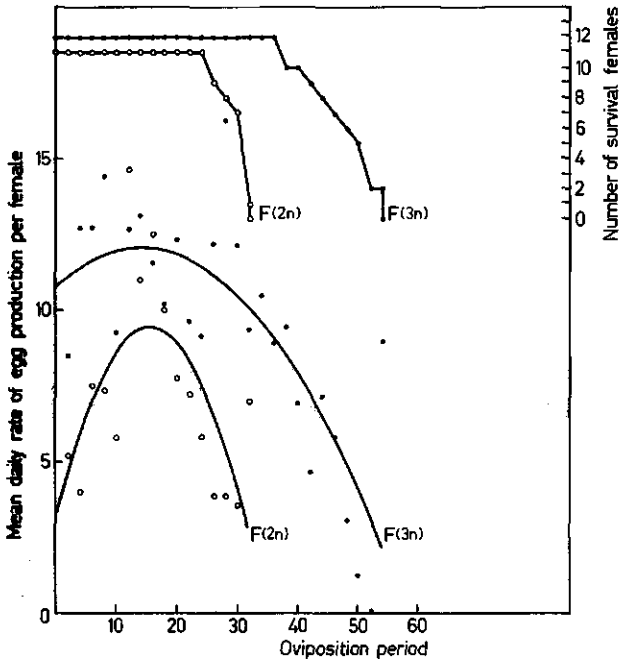


FIG. 23. *M. fairmairei*, 3n, 2n: relationship between 'mean daily rate of egg production' and the course of the oviposition period. ●, 3n; ○, 2n.

eggs by a certain number of females, and r are the correlation coefficients. There are no significant differences between the regression lines of each form of *M. fairmairei* ($F = 0.034$, $P > 0.05$) and *M. brevipennis* ($F = 0.036$, $P > 0.05$). Also, there are no differences between the regression lines of the brachypterous forms of the two species ($F = 0.0003$, $P > 0.05$).

The mortality of each female during the oviposition period is given at the top of each figure.

Comparing the two female biotypes of *M. fairmairei* at a similar manner as above (Fig. 23) the regression analysis shows:

M. fairmairei (2n) $Y = 3.29 + 0.788X - 0.003X^2$ ($r = 0.787$***)
M. fairmairei (3n) $Y = 10.80 + 0.179X - 0.063X^2$ ($r = 0.628$***)

There is a significant difference between the two regression lines ($F = 37.49$***).

10.6. ADULT LONGEVITY

The analysis of variance shows (Table 30) that there are no significant differences between the mean longevities of brachypterous females of the two sibling species ($F = 0.84$, d.f. 3 and 47, $P > 0.05$).

The mean longevity of both wing forms of males and females of *M. brevipen-*

nis does not differ significantly ($F = 1.08$, d.f. 3 and 43, $P > 0.05$).

Also the mean longevities of brachypterous males of *M. fairmairei* and those of both forms of males of *M. brevipennis* appeared not to differ significantly ($F = 0.34$, d.f. 2 and 32, $P > 0.05$).

In chapter 7 it was suggested that males of *M. fairmairei* might live longer in the field than females, and this is supported by the laboratory data ($F = 4.48$ d.f. 2 and 36, $0.01 < P < 0.025$).

10.7. Egg fertility

All deposited eggs of *M. brevipennis* were found to be fertile, even those of females which copulated only once. In the egg-groups of some single-mated females of *M. fairmairei*, a few eggs did not exhibit embryogenesis. These egg-groups had been oviposited during the last 5–7 days of the oviposition period. In such females of *M. fairmairei*, a shortage of sperm had been observed in their spermathecae. Thus, individual motile spermatozoids were noticed instead of a mass-flow of sperm.

The higher fecundity of those females, which probably are triploid, leads to exhaustion of the sperm supply. A similar phenomenon is observed in females crossed with males of the other species (See also chapter 15).

10.8. Discussion and conclusions

These experiments, in relation with studies of field population densities and phenology of the two species demonstrate that *M. fairmairei* is more prolific than *M. brevipennis*. The latter is characterized by a lower rate of reproduction, but which probably lasts longer than in the former species. These intrinsic differences might conceivable be due to the adaptation to different geographic regions and habitats by the two species.

Although there is not direct proof that the unisexual biotype of *M. fairmairei* is more prolific than the bisexual species, the author is of the opinion that the triploid biotype provides hybrid vigor over both bisexual species.

11. OVIPOSITION

11.1. Oviposition hosts

Hassan (1939) referred to the presence of eggs of *M. fairmairei* in the field from the second half of August to the first half of May. The eggs were oviposited in rushes. Morcos (1953) considered blackberry the most important oviposition site for *M. fairmairei*. Witsack (1971, 1973) reported oviposition of *M. brevipennis* in *Deschampsia caespitosa*, both in the field (overwintering eggs) and in laboratory rearings.

There are no records in the literature on the oviposition host of the two species during their first generation.

11.1.1. *Oviposition in the field*

Examination of grass samples from the K+ biotope in Leersum revealed that *M. fairmairei* oviposited in *Holcus lanatus* during the first generation and exclusively in *J. effusus* during the second one (Table 32). Large samples of *H. lanatus*, *H. mollis* and other plants taken in the G biotope, where *J. effusus* is not present, did not contain any overwintering egg. In this area females of *M. fairmairei* had been sampled until the end of October. In other localities, where *J. effusus* was in close vicinity, but not syntopically growing with *H. lanatus*, females were observed to oviposit as well in *J. effusus*. Thus, it is likely that *M. fairmairei* is searching for the presence of *J. effusus* within and probably in the vicinity of its habitat during its second generation.

At Langbroek, although *J. effusus* was growing within the habitat, examination of field samples revealed that the eggs of both generations of *M. brevipennis* had been deposited in *D. caespitosa* (Table 32). There were a few eggs deposited in *J. effusus*, but these eggs produced *M. fairmairei* adults. They were oviposited in that locality either by a few individuals of *M. fairmairei* occurring there or by migratory individuals of this species. Therefore, there is strong evidence that *M. brevipennis* oviposits only in *D. caespitosa*. The fact that this species has been found in habitats where *J. effusus* is not present, favours this conclusion. On the other hand *M. fairmairei* needs a second host for overwintering eggs. In several localities in Europe, where *M. fairmairei* was collected, such a host appeared to be *J. effusus*. Thus, in South and Central France, in South Greece, in South England and in Holland the close association of *J. effusus* and *H. lanatus* gave evidence for the presence of *M. fairmairei*.

11.1.2. *Oviposition in the laboratory*

When *M. fairmairei* was reared under long photoperiod (L.D = 18 : 6) it oviposited in *H. lanatus*, even when *J. effusus* was available (Table 33). When larvae had developed under short photoperiod (L.D = 10 : 14) the females laid their eggs only in *J. effusus*. When larvae had developed under short photoperiod

TABLE 32. Eggs deposited by *M. fairmairei* and *M. brevipennis* in the field.

Date	Sampled plants: Species	No of stems	No of stems with eggs	Total No of eggs	No of eggs/stem: Min	Max
M. fairmairei at Leersum						
20.8.74	*H. lanatus*	20	11	390	8	122
20.8.74	*J. effusus*	27	3	18	6	6
20.8.74	other grasses	32	–	–	–	–
Oct. 73–April 1974	*H. lanatus*	80	–	–	–	–
Oct. 73–April 1974	*J. effusus*	12	12	9893	84	1739
Oct. 74–April 1975	*H. mollis*	131	–	–	–	–
Oct. 74–April 1975	*H. lanatus*	164	–	–	–	–
Oct. 74–April 1975	*Rubus sp.*	*	–	–	–	–
M. brevipennis at Langbroek						
30.7.74	*D. caespitosa*	33	12	123	3	22
20.8.74	*D. caespitosa*	58	11	86	5	11
20.8.74	*F. arundinacea*	10	–	–	–	–
20.8.74	*D. glomerata*	8	–	–	–	–
20.8.74	*J. effusus*	50	12	95	4	40
20.8.74	other grasses	113	–	–	–	–
26.11.74	*D. caespitosa*	91	24	194	2	35
26.11.74	*J. effusus*	52	2	80	16	64
26.11.74	*Rubus sp.*	*	–	–	–	–

* One branch of one meter length with 8 subbranches of 80 cm length.

and the emerged females were exposed to long photoperiod, they oviposited in *J. effusus* for a period of about two weeks. After this period eggs had been deposited both in *H. lanatus* and in *J. effusus*. Thus, photoperiod is responsible for the choice of the oviposition host in *M. fairmairei*. It is likely, that the effect of photoperiod on the ovipositing females is a gradual process requiring a period of 2–3 weeks.

Considering that blackberry might be a short day oviposition host, females of *M. fairmairei* were placed either in cages containing *J. effusus*, *Rubus sp.* and *H. lanatus*, or *Rubus sp.* and *H. lanatus*. Table 33 shows that none of the deposited eggs had been oviposited in *Rubus sp.* when *J. effusus* was available. When *J. effusus* was not available, *M. fairmairei* oviposited a large number of eggs in its food-plant and a few in *Rubus sp.* Thus, it can be concluded that in the laboratory, caged females lacking the opportunity to find *J. effusus* can

TABLE 33. Eggs deposited by *M. fairmairei* and *M. brevipennis* in laboratory rearings.

L:D	Sampled plants			Total No. of eggs	No. of eggs/stem	
	Species	Total No. of stems	No. of stems with eggs		Min	Max
M. fairmairei						
18:6	*J. effusus*,	42	–	–	–	–
	H. lanatus.	101	53	963		
10:14	*J. effusus*,	8	6	2117	112	676
	H. lanatus	18	–	–	–	–
10:14	*J. effusus*,	5	5	1109	77	344
	H. lanatus.	14	2	26	8	18
	Rubus sp.	2	–	–	–	–
10:14	*H. lanatus*,	20	15	771	3	140
	Rubus sp.	1	1	18	18	18
10:14	*H. lanatus*,	20	–	–	–	–
↓	*J. effusus*.	6	5	1155	127	313
18:6	*H. lanatus*,	24	18	478	3	58
	J. effusus.	18	13	680	4	130
M. brevipennis						
18:6	*D. caespitosa*,	199	107	1238		
	J. effusus.	47	9	296		
18:6	*D. caespitosa*,	27	11	323	4	54
↓						
10:14	*J. effusus*.	10	8	904	18	258
10:14	*D. caespitosa*,	40	13	258	1	52
	J. effusus.	9	9	977	21	344

oviposit under short day conditions in their food plant. In the field, second generation females of *M. fairmairei* require to search for *J. effusus* as the most appropiate substrate for hibernating eggs.

M. brevipennis oviposited in *D. caespitosa* and *J. effusus* under both long and short photoperiodical conditions (Table 33). The number of eggs oviposited in *J. effusus* under short photoperiod was significantly larger than that under long photoperiod ($X^2 = 985{,}5^{***}$). This indicates that *M. brevipennis* preferred to oviposit in *J. effusus* under these conditions, while in the field the eggs were found only in *D. caespitosa*. Possibly, the closer association of the two plants in the laboratory than in the field could be a reasonable explanation for these contrary data. Whenever other grasses were offered separately as food plants to each species the number of deposited eggs was correlated with the suitability of the plant as a food-plant (Table 33). Therefore, *M. brevipennis* never oviposited in *H. lanatus*, and the diploid *M. fairmairei* never in *D. caespitosa*. The triploid biotype of *M. fairmairei*, which could be reared on *D. caespitosa* successfully oviposited in this plant.

11.2. Oviposition sites and mechanism

The leaf blade is referred to as leaf and the stem, including the covering leaf sheath as well, as the stem. Additionally there is a distinction made between hollow and not hollow stems. The leaves of *J. effusus* are considered as stems.

The two sibling species have the same oviposition site and mechanism. In the field eggs were found deposited in the lower part of the stems. Most eggs were laid under the epidermis and across the main venation of the leaf sheath forming an angle with the vein line (Fig. 24). The angle was approximately 45° for females that oviposited facing either upwards or downwards. Eggs deposited in hollow stems or in those of *J. effusus* were laid perpendicularly in the stem. In the egg-slits of *J. effusus* and *D. caespitosa* a gumlike secretion (Morcos 1951, Strübing 1956) could be easily observed. This secretion covered the eggs and glued them with the plant tissues. The white-like secretion indicated that the eggs had been oviposited recently. This secretion in old slits turned brownish.

Oviposition behavior in the laboratory was similar to that in the field. Under crowded conditions both species oviposited a few eggs also in the leaves. These eggs were mainly deposited across the central venation of the leaf corresponding to the pattern found in the leaf sheath (Fig. 24). In the leaves of *D. caespitosa* eggs were often found deposited in all sites of the leaf. At about the end of the oviposition period some eggs were found partly outside the plant tissues.

Although there was a preference for stems as oviposition sites, both species oviposited in many other parts of the plant. The structure of the plant site may influence the manner of oviposition.

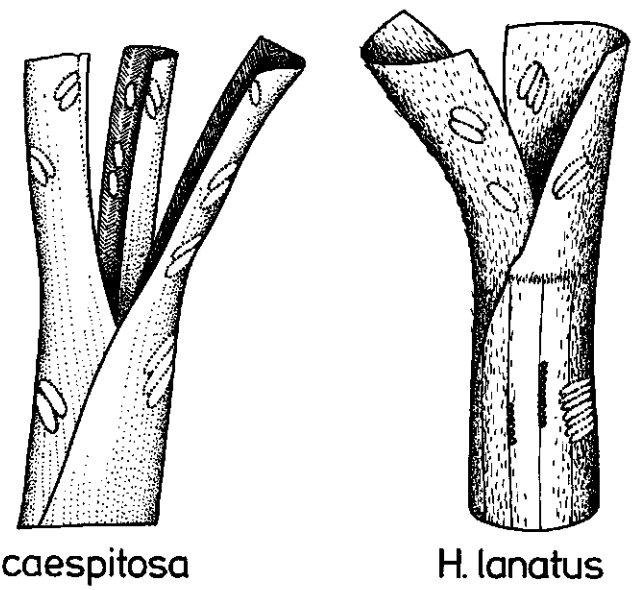


Fig. 24. Oviposition sites of *M. fairmairei* in *H. lanatus* and *M. brevipennis* in *D. caespitosa*.

11.3. Size of egg-groups

Both species oviposited most of their eggs in groups. The individual egg-groups were distinguishable even when several of them touched each other. The total numbers of egg-groups oviposited by each female during its oviposition period were counted in great detail. This was necessary since data on egg-group size of delphacids presented in the literature are somehow confused (Mochida 1964, 1973, Raatikainen 1960, 1967, Mitsuhashi and Koyama 1975). For example, the mean size of egg-groups laid by *Javesella pellucida* in stems of wheat, as recorded by Mochida (1973), is certainly different from the records given by Raatikainen (1967). Both authors referred to several factors influencing the egg-group size. Among these, Mochida has demonstrated statistically that the mean egg-group size laid by the brachypterous form of *J. pellucida* was significnatly larger than that laid by the macropterous form. Raatikainen noticed that the egg-group was apparently small-sized at the beginning of the oviposition period, becoming larger as time elapsed. In addition the same author concluded that several factors influence the egg-group size. Because of these problems the influence of some factors upon the egg-group size has been analyzed, in order to unravel the true differences, if any, between the two delphaicd species.

11.3.1. *Egg-group size and wing form*

Analysis of variance demonstrated (Tables 34 and 35) that there are significant differences among the mean egg-group sizes laid by individuals of each wing form of both species. However the mean egg-group sizes (Fig. 25) of the total number of brachypterous females do not differ significantly from that of the macropterous form, both in *M. fairmairei* ($F = 3.27$, d.f. 1 and 3852, $P > 0.05$) and in *M. brevipennis* ($F = 0.15$, d.f. 1 and 1665, $P > 0.05$). However, the mean size of egg-groups laid by the total number of *M. fairmairei* females was significantly smaller than that of *M. brevipennis*, both in the brachypterous forms ($t = 12.50^{***}$) and in the macropterous ones ($t = 13.70^{***}$) (Tables 34 and 35).

Thus *M. brevipennis* laid a larger average egg-group size in *D. caespitosa* than did *M. fairmairei* in *H. lanatus*.

11.3.2. *Egg-group size during the oviposition period*

The relationships between the mean egg-group size laid by each form of each species during the course of the oviposition period is shown in Fig. 26. The regression analysis has given:

M. fairmairei brachypterous (F.B.) $Y = 1.895 + 0.00995X$ ($r = 0.422$)
M. fairmairei macropterous (F.M.) $Y = 1.940 + 0.00580X$ ($r = 0.323$)
M. brevipennis brachypterous (B.B.) $Y = 2.750 - 0.00630X$ ($r = -0.350$)
M. brevipennis macropterous (B.M.) $Y = 2.610 + 0.00510X$ ($r = 0.068$)

where X is the oviposition period in days, Y the mean size of egg-groups laid by the total number of females of each form and species (recorded in Tables 34

TABLE 34. Number and size of egg-groups deposited per female by each wing form of *M. fairmairei*.

	Brachypterous					Macropterous				
♀	No. of eggs	No. of egg-groups	Eggs per group Mean ± s.d.	Min	Max	No. of eggs	No. of egg-groups	Eggs per group Mean ± s.d.	Min	Max
1	424	182	2.33 ± 0.973	1	5	564	247	2.28 ± 0.935	1	5
2	415	175	2.37 ± 0.959	1	5	189	86	2.20 ± 0.860	1	5
3	525	264	1.99 ± 0.894	1	5	492	259	1.90 ± 0.667	1	4
4	391	208	1.88 ± 0.832	1	5	244	116	2.10 ± 0.759	1	4
5	477	220	2.17 ± 0.876	1	5	386	180	2.14 ± 0.851	1	5
6	456	223	2.04 ± 0.768	1	4	235	123	1.91 ± 0.786	1	5
7	475	236	2.01 ± 0.789	1	5	550	312	1.76 ± 0.708	1	5
8	221	107	2.07 ± 0.812	1	4	243	105	2.31 ± 0.854	1	5
9	294	171	1.72 ± 0.728	1	4	83	37	2.24 ± 0.970	1	5
10	301	134	2.25 ± 0.885	1	6					
11	264	118	2.24 ± 1.079	1	5					
12	476	211	2.26 ± 0.785	1	4					
13	124	62	2.00 ± 0.984	1	5					
14	162	84	1.93 ± 0.753	1	5					
	5005	2395	2.09 ± 0.880	1	6	2986	1465	2.04 ± 0.824	1	5

F = 7.97***, d.f. = 13 and 2382 F = 11.62***, d.f. = 8 and 1456

TABLE 35. Number and size of egg-groups deposited per female by each wing form of *M. brevipennis*.

	Brachypterous					Macropterous				
♀	No. of eggs	No. of egg-groups	Eggs per group Mean ± s.d.	Min	Max	No. of eggs	No. of egg-groups	Eggs per group Mean ± s.d.	Min	Max
1	171	78	2.19 ± 0.934	1	5	100	46	2.17 ± 0.916	1	5
2	251	107	2.35 ± 0.958	1	6	96	44	2.18 ± 0.860	1	4
3	181	77	2.35 ± 0.864	1	5	86	31	2.77 ± 1.099	1	6
4	134	50	2.68 ± 0.926	1	5	37	18	2.06 ± 0.725	1	3
5	114	38	3.00 ± 1.214	1	6	143	52	2.75 ± 1.284	1	7
6	185	68	2.72 ± 1.211	1	6	86	30	2.87 ± 1.335	1	6
7	101	31	3.26 ± 1.586	1	7	266	94	2.83 ± 1.208	1	6
8	193	65	2.97 ± 1.654	1	9	245	90	2.72 ± 1.096	1	6
9	116	47	2.47 ± 1.069	1	5	197	69	2.86 ± 1.183	1	6
10	176	69	2.55 ± 0.753	1	4	175	65	2.69 ± 0.975	1	5
11	129	48	2.69 ± 0.939	1	5	242	86	2.81 ± 1.126	1	6
12	207	72	2.88 ± 1.053	1	6	210	91	2.31 ± 1.220	1	6
13	132	43	3.07 ± 1.065	1	5	179	67	2.67 ± 1.151	1	6
14						80	26	3.08 ± 1.294	1	6
15						181	65	2.78 ± 1.353	1	9
	2090	793	2.64 ± 1.130	1	9	2323	874	2.66 ± 1.176	1	9

F = 4.83***, d.f. = 12 and 780 F = 2.88***, d.f. = 14 and 859

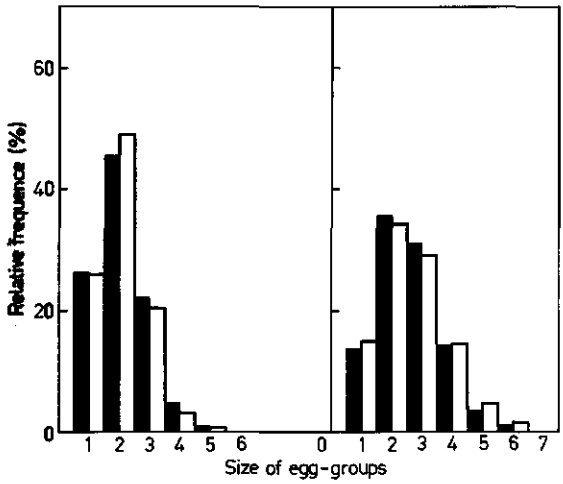


FIG. 25. Relative frequency of egg-group sizes of *M. fairmairei* (left) and *M. brevipennis* (right). Solid and open columns indicate the sizes of egg-groups laid by the brachypterous and the macropterous form, respectively.

and 35) and r the correlation coefficient. There was no significant difference between the regression lines. Thus the F test between

F.B. and F.M. was 0.207 $P > 0.05$,
B.B. and B.M. was 0.241 $P > 0.05$,
B.B. and F.B. was 2.087 $P > 0.05$,
B.M. and F.M. was 0.121 $P > 0.05$.

Obviously, there is no correlation between the size of the egg-group and the time-intervals within the oviposition in any wing form of the two species. The mean size of egg-groups remained constant as time elapsed in both wing forms of each species.

11.3.3. *Egg-group size and rate of egg-production*

Since differences between the mean sizes of egg-groups laid by each wing form of the two siblings were not found, the mean egg-group size laid by the individuals of *M. fairmairei* with a high rate of egg production was compared to those with a lower one. It was expected that in this way the possibly triploid biotype of *M. fairmairei* could be discriminated. The results in Fig. 27 represented in a similar manner to those of Fig. 26, show:

M. fairmairei (2n) $Y = 1.96 + 0.00759X$ ($r = 0.243$)
M. fairmairei (3n) $Y = 2.12 - 0.00345X$ ($r = -0.128$)

There is no significant difference between the two regression lines ($F = 0.481$, $P > 0.05$). Thus, here also the mean egg-group size remained constant during the oviposition period.

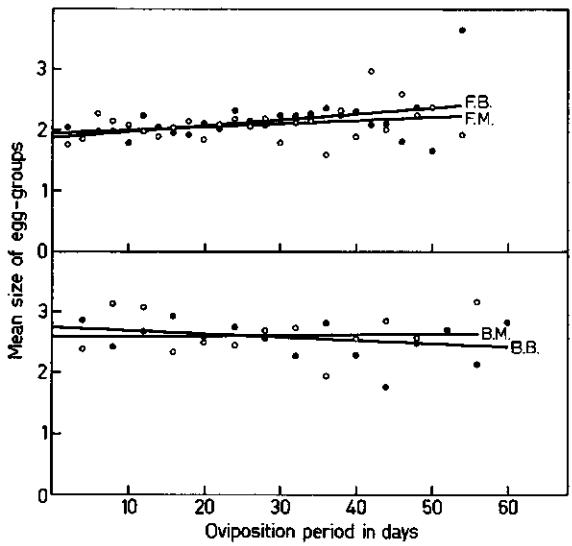


FIG. 26. Correlation between oviposition period and mean size of egg-groups laid by macropterous (○) and brachypterous (●) females of *M. fairmairei* (above) and *M. brevipennis* (below).

11.3.4. *Egg-group size and oviposition plant*

The mean size of egg-groups oviposited by each species in their field oviposition-hosts was compared to that in the respective hosts in the laboratory. The photoperiod used in the laboratory rearings corresponded with the field conditions.

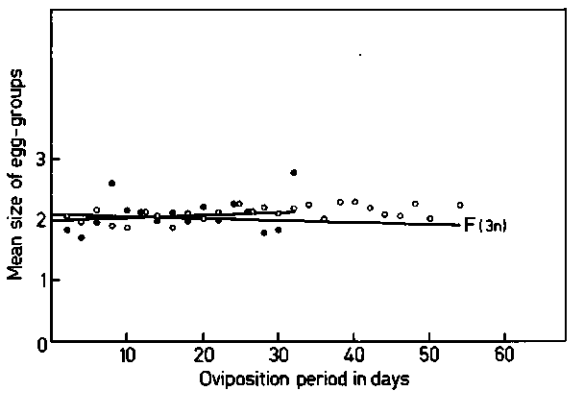


FIG. 27. Correlation between oviposition period and mean size of egg-groups laid by the possibly diploid (2n, ●) and triploid (3n, ○) biotypes of *M. fairmairei*.

11.3.4.1. Egg-group size in the field

Table 36 shows that there were no significant differences between the mean size of egg-groups deposited by *M. brevipennis* in *D. caespitosa* at different dates. The same was found for the mean size of egg-groups deposited by *M. fairmairei* in *J. effusus* at two localities (Table 37). Obviously, there were significant differences between the mean sizes of egg-groups deposited in *H. lanatus* and *J. effusus* (t = 29.59***).

TABLE 36. Size of egg-groups of *M. brevipennis* in the stems of *D. caespitosa* from samples taken at Langbroek.

Date of sampling	No. of eggs	No. of egg-groups	Eggs per group		
			Mean ± s.d.	Min	Max
30.7.74	123	40	3.08 ± 0.93	1	5
20.8.74	86	27	3.19 ± 0.92	1	5
26.11.74	194	69	2.81 ± 1.21	1	7

F = 1.445, d.f. 2 and 133, P > 0.05

TABLE 37. Size of egg-groups of *M. fairmairei* in the stems of *H. lanatus* and *J. effusus*. Samples a, b taken at Leersum and c, d taken at Langbroek.

No. of sample	Date of sampling	Plant	No. of eggs	No. of egg-groups	Eggs per group			
					Mean ± s.d.	Min	Max	
a.	20.8.74	*H. lanatus*	390	109	3.58 ± 1.19	1	7	
b.	15.10.73	*J. effusus*	9893	1378	7.18 ± 4.23	1	30	F = 0.382,
c.	20.8.74	*J. effusus*	95	12	7.92 ± 4.80	1	20	d.f. 2 and
d.	26.11.74	*J. effusus*	80	10	8.00 ± 2.26	1	12	1398
								P > 0.05

11.3.4.2. Egg-group size in the laboratory

The relative frequency of egg-group sizes oviposited by each species in different plants in the laboratory are represented in Fig. 28. It is evident that the mean sizes of egg-groups were significantly larger amongst those oviposited by each species in different plants, than by the two species in the same plant (Tables 38 and 39). For example, the mean size of egg-groups laid by each species in *J. effusus* was apparently larger than that laid in any other plant. However, the mean size of egg-groups laid by *M. fairmairei* in *A. sativa*, *H. vulgare* and *D. caespitosa* were significantly larger than those laid by *M. brevipennis* in the same grasses (t = 5.46***, t = 7.00*** and t = 3.90*** respectively). The same occurred when both species oviposited in *J. effusus* either under long day conditions (t = 2.88**) or under short day conditions (t = 15.50***). Evidently, the egg-groups in the respective food-plants were larger in the field than in the laboratory.

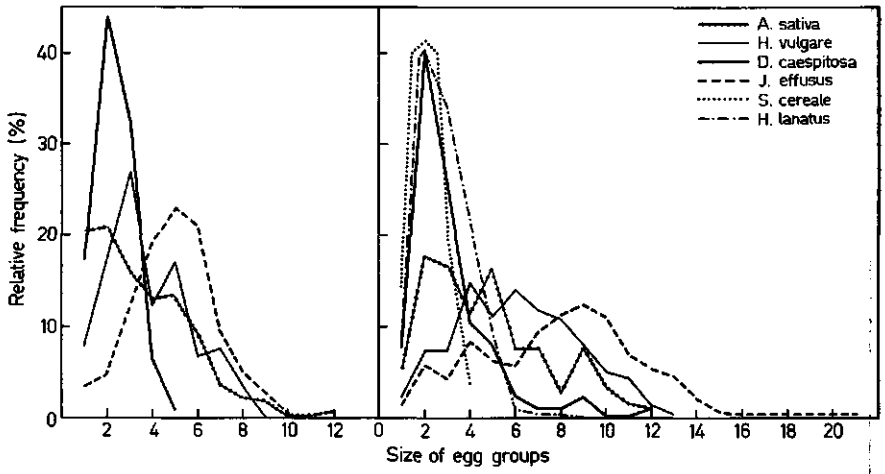


FIG. 28. Relative frequency of egg-group sizes oviposited by *M. brevipennis* (left) and *M. fairmairei* (right) in the stems of different plants in laboratory rearings.

In contrast to this, the mean egg-group sizes laid by *M. fairmairei* in *J. effusus* was larger in the laboratory than in the field ($t = 4.68^{***}$).

Egg-group size and photoperiod

The mean size of egg-groups oviposited by *M. fairmairei* under long and short day conditions in the laboratory did not differ significantly both in *H. lanatus* ($t = 1.75$ $P > 0.05$) and in *J. effusus* ($t = 0.79$ $P > 0.05$). The same was found for *M. brevipennis*, when the eggs were deposited in *D. caespitosa* ($t = 0.38$ $P > 0.05$). However, the mean size of egg-groups oviposited by *M. brevipennis* under short day conditions in *J. effusus* was significantly smaller than under long day conditions ($t = 5.34^{***}$).

TABLE 38. Size of egg-groups of *M. brevipennis* deposited in the stems of different plants in lab. rearings

L : D	Plant	No. of eggs	No. of egg-groups	Eggs per group			
				Mean ± s.d.	Min	Max	
18 : 6	*A. sativa*	824	237	3.48 ± 2.11	1	12	
18 : 6	*H. vulgare*	477	119	4.00 ± 2.13	1	12	
18 : 6	*D. caespitosa*	1561	676	2.31 ± 0.91	1	8	$t = 0.38$
10 : 14	*D. caespitosa*	258	110	2.35 ± 1.05	1	5	$P > 0.05$
18 : 6	*J. effusus*	1200	189	6.35 ± 2.98	1	16	$t = 5.34^{***}$
10 : 14	*J. effusus*	977	195	5.01 ± 1.77	1	10	

TABLE 39. Size of egg-groups of *M. fairmairei* in the stems of different plants in laboratory rearings.

L:D	Plant	No. of eggs	No. of egg-groups	Eggs per group		
				Mean ± s.d.	Min	Max
18:6	*A. sativa*	811	168	4.83 ± 2.67	1	12
18:6	*H. vulgare*	826	135	6.12 ± 2.69	1	13
18:6	*S. cereale*	117	55	2.13 ± 0.69	1	4
18:6	*D. caespitosa*	266	85	3.13 ± 1.91	1	12
10:14	*Rubus sp.*	18	15	1.20 ± 0.41	1	2
18:6	*H. lanatus*	1441	560	2.57 ± 0.92	1	8 | t = 1.75
10:14	*H. lanatus*	1096	443	2.47 ± 0.88	1	6 | P > 0.05
18:6	*J. effusus*	680	88	7.73 ± 4.01	1	25 | t = 0.73
10:14	*J. effusus*	4985	616	8.09 ± 3.80	1	26 | P > 0.05

11.3.5. *Egg-group size and oviposition site*

Table 40 shows that the mean size of egg-groups laid by *M. brevipennis* and *M. fairmairei* in the leaves of their respective host plants was significantly smaller than that laid in the stems ($t = 9.53^{***}$ and $t = 13.83^{***}$, respectively) (Table 39). Also, the mean size of egg-groups laid by *M. fairmairei* in hollow stems of *A. sativa* was significantly larger than that laid in not-hollow ones ($t = 6.46^{***}$).

11.3.6. *Discussion*

1. Apparently the egg-group size in delphacids is dependent upon a complex of factors.

Amongst the main factors, which somehow controlled the size of egg-groups, is the oviposition substrate. The mean size of egg-groups deposited by each species in those plants, which provided a difficult oviposition substrate may be reduced by two ways.

TABLE 40. Size of egg-groups of *M. fairmairei* and *M. brevipennis* deposited in different plant-sites in laboratory rearings.

Ovipositing species	Plant and its site	No. of eggs	No. of egg-groups	Eggs per group		
				Mean ± s.d.	Min	Max
M. fairmairei	*H. lanatus* (leaves)	626	375	1.67 ± 0.68	1	4
M. brevipennis	*D. caespitosa* (leaves)	21	17	1.24 ± 0.44	1	2
M. fairmairei	*A. sativa* (hollow stems)	361	46	7.85 ± 2.98	2	15
M. fairmairei	*A. sativa* (not hollow stems)	128	29	4.41 ± 1.62	2	9

a) When the oviposition substrate is hard, as in stems of *Rubus sp.* oviposition can be difficult. Then the insect ovipositing one or two eggs searches for an easier site. Slits either without eggs or with eggs half deposited in them were observed in hard plant sites.

b) When substrates had not completed development, as stems of young seedlings and leaves, oviposition in long slits was never observed. Probably, water contents in such plant tissues influences oviposition behavior.

2. In the present work a uniform oviposition substrate was offered to each wing form of the two siblings, provided by cut-stems of each respective host-plant. In the work of MOCHIDA (1973) seedlings with an uncertain developmental stage were offered as oviposition substrates to *J. pellucida*. Therefore the statistical analysis MOCHIDA employed, without considering the oviposition substrate as the most important factor determining the size of egg-groups laid by each wing-form of *J. pellucida*, has resulted in wrong conclusions. The mean size of egg-groups in *J. pellucida* recorded by MOCHIDA was approximately 2 eggs per egg-group, while the record of RAATIKAINEN, referring to the same species on the same grass (*Triticum aestivum*), was 16.0.
3. Another factor, which probably had an influence on the size of egg-groups is the rate of egg-deposition. *J. effusus* was an 'easy' oviposition substrate for both siblings, since the range and mean size of the egg-groups were larger than in other plants (Tables 38 and 39). Both species utilized this plant only for oviposition. The rate of egg-production during the oviposition period was larger in *M. fairmairei* than in *M. brevipennis* (see chapter 10). Consequently, the mean size of egg-groups oviposited in *J. effusus* by *M. fairmairei* was larger than that laid in the same plant by *M. brevipennis*. This also occurred, when both species oviposited on *A. sativa*, *H. vulgare* and *D. caespitosa*.
4. Since the mean size of egg-groups oviposited by *M. brevipennis* in *J. effusus* was larger under long day than short day conditions, it may be that the egg production rate of this species is larger in long photoperiods. Different results were obtained for *M. fairmairei* (Tables 38 and 39).
5. It is likely that in an 'easy' oviposition substrate the mean size of egg-groups will decrease at the end of the oviposition period (see chapter 10). The egg-group size might become larger as oviposition period elapses, only in the case when progressing developmental growth of the plant provides a more proper oviposition substrate. The mean sizes of egg-groups oviposited by each species in their respective host-plants, were lower than the egg deposition rates during the oviposition period. Therefore the egg-group size remained constant during the oviposition period in both wing forms of the two species.
6. Another factor, which possibly influences the egg-group size, is inhibited oviposition, due to the delay of mating or failure to find a suitable oviposition host. In these cases, females with abdomens full of eggs, may oviposit large egg-groups in an 'easy' oviposition substrate.

12. EMBRYONIC DEVELOPMENT AND EGG-DIAPAUSE

An extensive study of the type and induction of the embryonic dormancy in *M. brevipennis* from East Germany has been published by WITSACK (1971). MÜLLER (1972, 1976) stated that a highly developed form of 'oligopause' with egg-dormancy is established in *M. brevipennis*, which in Central Europe is bivoltine and overwinters in the egg stage.

There are no previous studies on the embryonic dormancy of *M. fairmairei*.

Several studies on diapause of closely related species and races of insects from different geographic regions (*e.g.* GOLDSCHMIDT 1938, ANDREWARTHA 1952, BIGELOW 1960, DANILEVSKY 1965, MASAKI 1965, ANKERSMIT and ADKISSON 1967, FRAENKEL and HSIAO 1968, DE WILDE 1969, TAUBER and TAUBER 1972) have shown that such insects have displayed marked differences in initiation, intensity and termination of diapause. Therefore a comparative study on the embryonic dormancy of the *Muellerianella* complex (originating from the same locality in the Netherlands) was undertaken in order to investigate 1. whether there are differences in the expression of egg-diapause between the two species, and 2. whether such differences are related with other aspects of the biology and geographic distribution of each species.

12.1. METHODS AND MATERIAL

Since it is known that embryogenesis in *M. brevipennis* is inhibited only before embryo rotation (WITSACK, 1971), three stages of the egg life of *Muellerianella* have been considered in the present study: a. Embryonic development before embryo rotation, b. the stage after embryo rotation and c. the eclosion. These stages are well distinguishable without embryo staining. Thus, when embryos are unrotated their yellowish mycetome is visible in the anterior pole of the egg (Fig. 29, A–E) 2–3 days after the egg is laid. In rotated embryos the mycetome is located at the posterior pole and at the anterior pole the embryonic eye is visible (Fig. 29F). A few eggs with embryonic eye located in the posterior pole failed to hatch. When eggs are hatched their chorions remain in the oviposition-slit. Eggs which do not exhibit a visible mycetome are considered unhealthy or unfertile.

The material studied comprised *M. fairmairei* (mixture of diploid and triploid females from the K+ biotope at Leersum), *M. fairmairei* (Greek diploid colony) and *M. brevipennis* from Langbroek. In addition, all other material of *M. fairmairei* from C. France, S. France, S. England was successfully reared under long day conditions.

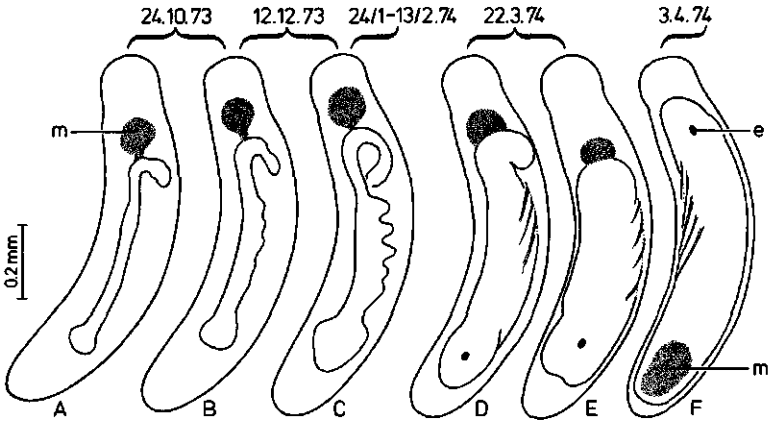


FIG. 29. Embryonic development of *M. fairmairei*. Samples taken at Leersum during October 1973 till April 1974. e. embryonic eye, m. mycetome.

12.2. FIELD OBSERVATIONS

Field data on the embryonic development during the first and second generation of *Muellerianella* are included in Table 41. As was expected from the phenological data, the embryonic development in first generation eggs of *M. fairmairei* preceded that of *M. brevipennis*. On 20 August 1974 first generation eggs of *M. brevipennis* were found with unrotated embryos, while second generation eggs of *M. fairmairei* were in the same embryonic stage. The data

TABLE 41. Embryonic development of *M. fairmairei* and *M. brevipennis* in field samples.

M. fairmairei at Leersum					
Date	oviposition host	Egg development (No.)			
		dead	with unrotated embryo	with rotated embryo	hatched
20.08.74	*H. lanatus*	–	–	40	350
20.08.74	*J. effusus*	–	18	–	–
Oct. 1973–March '74	*J. effusus*		9893	–	–
M. brevipennis at Langbroek					
30.07.74	*D. caespitosa*	–	123	–	–
20.08.74	*D. caespitosa*	–	18	7	50
26.11.74	*D. caespitosa*	2	194	–	9

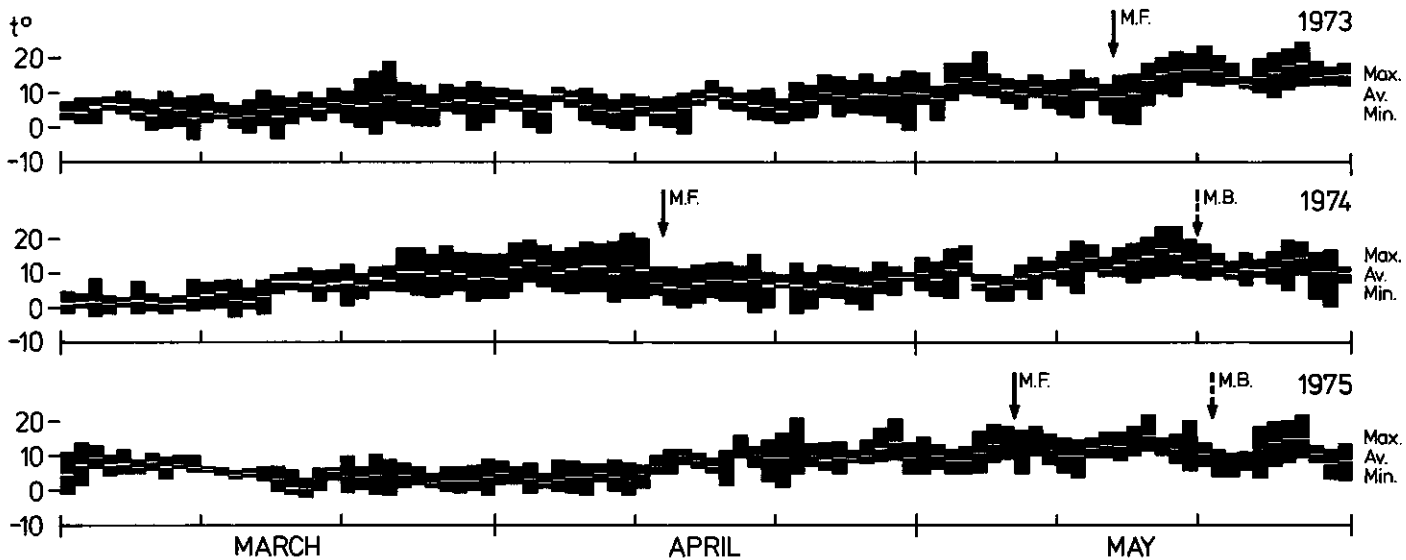


FIG. 30. Daily temperature during March-May of 1973–1975.
The arrows indicate the time of larval hatching of *M. fairmairei*, (MF) and *M. brevipennis*, (MB).

show, that some first generation eggs of *M. brevipennis* did not result in a second generation. In addition, eggs were found in samples taken on 26 November 1974 in slits comprising a mixture of chorions and eggs with unrotated embryos suggesting that *M. brevipennis* is partly univoltine.

Egg-samples of both species taken after the second adult generation (26.11.74) have shown that the embryo remained during the autumn at the prerotation stage (Table 41). Embryogenesis during hibernation has been studied only in *M. fairmairei*, because its eggs are readily obtained from *J. effusus*. Stained embryos of these eggs (Fig. 29) show that during overwintering their embryo had developed slightly, but never reached the stage of rotation. Some eggs exhibited embryo rotation on 22nd March. All the eggs had completed embryo rotation on 1st of April 1974. The temperature during that period is illustrated in Fig. 30. In the samples, obtained between October and March, the eggs hatched at 20°C in the laboratory after 8–15 days, while those from April after 1–4 days.

Some comparative data between the two species were taken from samples at Langbroek on 26th November, 1974. Eggs of both species after extraction from the plant tissue were incubated in embryo blocks at 20°C. Fig. 31 shows that 92.9% (a total of 99) eggs of *M. brevipennis* exhibited embryo rotation after a period of 8.20 $\pm$ 1.49 days, while 93.9% (a total of 33) eggs of *M. fairmairei* after a period of 7.23 $\pm$ 0.49 days. There is a significant difference ($t = 5.43^{***}$, d.f. 121) which indicates that eggs of *M. brevipennis* required a longer time before embryo rotation, than those of *M. fairmairei*. After embryo rotation, all the eggs of *M. brevipennis* and *M. fairmairei* hatched after 6.61 $\pm$ 0.63 and 6.35 $\pm$ 0.54 days, respectively (Fig. 32). This difference is also significant ($t = 2.14^{*}$, d.f. = 118).

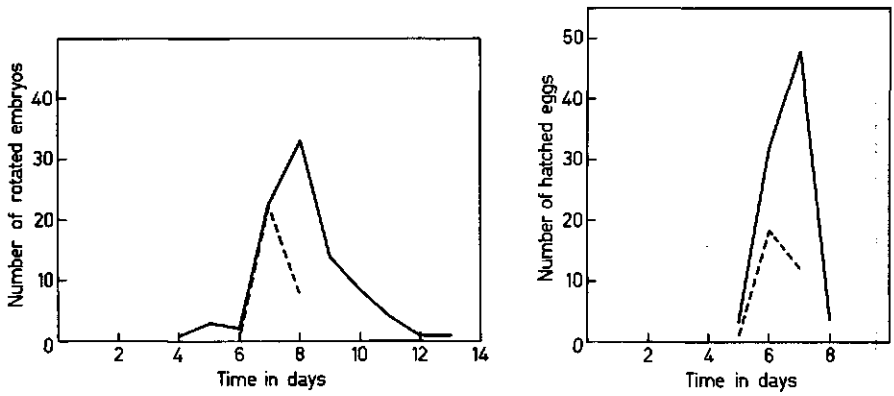


FIG. 31. (left) Number of field eggs of *M. fairmairei* (dashed line) and *M. brevipennis* (solid line), which showed embryonic rotation at 20°C. Egg-samples taken on 26.11.74.
FIG. 32. (right) Number of eggs of *M. fairmairei* (dashed line) and *M. brevipennis* (solid line), which hatched after embryonic rotation had occurred. Same material as in Fig. 31.

12.3. Laboratory experiments

12.3.1. *Effect of photoperiod*

Witsack (1971) has shown that there is a continuous embryonic development in the eggs of *M. brevipennis* deposited under long day conditions. In those deposited under short day conditions (L.D. = 10 : 14) the embryogenesis is inhibited before the embryo rotates.

Similar experiments were made in order to investigate the effect of photoperiod on the embryonic development of both species.

a. Long photoperiod (L : D = 18 : 6). The embryo development of both species was continuous in the total number of eggs deposited in their respective food plants. At temperatures of 20°–25°C the embryos started to rotate on the seventh day after oviposition (Fig. 33). Eggs of *M. fairmairei* deposited at the beginning and the end of the oviposition period, required a mean time of 8.33 ± 0.98 and 8.14 ± 0.79 days, respectively for their embryo rotation. The difference is not significant ($t = 1.50$, d.f. = 213, $P > 0.05$). The total number of eggs of *M. brevipennis* oviposited at the end of the oviposition period exhibited embryo rotation after 8.14 ± 1.07 days, which does not differ significantly from the corresponding period of *M. fairmairei* ($t = 0.01$, d.f. 144, $P > 0.05$).

The eggs of both species started hatching on the sixth day after their embryo had been rotated (Fig. 34). This period was 6.92 ± 0.68 days in *M. fairmairei* and 6.95 ± 0.56 in *M. brevipennis*. The difference is not significant ($t = 1.57$, d.f. = 120, $P > 0.05$). It is clear then that there are no differences in embryonic development of these species under long day conditions at 20°–25°C.

b. Short photoperiod (L : D = 10 : 14). Females of the two species 2–5 days old were collected in the field on 19th September 1974 and were caged under

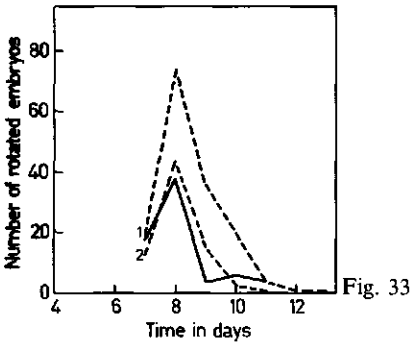


Fig. 33

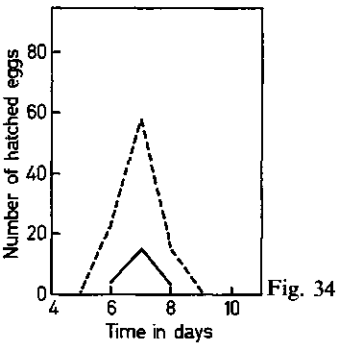


Fig. 34

Fig. 33. Number of rotated embryos of eggs which had been deposited under long photoperiod at 20°C.
Dashed lines no. 1 and 2, represent eggs of *M. fairmairei* laid at the beginning and the end of the oviposition period, respectively; solid line represents eggs of *M. brevipennis* laid at the end of the oviposition period.

Fig. 34. Number of eggs of *M. fairmairei* (dashed line) and *M. brevipennis* (solid line), which hatched after embryonic rotation had occurred. Part of material of Fig. 33.

TABLE 42. Embryonic development of *M. fairmairei* and *M. brevipennis* under different photoperiods and a constant temperature of 20°C.

Exper. No.	L:D	Oviposition host	Oviposition period*	Total oviposited eggs	Egg development							
					Dead		With unrotated embryo		With rotated embryo	Hatched	With rotated embryo and hatched	
					No.	%	No.	%	No	No	No	%
				M. fairmairei								
A.	18:6	*H. lanatus*		963					–	963	963	(100.0)
B.	10:14	*J. effusus*	41	2117	29	(1.4)	2062	(97.4)	4	22	26	(1.2)
C.	10:14	*J. effusus*	45	1109	63	(5.7)	957	(86.3)	7	82	89	(8.0)
D.	10:14	*H. lanatus* and	42	771	80	(10.4)	398	(51.6)	203	90	293	(38.0)
		Rubus sp.	42	18	7	(38.9)	9	(50.0)	2	–	2	(11.1)
E.	10:14→18:6	*J. effusus*	12	1155	–	(–)	1155	(100.0)	–	–	–	(–)
	10:14→18:6	*H. lanatus*	13–42	478	–	(–)	–	(–)	87	391	478	(100.0)
	10:14→18:6	*J. effusus*	13–42	680	28	(4.1)	102	(15.0)	45	505	550	(80.9)
				M. brevipennis								
F.	18:6	*D. caespitosa*		1238		–		–	–	1238	1238	(100.0)
	18:6	*J. effusus*		296		–		–	–	296	296	(100.0)
G.	10:14	*D. caespitosa*	90	297	39	(13.1)	258	(86.9)	–	–	–	(–)
	10:14	*J. effusus*	90	977	348	(35.6)	629	(64.4)	–	–	–	(–)
H.	10:14→18:6	*D. caespitosa*	53	323	–	(–)	73	(22.6)	40	210	250	(77.4)
	10:14→18:6	*J. effusus*	53	904	142	(15.7)	310	(34.3)	28	424	452	(50.0)

*The period of the last surviving female.

short day condition (L.D. = 10 : 14) at 20 °C. Table 42 (B, C) shows that 1.2 and 8.0% of the total number of eggs deposited in *J. effusus* (oviposition host under short photoperiod) by *M. fairmairei* had either exhibited embryo rotation or they had hatched. Those eggs deposited in *H. lanatus* had 38% non-diapausing eggs (Table 42D). In contrast to *M. fairmairei*, none of the deposited eggs of *M. brevipennis* had exhibited embryo rotation, although the period after the eggs were extracted from the plant was twice that of *M. fairmairei* (Table 42 G).

The number of dead eggs of both species was larger under short than under long photoperiod. In addition, this number was larger in *M. brevipennis* than *M. fairmairei*. It is evident, that dead eggs were more common, when both species had not oviposited in their natural oviposition plant of the second generation. Thus, in *M. faimairei*, 10.4 and 38.9% of the eggs laid in *H. lanatus* and *Rubus sp.*, respectively, died, while the corresponding number of dead eggs laid in *J. effusus* was 1.4 and 5.7%. Similar effects were found for *M. brevipennis* (13.1 and 35.6% dead eggs in *D. caespitosa* and *J. effusus* respectively) (Table 42 G). Probably, this egg-mortality was higher as time elapsed, since the eggs of *M. brevipennis* had remained at 20 °C for a longer time than those of *M. fairmairei*.

c. Short → long photoperiods. The same material used in short-photoperiod experiments was caged under long photoperiod at 20 °C. All caged females of *M. fairmairei* were removed 12 days later to a new cage containing also *H. lanatus* and *J. effusus* (Table 42 E). Two weeks later none of the 1155 eggs, which had been deposited only in *J. effusus*, exhibited embryo rotation. The rest of the eggs deposited (30 days later) in *H. lanatus* resulted in 100% non-diapausing eggs, while of those in *J. effusus* 19.1% had unrotated embryos. It is likely, that under the influence of long photoperiod the females in the new cage oviposited a considerable number (550) of non-diapausing eggs in *J. effusus*. However, in this culture such eggs should have been deposited only in *H. lanatus*.

With material and treatment of females of *M. brevipennis* similar to that of *M. fairmairei* it was shown (Table 42H) that 31.2% of the total deposited eggs were in diapause. The corresponding relative number of *M. fairmairei* (54.3%) was significantly larger ($X^2 = 171.69^{***}$) (Table 42E, H). This result strongly indicates that *M. brevipennis* reacted more rapidly than *M. fairmairei* to its exposition to long photoperiod by depositing larger numbers of non-diapausing eggs. The reverse situation (long-short photoperiod) probably has the same effect on the earlier induction of diapause in *M. brevipennis* than *M. fairmairei*, as is found in the field.

12.3.2. *Effect of the oviposition plant*

It has been mentioned above that eggs of *M. fairmairei* deposited under short day conditions in *H. lanatus*, exhibited a relatively larger number of eggs with rotated embryos than those deposited in *J. effusus*. Different structure and water relation in the tissues of the oviposition plants might have an influence on this phenomenon. Therefore, egg-samples with unrotated embryos, deposit-

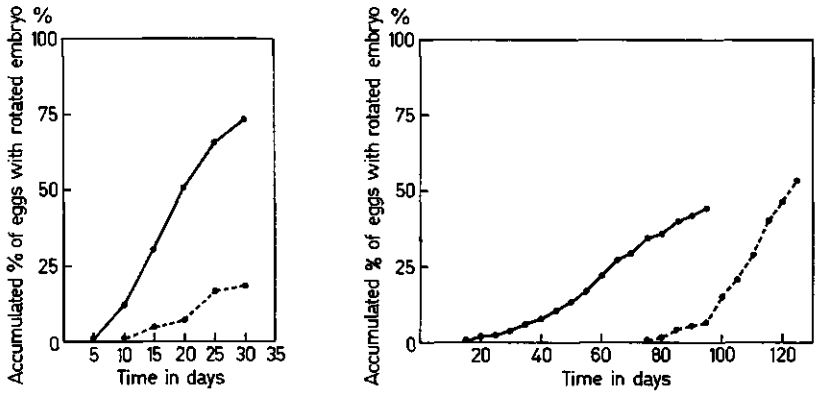


FIG. 35. Embryonic rotation of eggs laid by *M. fairmairei* which had been collected at Leersum (19.9.74) and left to oviposit under short day conditions at 20°C for a period of 42 days. Dashed line indicates rotated embryos of eggs which had been deposited in *J. effusus* and solid line those deposited in *H. lanatus*.

FIG. 36. Embryonic rotation of eggs laid by *M. brevipennis* which had been collected at Langbroek (19.9.74) and left to oviposit under long day conditions at 20°C. Dashed line represents rotated embryos of 74 eggs which had been deposited in *J. effusus* (3 eggs died), and solid line those of 114 eggs which had been deposited in *D. caespitosa* (23 eggs died).

ed under the same short day conditions by the same individuals, at the same period, but in the two different oviposition hosts, were transferred to embryo-blocks at 20°C. The results are shown in Fig. 35 from which it appears that the relative number of eggs with rotated embryo in *H. lanatus* was larger than that in *J. effusus*. Thus, from 59 eggs, which had been oviposited in *H. lanatus*, 72.9% exhibited embryo rotation in a period of 30 days (the remaining eggs being dead). Only 18% of the 43 eggs from *J. effusus* had shown embryo rotation in the same period (3 eggs dead).

Since none of the eggs deposited by *M. brevipennis* under short photoperiod had exhibited embryo rotation, egg-samples deposited in *J. effusus* and *D. caespitosa* under short → long photoperiod were compared. In addition, it should be mentioned that 77.4 and 50.0% of the eggs in *D. caespitosa* and *J. effusus*, respectively, had exhibited embryo rotation (Table 42H). Thus, egg-samples, similar to those for the experiments of *M. fairmairei* (Fig. 35), were made. Each sample was kept at 20°C until about 50% of the total number of eggs had exhibited embryo rotation. Fig. 36 shows that there was an interval of one month difference between both species.

The nature of the mechanisms controlling this phenomenon is outside the scope of the present work. However, it is clear, that ovipositing females develop the appropriate mechanism during pre-oviposition, because non-diapausing eggs were oviposited in *J. effusus* by both species. In other words, postoviposition influence is not favouring this phenomenon. In nature, *J. effusus* as a selective oviposition host for *M. fairmairei* and other leafhoppers, is of great importance.

12.3.3. *Effect of low temperature ('chilling')*

Eggs in diapause oviposited by each of the two species in *J. effusus* under short photoperiod at 20°C, were extracted from the plant tissues and placed at 3°C, except one egg-sample which was kept continuously at 20°C. After intervals of cold application the egg-samples were transferred from the low temperature to 20°C, and each day the number of eggs with rotated embryos was recorded. The results are illustrated in Figs. 37 and 38. In *M. fairmairei* (Fig. 37) it appeared that about 2–3 weeks chilling had no influence on the relative number of eggs with rotated embryos, because egg-samples with and without chilling had approximately the same result. Obviously, in all samples the relative number of eggs with rotated embryo increased, as time elapsed.

Cold application inhibited embryo rotation. Therefore, a critical amount of rotated embryos accumulated within a short period, after the termination of the cold application, while the remainings eggs of each sample exhibited a gradual embryo rotation as time elapsed (Fig. 37).

Increasing the period of chilling up to 35 days (Fig. 39) resulted in an embryo rotation of all eggs in the samples. Apparently, prolonged chilling (→20 days) accelerated the subsequent embryo rotation in *M. fairmairei* at 20°C.

Comparative experiments with eggs of *M. brevipennis* have demonstrated that embryo rotation does not occur without chilling (Fig. 38). Cold application is absolutely necessary for embryo rotation to occur. The required period

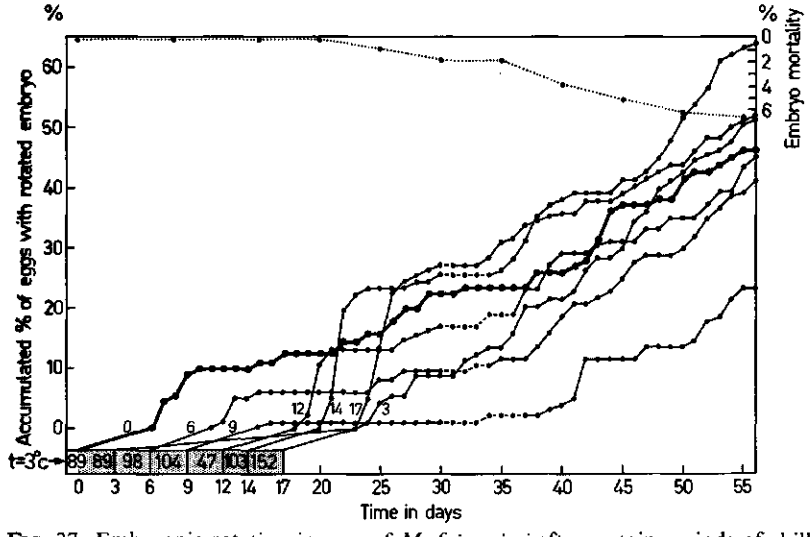


FIG. 37. Embryonic rotation in eggs of *M. fairmairei* after certain periods of chilling. The material comprises egg-samples, which had been deposited during 42 days in *J. effusus* under L.D. = 10:14 at 20°C. The number on each line represents the period of chilling of the respective egg sample. The numbers in the column of temperature indicate the size of each egg-sample. The dashed part of each line represents a daily chilling during 17 hours. Dotted line represents the total mortality of the samples (682 eggs).

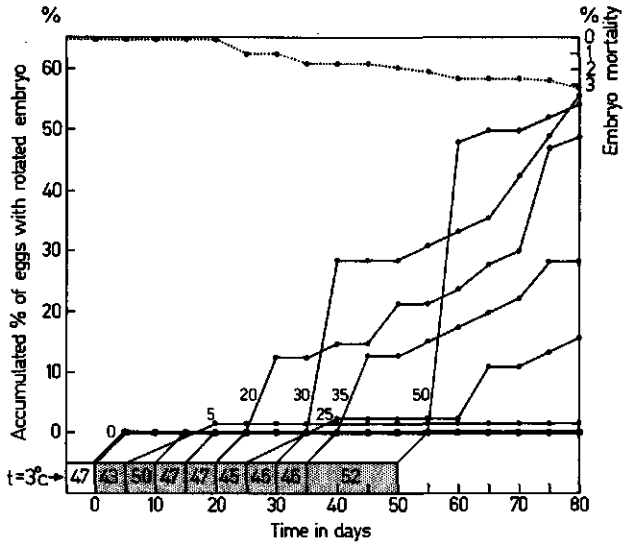


FIG. 38. Embryonic rotation in eggs of *M. brevipennis* after certain periods of chilling. The material comprises egg-samples which had been deposited during 90 days in *J. effusus* under L.D = 10 : 14 at 20 °C. The results have been drawn in the same manner as in Fig. 37.

of chilling is certainly longer than that in *M. fairmairei*, in order to obtain the same relative numbers of embryo rotation. Thus, the cold application period, which was required to obtain embryo rotation in 50% of the eggs in *M. brevipennis*, was 3–4 weeks longer than that in *M. fairmairei* (Fig. 39).

Thus, it is clear that egg-dormancy in *M. brevipennis* is stronger than in *M. fairmairei*, when both species have oviposited under short photoperiod and temperature of 20 °C.

Temperatures of 3 °C during 17 hours and 20 °C for 7 hours daily, had not influenced the process of the embryo rotation in eggs of *M. fairmairei*. Some eggs exhibited embryo rotation during that period (Fig. 37). The total relative number of dead eggs of both species at 20 °C was small (Figs. 37 and 38). However, that number was larger in *M. fairmairei* than in *M. brevipennis* (6.5 and 3.1% of the total number of eggs, respectively).

In addition, experiments were made to investigate the effect of low temperature on ovipositing females of *M. fairmairei* under short day conditions. Thus, females collected in the field were caged to oviposit outdoors between 30 October and 6 November 1974. A total number of 30 eggs (Fig. 40, Sample A), oviposited in *J. effusus*, were placed in an embryo-block at 20 °C on 13 November. On the other hand, some females were caged to oviposit indoors for 12 days (19.9–2.10.1974) under long photoperiod at 20 °C. The stems of *J. effusus* containing oviposited eggs, were placed outdoors on 14 November. After 14 and 21 days two samples (sample a = 177 and b = 144 eggs, respectively, Fig. 40) were placed at the same manner and conditions as sample (A).

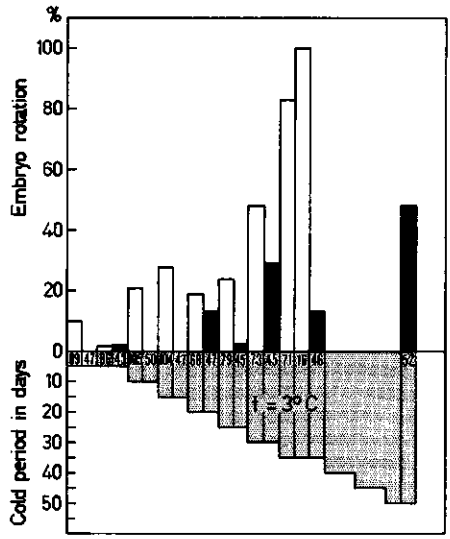


FIG. 39. Embryonic rotation in eggs of *M. fairmairei* (open columns) and *M. brevipennis* (solid columns) deposited during 42 and 90 days respectively, in *J. effusus* under L:D = 10:14 and t = 20°C. Each egg-sample has undergone chilling for certain intervals of 0, 5, 10... 50 days. The relative embryo rotation has been measured at 20°C during 12 days after the period of chilling.

The maximum and minimum daily temperatures during 30 October till 4 December are drawn in Fig. 41. Daily recordings of the embryo rotation of these samples (Fig. 40) show that the eggs of samples (a) and (b) had similar embryo rotation of those at 3°C (Fig. 37), but those of sample (A) exhibited embryo rotation similar to eggs oviposited under long day condition (Fig. 33).

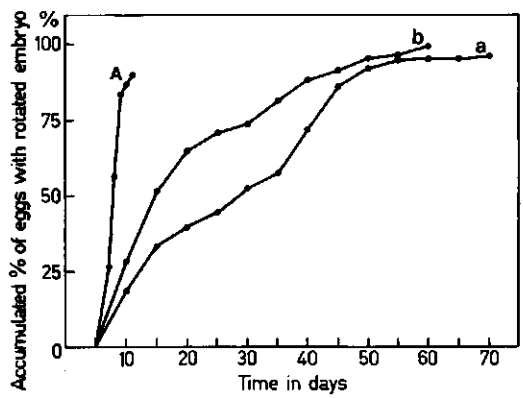


FIG. 40. Embryonic rotation in three egg-samples of *M. fairmairei* at 20°C. Sample A, eggs deposited outdoors; samples a and b are eggs which had been deposited indoors at 20°C and afterwards transferred outdoors for 2 and 3 weeks, respectively.

penetrate into more northern regions, because the long summer-photoperiods are associated with higher temperatures, although the favourable season is shorter. On the other hand, the longer duration of the favourable season in more southern regions may have enabled polyvoltinism in southern strains of this species.

c. However, the allopolyploid female biotype of *M. fairmairei* shows no inheritance in diapause of the two parental species. This biotype acquiring a favourable new combination of the different physiological characteristics of its diploid ancestors occupies a wide range (Middle and West Europe) with high population densities.

As has been reported for plants (STEBBINS, 1940) such allopolyploid species appear to have acquired tolerance to only a mild winter from one parent and to a cold winter from the other. The exception in the *Muellerianella* biotype is its dependence on males of its 'host species' which forces this biotype to follow the seasonality and biological rhythms of the bisexual species.

13. LARVAL DEVELOPMENT

13.1. Field observations

Since the larvae of the two *Muellerianella* species are easily distinguishable from those of other Delphacidae, this has facilitated observations on their emergence and development in the field.

First instar larvae of *M. fairmairei* emerged at Leersum during the first half year of May in 1973 and 1975 (Fig. 30), while during a warm period from 21st March to 1st April 1974 with a mean temperature of 15°C larvae emerged on 3rd April. Larvae of *M. brevipennis* emerged at Langbroek during the last week of May in 1974 and 1975. Simultaneous observations on these latter dates at Leersum showed that the larvae of *M. fairmairei* had reached the second and third instar. Apparently the eggs of *M. brevipennis* required a larger number of day-degrees to hatch than those of *M. fairmairei*. In both species the larvae required 50–60 days to reach the adult stage. The mean field temperature of that period was about 15°C (Table 4).

The second generation larvae of *M. fairmairei* started emerging during the second week of July, those of *M. brevipennis* during the first week of August. Thus, whenever observations have been made in the field the development of the larvae of *M. fairmairei* was 2–3 instars more advanced than that of *M. brevipennis*.

13.2. Laboratory experiments

There are several studies on rearing methods and ecological factors affecting the larval development of leafhoppers and planthoppers (*e.g.* Hassan 1939, Morcos 1953, Kisimoto 1956 a and b, Raatikainen 1960 and 1967, Kouskolekas and Decker 1966, Mason and Yonke 1971, Mochida 1973 and Ammar 1976). According to the data reported by these authors the plant species and its developmental stage, temperature, relative humidity, photoperiod, and crowding during larval development are of great importance. Failure to control each of these factors may result in lack of success.

In the present investigation these factors have been experimentally analysed in *M. fairmairei* and, to a lesser extent, in *M. brevipennis*.

13.2.1. *Material and methods*

Field observations revealed that young larvae of the two sibling species were feeding on the lower parts of the stems and often on the dead leaves of their respective plant-hosts. In their microhabitats the density of the plant stems and the moist soil established a higher relative humidity than that measured at 1.5

meter above the soil level of about 80%. Therefore cuttings of the lower parts of the stems of *H. lanatus* and *D. caespitosa* were offered as a food to the larvae in the laboratory. These cut stems have often rooted in the test-tubes. New test-tubes were offered to the larvae at intervals of 2–5 days. Young larvae, which had been hatched in a moist petri-dish over a period of not longer than 15 hours, were released to hop into the test-tube. Care was taken not to touch them during their development. In each test-tube usually one larva was reared at a time.

Observations on larval development were made daily, mostly at about 9 a.m. The larval exuvia were used as a criterion in measuring the duration of each larval instar. In order to maintain a relative humidity of about 100% the test-tubes were enclosed in a transparent plastic sack together with a wet filter paper. A black plastic sack was used for larval rearing under short day (L.D. = 8 : 16) and it was removed each day during the photophase.

13.2.2. *Effect of humidity (M.f.)*

Larvae reared in cellulose cylinders fixed to one grass stem at a relative humidity of about 60%, were compared with those in test-tubes at a relative humidity of about 100%. Both groups of larvae were reared at the same temperature (26 °C) and a photoperiod of L.D. = 18 : 6 (Table 44). The duration of each larval instar as well as the total duration of larval development was significantly longer in low humidity as compared to a higher one ($t_I = 2.72^{**}$, $t_{II} = 4.59^{***}$, $t_{III} = 4.41^{***}$, $t_{IV} = 7.94^{***}$, $t_V = 4.70^{***}$ and $t_{I-V} = 7.94^{***}$).

13.2.3. *Effect of temperature (M.f.)*

Table 44 shows that there were differences between the duration of each nymphal stage and the total larval development when photoperiod and relative humidity were kept constant and the temperature varied from 15, 20 and 26 °C.

The data of Fig. 42 show that the rate of larval development had increased more rapidly from 15–20 °C than from 20–26 °C. It is likely that the mean duration of each larval instar under these temperatures fits a hyperbolic curve. Apparently the slope of each curve increases with the progress of the larval instars. The different rate of development of each larva at 15 °C is remarkable. The mean period of 54.8 days required for the larval development at 15 °C is close to that in the field under a similar mean temperature which prevails in May-June (Table 4). However, the 'treshold of larval development' lies at a lower temperature than 15 °C. About 100 larvae of the second instar caged outdoors at the beginning of October 1974, produced a few adults at the beginning of March 1975. The mean monthly temperature at that period was from 3.1–7.3 °C (Table 4).

Fig. 43 shows that when the temperature was 32 °C larvae did not survive longer than two weeks. From 36 harched larva 29 died during their first larval instar and the rest during the second one. The seven larvae required a mean period of 9.57 ± 0.79 days to develop up to the second instar.

It is very likely that the rate of larval development of *M. fairmairei* at tem-

peratures fluctuating between 20–25°C is approximately the same as at a constant temperature of the mean value. These temperatures were further used for measuring the larval development of both species.

13.2.4. *Effect of larval material (M.f.)*

Hatched larvae from laboratory and field eggs respectively were compared under the same temperature (20–25°C), photoperiod (L : D = 18 : 6) and relative humidity (100%). Table 44 shows that there were no significant differences between the duration of each larval instar and the total duration of larval development of the two groups ($t_I = 0.62$, $t_{II} = 1.55$, $t_{III} = 0.93$, $t_{IV} = 0.30$, $t_V = 2.12$ and $t_{I-V} = 1.93$. Probabilities $\geqslant 0.05$). From other studies (*e.g.* AMMAR 1976) it appeared, however, that the rate of larval development was higher in field populations than in laboratory cultures. We did not find such differences and this may be due to the fact that our colonies were renewed each year and large numbers of females from the field were used for establishing colonies.

13.2.5. *Effect of photoperiod (M.f.)*

The effect at long (L : D = 18 : 6) and short (L : D = 8 : 14) photoperiod was compared in experiments with the same temperature and humidity. Under these conditions the duration of the first four larval instars did not show any significant difference ($t_I = 0.71$, $t_{II} = 1.80$, $t_{III} = 1.70$, $t_{IV} = 1.55$; probabilities > 0.05), while the duration of the fifth instar at short photoperiod was significantly longer than at long photoperiod ($t = 9.12$***).

13.2.6. *Effect of crowding (M.f.)*

Two groups (A and B) each of about 30 field-hatched larvae were studied simultaneously under the same conditions (Table 45). Each larva in group A was reared in a single test-tube: those from group B were reared together in one test-tube for 11 days (the mean period required for the larvae in group B to reach the fourth instar). After that period each larva of group B was reared in the same way as those in group A. Table 45 shows that a significant retardation in development (3.13 days) occurred from the first to third larval instar in group B as compared with group A ($t = 10.64$***). The duration of the 5th larval instar in both groups did not differ significantly ($t = 1.18$, $P > 0.05$). Apparently 11 days of crowding reduced the total period required for larval development significantly whith 4.4 days ($t = 6.52$***). The total mean relative retardation of larval development (19.0%) in group B was partly induced also during the 4th larval instar ($t = 3.16$**). This phenomenon emphasizes that the rate of development of a certain instar is influenced by the development in the previous instar.

13.2.7. *Comparison of the larval development of the two species*

It has been demonstrated that several factors influenced the larval development of *M. fairmairei*. Comparison with *M. brevipennis* has been made only

TABLE 44. Time (in days) of larval development of each sex of *M. fairmairei (M.f.)* and *M. brevipennis* Number (1) indicates two times of daily observations; the asterisk refers to field material.

					No. observations and duration of larval instars			
				larval instars	I		II	
Spec.	sex	L:D (hours)	T (C°)	R.H. (%)	No.	Mean ± S.D.	No.	Mean ± S.D.
M.f.	♂	18:6	26°	±60	3	3.67 ± 0.58	3	3.67 ± 0.58
M.f.	♀	18:6	26°	±60	7	5.14 ± 1.86	7	4.57 ± 1.13
M.f.	♂,♀	18:6	26°	±60	16	4.75 ± 1.44	15	4.26 ± 1.03
M.f.(1)	♂	18:6	26°	±100	3	4.00 ± 0.00	3	2.67 ± 0.29
M.f.(1)	♀	18:6	26°	±100	50	3.74 ± 0.54	50	3.02 ± 0.49
M.f.(1)	♂,♀	18:6	26°	±100	53	3.75 ± 0.53	53	3.00 ± 0.49
M.f.	♂	18:6	20°	±100	1	5.00	1	4.00
M.f.	♀	18:6	20°	±100	9	5.11 ± 0.78	9	3.78 ± 0.44
M.f.	♂,♀	18:6	20°	±100	10	5.10 ± 0.74	10	3.80 ± 0.42
M.f.	♂	18:6	15°	±100	2	11.0 ± 0.00	2	9.50 ± 0.70
M.f.	♀	18:6	15°	±100	16	11.38 ± 0.72	16	10.19 ± 1.11
M.f.	♂,♀	18:6	15°	±100	26	11.50 ± 0.76	25	10.12 ± 0.93
*M.f.**	♂	18:6	20–25°	±100	6	4.50 ± 0.55	6	3.33 ± 0.51
*M.f.**	♀	18:6	20–25°	±100	28	4.64 ± 0.49	28	3.25 ± 0.44
*M.f.**	♂,♀	18:6	20–25°	±100	34	4.62 ± 0.48	34	3.26 ± 0.44
M.f.	♂	18:6	20–25°	±100	4	5.00 ± 0.82	4	3.50 ± 0.58
M.f.	♀	18:6	20–25°	±100	26	4.69 ± 0.68	6	3.42 ± 0.50
M.f.	♂,♀	18:6	20–25°	±100	32	4.71 ± 0.67	32	3.44 ± 0.50
*M.f.**	♂	8:16	20–25°	±100	2	4.50 ± 0.71	2	3.00 ± 0.00
*M.f.**	♀	8:16	20–25°	±100	29	4.72 ± 0.45	29	3.31 ± 0.54
*M.f.**	♂,♀	8:16	20–25°	±100	37	4.70 ± 0.46	35	3.49 ± 0.61
*M.b.**	♂	18:6	20–25°	±100	22	4.41 ± 0.50	22	3.50 ± 0.60
*M.b.**	♀	18:6	20–25°	±100	24	4.79 ± 0.98	24	3.54 ± 0.66
*M.b.**	♂,♀	18:6	20–25°	±100	56	4.70 ± 0.78	53	3.47 ± 0.60
M.b.[1]*	♂	8:16	20–25°	±100	16	5.75 ± 0.62	16	3.66 ± 0.68
M.b.[1]*	♀	8:16	20–25°	±100	22	5.77 ± 0.58	22	3.60 ± 0.82
M.b.[1]*	♂,♀	8:16	20–25°	±100	40	5.80 ± 0.60	40	3.69 ± 0.77

under short and long photoperiod, temperature of 20–25°C and a relative humidity of about 100% (Table 44).

Under long photoperiod the duration of each larval instar and of the total larval development of *M. brevipennis* does not differ significantly from *M. fairmairei* ($t_I = 0.60$, $t_{II} = 1.88$, $t_{III} = 2.68$*, $t_{IV} = 0.71$, $t_V = 1.31$ and $t_{I-V} = 0.13$. Probabilities $\geqslant 0.05$).

(M.b.) under certain photoperiods (L : D), temperatures (T) and relative humidities (R.H.).

III		IV		V		I–V		Total larval mortality (%)
No.	Mean ± S.D.	No.	Mean ± S.D.	No.	Mean ± S.D.	No.	Mean ± S.D.	
3	3.33 ± 0.58	3	5.00 ± 1.00	3	9.33 ± 3.21	3	25.00 ± 4.36	
7	5.28 ± 1.60	7	5.29 ± 1.25	7	7.86 ± 1.86	7	28.14 ± 3.34	
15	4.47 ± 1.41	15	5.20 ± 0.94	10	8.30 ± 2.26	10	27.20 ± 3.74	37.5
3	3.33 ± 0.76	3	3.17 ± 0.29	3	4.67 ± 0.29	3	17.83 ± 1.04	
50	2.82 ± 0.30	50	3.23 ± 0.38	50	4.95 ± 0.46	50	17.76 ± 0.93	
53	2.85 ± 0.34	53	3.23 ± 0.37	53	4.93 ± 0.46	53	17.76 ± 0.92	0.0
1	6.00	1	4.00	1	8.00	1	27.00	
9	4.11 ± 0.33	9	4.22 ± 0.44	9	6.78 ± 0.44	9	23.89 ± 1.27	
10	4.30 ± 0.67	10	4.20 ± 0.42	10	6.90 ± 0.57	10	24.20 ± 1.55	0.0
2	9.00 ± 0.00	2	9.00 ± 0.00	2	12.00 ± 0.00	2	50.50 ± 0.70	
16	9.31 ± 0.95	16	10.50 ± 1.03	16	14.00 ± 1.97	16	55.38 ± 3.14	
25	9.32 ± 0.80	25	10.36 ± 0.95	18	13.78 ± 1.96	18	54.83 ± 3.35	30.8
6	3.50 ± 0.55	6	4.00 ± 0.00	6	5.00 ± 0.00	6	20.33 ± 0.51	
28	3.54 ± 0.51	28	4.04 ± 0.51	28	5.07 ± 0.26	28	20.54 ± 0.74	
34	3.53 ± 0.50	34	4.03 ± 0.45	34	5.06 ± 0.24	34	20.50 ± 0.70	0.0
4	3.75 ± 0.50	4	4.00 ± 0.00	4	5.25 ± 0.95	4	21.50 ± 1.29	
26	3.38 ± 0.57	26	4.08 ± 0.27	26	5.35 ± 0.63	26	20.92 ± 1.26	
32	3.41 ± 0.55	32	4.00 ± 0.35	30	5'33 ± 0.66	30	21.00 ± 1.26	6.2
2	3.50 ± 0.71	2	3.50 ± 0.71	2	7.00 ± 0.00	2	21.50 ± 0.71	
29	3.31 ± 0.60	29	4.38 ± 0.94	29	7.52 ± 1.53	29	23.45 ± 2.23	
35	3.31 ± 0.57	33	4.30 ± 0.90	31	7.48 ± 1.46	31	23.32 ± 2.18	10.8
22	3.23 ± 0.43	22	3.82 ± 0.73	22	5.18 ± 0.73	22	20.14 ± 1.98	
24	3.25 ± 0.44	24	3.92 ± 0.65	24	5.25 ± 0.85	24	20.75 ± 2.03	
52	3.25 ± 0.43	49	3.94 ± 0.71	46	5.22 ± 0.78	46	20.46 ± 1.99	12.5
16	3.77 ± 0.51	16	3.75 ± 0.51	16	5.83 ± 0.66	16	22.77 ± 1.68	
22	3.91 ± 0.68	22	3.98 ± 0.42	22	6.71 ± 1.43	22	23.98 ± 2.43	
38	3.85 ± 0.61	38	3.88 ± 0.46	38	6.34 ± 1.22	38	23.47 ± 2.18	7.3

Under short photoperiod the total duration of larval development was not significantly different between the species ($t_{I-V} = 0.32, P > 0.05$), but significant differences occurred between the duration of some larval instars ($t_I = 9.07^{***}$, $t_{II} = 1.25, P > 0.05$, $t_{III} = 3.91^{***}$, $t_{IV} = 2.42^{*}$ and $t_V = 3.47^{***}$).

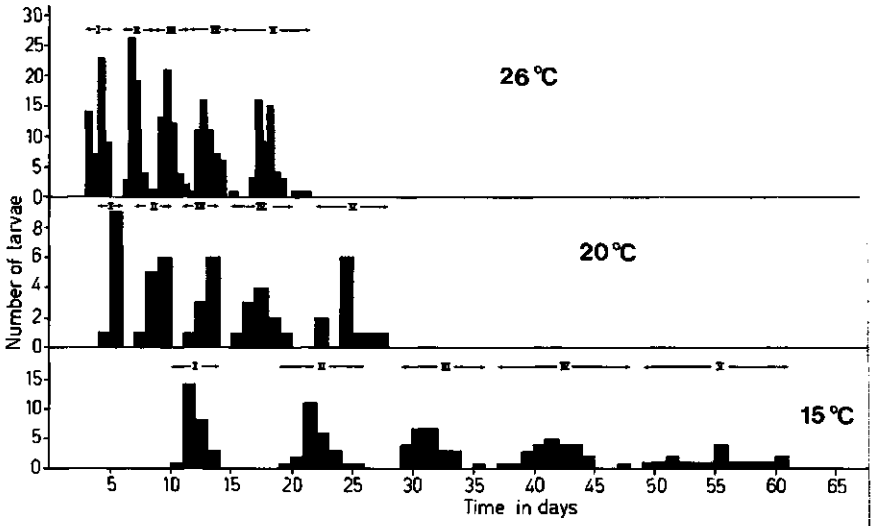


FIG. 42. Development of larval instars of *M. fairmairei* under different temperatures, constant photoperiod (L:D = 18 : 6) and relative humidity (R.H. = ±100%).

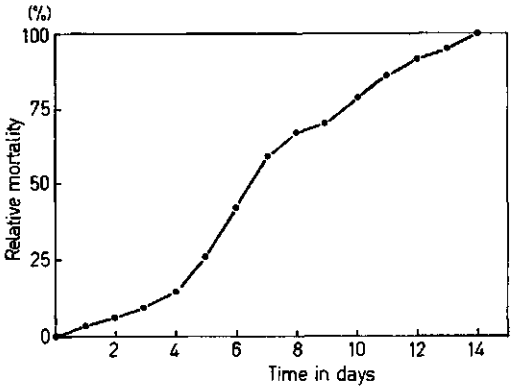


FIG. 43. Relative mortality of the first instar larvae of *M. fairmairei* at 32°C.

TABLE 45. Time (in days) of larval development of two groups of *M. fairmairei* under short photoperiod (L:D = 8 : 16), temperature of 20–25°C, relative humidity of about 100% and different densities of rearing (for explanation of the different treatment of Group A and B, see text).

Group	No. of observations and duration of larval instars								Total larval mortality (%)
	I–III		IV		V		I–V		
	No.	Mean ± S.D.	No.	Mean ± S.D.	No.	Mean ± S.D.	No.	Mean ± S.D.	
A.	35	11.46 ± 0.91	33	4.30 ± 0.90	31	7.48 ± 1.46	31	23.32 ± 2.18	10.8
B.	29	14.59 ± 1.35	29	5.28 ± 1.44	29	7.90 ± 1.29	29	27.76 ± 3.00	6.5

13.2.8. *Duration of larval development in both sexes*

In *M. fairmairei* the number of males which emerged was not sufficiently large to compare their duration of larval development with that of the females (Table 44). The male larvae which developed at a temperature of 15 °C required a mean period of about 5 days less that of the female larvae.

In *M. brevipennis* the relative number of emerged males was somewhat below the theoretical sex-ratio of 1:1. In males which emerged under long photoperiod the duration of each larval instar as well as the total was not significantly different from that of the females ($t_I = 1.68$, $t_{II} = 0.22$, $t_{III} = 0.16$, $t_{IV} = 0.75$, $t_V = 0.30$ and $t_{I-V} = 1.03$, Probabilities > 0.05). Similar results were obtained under short photoperiod ($t_I = 0.10$, $t_{II} = 0.25$, $t_{III} = 0.73$, $t_{IV} = 1.48$ and $t_{I-V} = 1.81$, Probabilities > 0.05). However, the period required for the fifth larval instar of the males was significantly shorter than that in the females ($t_V = 2.54$*).

Since similar results were obtained in *M. fairmairei* under short photoperiod, it is very likely that a retardation of the development in the fifth female larval stage occurs under these conditions for both species. It is possible, that this retardation is related to the ovarial maturation, because the preoviposition period in females of *M. brevipennis* lasted 8.20 ± 1.15 days, while in long photoperiods (Table 30) the duration was 7.00 ± 1.71 ($t = 2.29$*, d.f. = 34).

13.2.9. *Duration of each larval instar*

In both species the duration of the fifth larval instar was longer than that of the first one. The duration of the other three larval instars varied dependent of the experiments. Thus, at a temperature of 15 °C, causing a low rate of larval development, the third instar was significantly shorter than the second ($t = 3.26$**) and the fourth ($t = 4.17$***), while the fourth and second larval instar did not differ significantly ($t = 0.90$, $P > 0.05$). In experiments with *M. fairmairei* at a constant temperature of 26 °C, in which larval development was checked twice per day, the duration of the third larval instar was not found to differ significantly from that of the second ($t = 1.83$, $P > 0.05$), while that of the fourth larval instar differed significantly both from the third ($t = 5.51$***) and the second ($t = 2.73$**).

However, the duration of the second, third and fourth larval instars may be considered approximately the same, but some tendencies are shown that the third larval instar is the shortest and the fourth somehow more prolonged than the second.

13.2.10 *Time and site of larval emergence*

In those experiments in which larval development was checked twice daily (Table 44), larvae emerged both during the scotophase and the photophase. Thus, in long day experiments with *M. fairmairei*, from a total number of 265 exuvia 57 were found during 9–21 h, while 198 during 21–9 h. In the short-day experiment with *M. brevipennis* 102 exuvia were found during the scotophase (17.15–9.15 h), and 92 during the photophase.

The larvae moulted mainly on the host plant, but often exuvia were also found at all sites of the test-tubes.

13.2.11. *Larval mortality*

In the recorded larval mortality (Tables 44 and 45) accidental mortality (lost or killed larvae) has not been included. A relative high mortality occurred in larvae of *M. fairmairei* reared either at low relative humidity or at low temperatures. In all other cases a relatively low mortality was not correlated with certain developmental instars.

14. HOST PLANT DISCRIMINATION

14.1. METHODS AND MATERIAL

Some experiments were made under long photoperiod at 20–25°C to test longevity of larvae and young adults and reproduction of *M. fairmairei* and *M. brevipennis* on grass species other than their natural food plants in Holland. In addition some experiments on food-plant preference were made to investigate whether *H. lanatus* (the food plant of *M. fairmairei*) must be in contact with *J. effusus* (its winter oviposition plant) when first generation larvae are hatched from overwintering eggs.

In all experiments seedlings and mature grasses were considered. The material studied is Dutch colonies of *M. fairmairei* (2n + 3n) and *M. brevipennis*.

14.2. EXPERIMENTS WITH LARVAE

a. Larval longevity. Table 46 shows that larvae, just hatched from the eggs, had approximately the same longevity either on a substrate of a wet filter paper or when reared on the food-plant of the other species. The exception, of course, was the triploid biotype of *M. fairmairei*, which can develop on both grass-species. Also, one larva survived on *J. effusus* till the second day of the second instar.

These experiments indicate that lack of the proper nutrition is the main factor causing larval mortality, because water was supplied by both substrates offered to them (filter-paper and cut-stems). Also, it should be mentioned here that the mean time required for larvae of both species to emerge to the second larval instar was 4.7 days. The hairs of the stems of *H. lanatus* had no effect on the larval mortality of *M. brevipennis*.

TABLE 46. Longevity of first instar larva to which was offered either a moist filter paper or some plant species. The asterisk indicates stems of *H. lanatus* from which the hair-layer was removed.

Species	Substrate	Larval longevity in days			
		No.	Mean ± S.D.	Min	Max
M. fairmairei	Moist filter paper	27	2.78 ± 0.93	1	5
M. brevipennis	Moist filter paper	14	2.64 ± 0.74	2	4
M. fairmairei	*D. caespitosa*	25	2.80 ± 1.75	1	6
M. brevipennis	*H. lanatus*	18	3.22 ± 1.59	1	6
M. fairmairei	*J. effusus*	12	– –	1	7
M. brevipennis	*H. lanatus**	19	– –	1	5

On other grass-species (*A. sativa*, *H. vulgare*, *S. cereale* and *T. aestivum*) a few larvae of *M. brevipennis* succeeded in reaching adulthood. Mature stems (more than 30 days old) caused less mortality than seedlings of the same grass-species. In all experiments made by P. KOSTENSE (internal student report) *M. fairmairei* revealed lower larval mortality than *M. brevipennis*. The latter species tended to develop better on *A. sativa* than on *S. cereale*, whereas *M. fairmairei* responded in the reverse way.

In an experiment with 30 larvae of *M. brevipennis* (3rd and 4th instar) which were transferred from *D. caespitosa* to *A. sativa* (mature stems, 6 weeks old), only 8 larvae died and the remainders emerged to adults. Similar experiments with seedlings of the same grass-species resulted in 100% larval mortality.

b. The association of *J. effusus* and *H. lanatus*. In order to investigate the behaviour of first generation larvae when *J. effusus* is not in contact with *H. lanatus*, two cages A and B (Fig. 44) were made. *J. effusus* containing the hibernating eggs of *M. fairmairei* was extracted from the field on 5.1.75 and replanted in one side of each cage; three grass-species were interposed at the other side of the cage, where *H. lanatus* was growing.

In cage A a total of 8 hatched larvae were observed continuously until they reached the grass. Their behaviour on *J. effusus* mainly consisted of moving across all sides from the top to the base of the stem. One larva was observed to walk a small distance on the soil but rapidly returned to the stem and moved to the top of it. Often these larvae were remaining immobile for a period of 3 seconds to 35 minutes. Most of them remained on the stem longer than the 6 hour period of observation. During this period 6 larvae had fallen on the soil. Five of them reached the wire, which was supporting *S. cereale* and through it made contact with the grass. The way two of these larvae reached the grass is shown in Fig. 44. The time required for the larvae to reach the grass was 30–60 minutes. The distance between *J. effusus* and *S. cereale* was 12 cm.

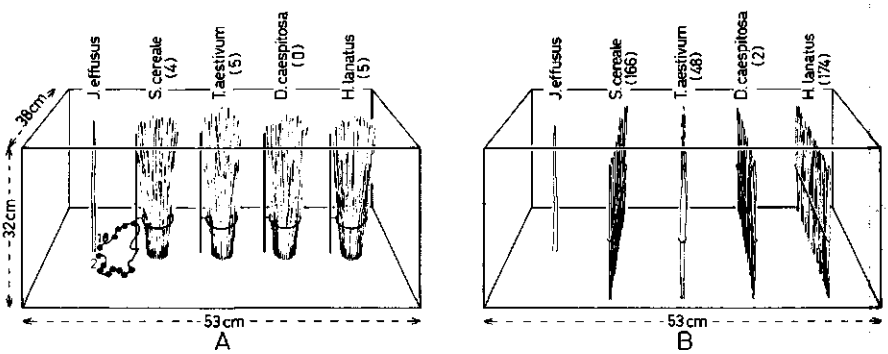


FIG. 44. Two cages (A, B) each containing four grass species and one stem of *J. effusus* collected in the field and containing eggs of *M. fairmairei*. Numbers in brackets indicate 4th-5th instar larvae removed from each grass species after 21 days. Numbers 1, 2 in cage A indicate the pathway of two larvae from *Juncus* towards the wire which was supporting the stems of the grasses. The dots on each line indicate how often the larva remained immobile.

Two other larvae moved away from *S. cereale* and walked out of the cage. It was remarkable that no larvae hopped during the time they were observed.

These experiments strongly suggest that the larvae employ only random searching in finding suitable grasses. The role of the wire, which could be in place of *J. effusus*, showed that hatched larvae will come in contact through this plant with its neighbouring grasses. Thereafter, the stem of *J. effusus* was removed out of the cage. One week later 14 larvae were observed only on *S. cereale* while after two weeks 9 were on *S. cereale*, 3 on *T. aestivum* and 2 had reached *H. lanatus* (cage A). In both cages the 4th-5th instar larvae were isolated on each grass-species and were counted as is shown in Fig. 44.

The experiment with cages A and B demonstrated: a. Larvae of *M. fairmairei* can develop on other grasses closely associated with *H. lanatus* and *J. effusus*. b. It is probable that 2–3 instar larvae begin to disperse in order to find their favoured host plant. c. *D. caespitosa* was entirely unsuitable as a food plant for *M. fairmairei*.

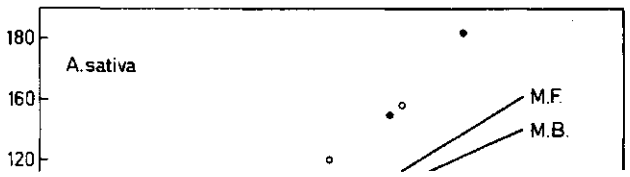
14.3. Experiments with adults

Table 47 shows that four cereal species were more or less suitable for establishing colonies of *M. fairmairei*. On the same grass material the same number of males and females of *M. brevipennis* failed to produce progeny. These females exhibited considerable locomotor activity and after one week all individuals had perished.

Similar experiments with more plant-species have demonstrated (Table 48) that *M. fairmairei* possesses, so far, the ability to develop and reproduce on considerably more grass-species than *M. brevipennis*. The latter species produced few progeny (5–15 adults) only when it was reared on mature grass-species (50 days old) of *A. sativa* and *H. vulgare*. On the other hand, while *M. fairmairei* was successfully reared on *H. mollis* and the ornamental *H. lanatus* var. *albovariegatus* (leaves with parallel stripes lacking chlorophyl), *M. brevipennis* failed on *D. flexuosa* (a congener of its host plant). *Oryza sativa*,

Table 47. Adult progeny of 7 females and 2 males of *M. fairmairei* obtained on different grass species. Initial age of all grass species was 15 days.

Plant species	Adult progeny						Total
	Brachypterous			Macropterous			
	♂♀	♂♂	♀♀	♂♀	♂♂	♀♀	♂♀
S. cereale	191	12	179	83	5	78	274
T. aestivum	47	–	47	10	–	10	57
H. vulgare	35	3	32	1	–	1	36
A. sativa	59	–	59	1	–	1	60



15. HYBRIDIZATION STUDIES

15.1. Material and methods

Hybridization material was provided by colonies of *M. brevipennis* (Dutch population) and *M. fairmairei* (Dutch and Greek populations). Other colonies of *M. fairmairei* (two populations from France and one from England) were not crossed with *M. brevipennis*.

It is known that *M. fairmairei* females of the Dutch population are a mixture of diploid and triploid females (Drosopoulos, 1976). Several crosses between females of this population with males of *M. brevipennis* have been made previously (Drosopoulos, 1975) and do not need to be included here. In addition, the diploid Greek population and the triploid Dutch population were crossed with *M. brevipennis* males, while in the reciprocal cross males of both Greek and Dutch populations were crossed with *M. brevipennis* females.

The method of crossing the two species was by placing unmated females of one species with males of the other in cages or test-tubes containing *D. caespitosa* and *H. lanatus*. Oviposition, reproduction and egg-fertility of the crossed females were measured as previously described (Chapter 10.1). In order to test whether the female had been successfully inseminated, its abdomen was dissected in physiological solution (Levy) and the bursa copulatrix and the spermatheca were examined under the microscope. Motile sperm was always observed in the spermatheca, when the female had mated.

15.2. Fertility of the parent crosses

15.2.1. *Group crosses in cages*

Table 50 shows the number of females successfully mated with males of the other species. In all these crosses some females had motile sperm in their spermathecae, even when females of one species were caged with males and females of the other species. However, it is not known whether the males mated first with females of their own species and afterwards with females of the other species. The quantity of the observed sperm in the spermatheca of the females mated with their own species was certainly larger than that in females mated with the males of the other species.

15.2.2. *Paired crosses in test tubes*

In order to test whether presence of sperm in the spermatheca of the female is always associated with egg fertility, paired crosses between *M. fairmairei* and *M. brevipennis* (Dutch populations) were made. In each cross, the mating behaviour, oviposition and egg fertility was followed at intervals of 2–5 days. All females of *M. brevipennis* and three of *M. fairmairei* were dissected, after

TABLE 50. Number of crossed females having successfully copulated within a period of one month after adult moult.

Number of crossed ♀ × ♂	Number of ♀ tested	Number of ♀ with sperm in their spermathecae
1. *M. fairmairei* ♀ (Greek diploid population) × *M. brevipennis* ♂ (Dutch diploid population)		
11 × 13	10	3
2. *M. brevipennis* ♀ (Dutch diploid population) × *M. fairmairei* ♂ (Greek diploid population)		
10 × 10	8	2
3. *M. brevipennis* ♀ (Dutch diploid population) × *M. fairmairei* ♂ (Dutch population)		
8 × 8	8	2
4. *M. fairmairei* ♀ (Dutch diploid and triploid population) × *M. brevipennis* ♀ (Dutch population)		
12 × 12	9	3
5. *M. fairmairei* ♀ (Dutch triploid population) × *M. brevipennis* ♂ (Dutch diploid population) × *M. brevipennis* ♀		
6 × 6 × 6	6 *M.f.* (3n)	3
	6 *M.b.* (2n)	6
6. *M. fairmairei* ♀ (Dutch diploid population) × *M. brevipennis* ♂ (Dutch diploid population) × *M. brevipennis* ♀		
6 × 6 × 6	6 *M.f.* (2n)	3
	6 *M.b.* (2n)	6
7. *M. brevipennis* ♀ (Dutch diploid population) × *M. fairmairei* ♂ (Dutch diploid population) × *M. fairmairei* ♀		
6 × 6 × 6	6 *M.f.* (2n)	6
	6 *M.b.* (2n)	3

they had deposited a considerable number of eggs (Table 51).

a. Mating behaviour. This part of the study will be given as a separate paper including the accoustical communication of the *Muellerianella* complex. At present, it can be briefly mentioned that 4–5 days old females brought together with males of the other species for a period of 3–4 hours per day did not copulate. During this period these males and females made contact with each other, but several efforts of the males to copulate with the female were unsuccessful, and finally the females in various ways refused to mate. In other experiments, copulations did occur occasionally but they were shorter in duration than copulations between males and females of the same species. In general, males and females of *M. fairmairei* came in contact with females and males respectively of *M. brevipennis* more frequently than the other way round.

TABLE 51. Fertility of interspecific parent crosses (Dutch populations). The symbols ⊕, ⊖, indicate whether females had or had no sperm in their spermathecae, respectively.

No. ♀ × ♂	Preovi-position period	Ovi-position period	No. of eggs deposited	Fertile eggs	
				No.	%
M. fairmairei ♀ × *M. brevipennis* ♂					
$♀_1 \times ♂_1$	9	17	158	113	71.5
$♀_2 \times ♂_2$	10	42 ⊖	218	38	17.4
$♀_3 \times ♂_3$	12	35	229	73	31.9
$♀_4 \times 2♂_{4,5}$	20	29 ⊕	236	166	70.3
$♀_5 \times ♂_6$	5	47 ⊕	226	32	14.2
$♀_6 \times ♂_7$	10	35	197	40	20.3
$♀_7 \times ♂_8$	10	5	27	0	0.0
$♀_8 \times ♂_9$	11	20	69	0	0.0
$♀_9 \times ♂_{10}$	10	22	184	133	72.3
Mean ± S.D.	10.78 ± 3.96,	28.00 ± 13.26			
			Total 1544	595	38.5
M. brevipennis ♀ × *M. fairmairei* ♂					
$♀_1 \times ♂_1$	10	42 ⊖	337	0	0.0
$♀_2 \times ♂_2$	6	30 ⊖	265	22	8.3
$♀_3 \times ♂_3$	27	25 ⊕	173	92	52.3
$♀_4 \times ♂_4$	22	24 ⊖	184	0	0.0
$♀_5 \times 2♂_{5,6}$	18	29 ⊖	105	0	0.0
$♀_6 \times ♂_7$	18	29 ⊖	151	0	0.0
$♀_7 \times ♂_8$	14	33 ⊖	180	0	0.0
$♀_8 \times ♂_9$	18	29 ⊖	109	0	0.0
$♀_9 \times ♂_{10}$	10	37 ⊖	164	0	0.0
Mean ± S.D.	15.89 ± 6.57	30.89 ± 5.69			
			Total 1668	114	6.8

b. Preoviposition period. Both in the brachypterous females ($♀_1$–$♀_9$) of *M. fairmairei* and in the brachypterous ($♀_1$–$♀_2$) and macropterous ($♀_3$–$♀_9$) females of *M. brevipennis* the preoviposition period compared with those recorded in Table 30 were evidently prolonged by cross mating (Table 51).

c. Oviposition. The number of eggs deposited by each of the two species was considerable (Table 51). The mean number of deposited eggs (183.33 ± 73.69) was not statistically different from that recorded in Table 30 of *M. brevipennis* (macropterous form) ($t = 0.99$, $P > 0.05$).

d. Egg fertility. There was no embryogenesis in eggs laid by females, which had not mated successfully (Table 51). This conclusion is based on dissections of females, that were still depositing fertile eggs and dissection of other females which were no longer depositing fertile eggs. In the former case the dissected females of *M. fairmairei* ($♀_5$ and $♀_4$) and of *M. brevipennis* ($♀_3$) had sperm in their spermatheca, while in the latter case sperm was absent in the spermatheca of the females of *M. fairmairei* ($♀_2$) and *M. brevipennis* ($♀_2$) (Fig. 46). In addition, 7 females of *M. brevipennis* did not produce any fertile egg and when

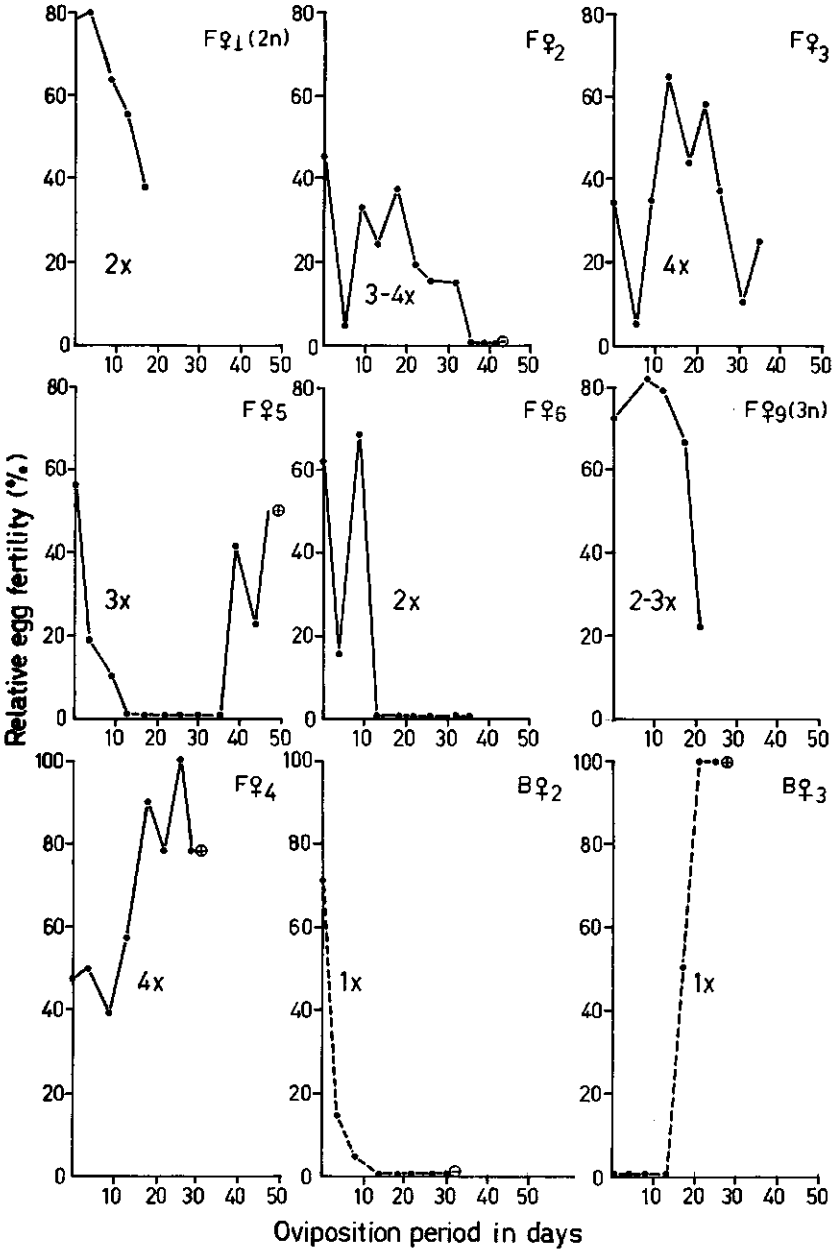


FIG. 46. The fertility of eggs deposited by each female of *M. fairmairei* (solid line) and *M. brevipennis* (dashed line) during its oviposition period, when each female was reared in a test tube together with a male of the other species. The symbols (⊖, ⊕) and the material are the same as in Table 51.
x indicates times of possibly successful copulations.

dissected they had no sperm in their spermatheca (Table 51). Under these conditions, more females of *M. fairmairei* successfully copulated interspecifically than females of *M. brevipennis*. The relative number of fertile eggs deposited by each female was variable.

The egg fertility of each female during its oviposition period is shown in Fig. 46. It is evident that most females deposited fertile eggs at the beginning of their oviposition period. Egg fertility appeared to fluctuate rapidly during the oviposition period. This phenomenon and the fact that large quantities of sperm were never observed in the spermatheca of these females suggest that males of one species do not inseminate adequately the females of the other species. This could be due either to the different structure of the genitalia of each of the two species, or to other factors which have been suggested for the pseudogamous beetle *Ptinus clavipes*, form *mobilis* (SANDERSON, 1960). It is likely, that most females copulated successfully several times during their close coexistence with males, because egg fertility often decreased and then suddenly increased. With this criterion the approximate number of successful copulations (x) of each female is given in Fig. 46.

e. Embryonic development. Embryonic development was followed in the eggs laid by $♀_1$ and $♀_9$ of *M. fairmairei* and $♀_2$ and $♀_3$ of *M. brevipennis* (Table 51). Progeny of males and females obtained from hatched eggs of $♀_1$ revealed that it was diploid, while all female progeny obtained from hatched eggs of $♀_9$ revealed that it was triploid. All fertile eggs laid by the two females of *M. brevipennis* and the triploid *M. fairmairei* exhibited normal embryogenesis, while only 17.6% of the fertile eggs oviposited by the diploid *M. fairmairei* female hatched. The rest showed embryonic development similar to that of eggs in diapause. The time embryogenesis was observed was one month after the eggs had been deposited. However, a similar phenomenon was observed also in eggs laid by other females of *M. brevipennis* crossed with *M. fairmairei* males and in eggs deposited by back-crossed hybrid females (DROSOPOULOS 1977).

15.3. LARVAL DEVELOPMENT OF THE HYBRIDS

Althoug the number of first instar hybrid larvae reared was small, it is clear that all of them can develop till adult stage, both on *H. lanatus* and *D. caespitosa* (Table 52). The time which a hybrid larvae required to develop does not appear to be dependent on the food plant. Also there is not much difference between the larval duration of the hybrids and that of the two species (Tables 52 and 44).

15.4. THE ADULT HYBRID PROGENY

Male and female hybrids have been often produced by crosses between the two species. However the hybrid progeny was always lower in density and produced over longer periods than the progeny of either of the two species

TABLE 52. Larval development (in days) of the hybrid progeny on *D. caespitosa* and *H. lanatus* at 18–20 °C.

Plant	Sex	No. of observations and total larval duration (Mean ± S.D)	
1. Progeny of *M. fairmairei* (2n)♀ × *M. brevipennis* ♂			
H. lanatus	♂	2	31.50 ± 0.71
	♀	2	27.50 ± 0.71
D. caespitosa	♂	2	32.50 ± 4.95
	♀	1	30.00
2. Progeny of *M. brevipennis* ♀ × *M. fairmairei* ♂			
D. caespitosa	♂	1	25.00
	♀	1	28.00
3. Progeny of *M. fairmairei* (3n)♀ × *M. brevipennis* ♂			
H. lanatus	♀	10	26.10 ± 2.23
D. caespitosa	♀	12	27.67 ± 2.15

(Table 53). The sex ratio of the hybrid progeny appeared to be variable, and in several crosses between the two species the chromosomal constitution of the parents and the hybrids is known. *M. fairmairei* ♀♀ (Greek diploid population) crossed with *M. brevipennis* ♂♂ (Dutch diploid population) produced a low progeny, with males being more frequent than females (Table 53, A). From the reciprocal cross low progeny was obtained but the females were more frequent than males (Table 53B). Similar to these two crossings were the results obtained (respectively) by crossing *M. fairmairei* (2n) ♀♀ × *M. brevipennis* ♂♂ (Dutch populations) and its reciprocal cross (Table 53C and D).

The fact that both the Greek and Dutch diploid populations of *M. fairmairei* females crossed with males of *M. brevipennis* resulted in more hybrid males than females, whereas in the reciprocal crosses more hybrid females than males were produced, may be due to one of the following causes: (1) either there is switch in a genically balanced sex determination mechanism between the two species (WHITE, 1973), even between different populations of the same species (see Tables 12–15), (2) selection operates against the less frequent sex of each cross due to a failure of zygote formation by the males sperm and the female nuclei, or (3) there is embryonic mortality during embryogenesis. However, additional cytological studies on the sex-chromosome evolution and sex determination mechanism are necessary for elucidation of this phenomenon.

Hybrid males of all types of crosses are sterile, but hybrid females of the crosses *M. fairmairei* (2n) ♀♀ × *M. brevipennis* ♂♂ often produce viable eggs when mated with *M. fairmairei*♂♂. All female hybrids mated readily with males of the two species. From some back-crossed female hybrids the triploid pseudogamous biotype of *M. fairmairei* was produced (DROSOPOULOS, 1977). It is not surprising that this pseudogamous biotype produces all-female progeny, even when crossed with *M. brevipennis* ♂♂ (Table 53E). Back-crosses of this progeny either with *M. brevipennis* ♂♂ or *M. fairmairei* ♂♂ also produced

TABLE 53. Adult progeny of some crossings (in cages) between *M. fairmairei* and *M. brevipennis*. The asterisk indicates pair-crosses. L:D = 18:6, T = 20–25 °C.

Number of ♀ × ♂	Progeny ♂	Progeny ♀	Required time for the hybrid progeny (in months)
A. *M. fairmairei* ♀ (Greek diploid population) × *M. brevipennis* ♂ (Dutch diploid population)			
1. 11 × 10	–	–	
2. 11 × 13	4	3	3.5
3. 11 × 13	10	5	3.5
4. 9 × 10	6	6	3.0
B. *M. brevipennis* ♀ (Dutch diploid population) × *M. fairmairei* ♂ (Greek diploid population)			
1. 6 × 11	5	19	3.0
2. 11 × 12	–	2	3.5
3. 10 × 10	–	–	–
C. *M. fairmairei* ♀ (Dutch diploid population) × *M. brevipennis* ♂ (Dutch diploid population)			
1. 1 × 1	4	3	
D. *M. brevipennis* ♀ (Dutch diploid population) × *M. fairmairei* ♂ (Dutch diploid population)			
1. 9 × 6	7	21	3.5
2.* 1 × 1	8	136	3.0
1 × 1	–	6	3.0
7 × 7	–	–	3.0
3. 6 × 6	–	25	3.5
4. 6 × 6	7	10	3.5
5. 6 × 6	–	84	3.0
6. 6 × 6	11	12	3.0
E. *M. fairmairei* ♀ (Dutch triploid population) × *M. brevipennis* ♂ (Dutch diploid population)			
1. 6 × 6	–	88	2.5
2. 6 × 6	–	78	2.5
F. Back-crossings of the progeny E_1 × *M. brevipennis* ♂♂ (Dutch diploid population)			
1. 6 × 6	–	42	2.5
G. Back-crossings of the progeny E_1 × *M. fairmairei* ♂♂ (Dutch diploid population)			
1. 6 × 6	–	791	2.5

all-female progeny. However the progeny of the females backcrossed with *M. fairmairei* ♂♂ were evidently more numerous than the progeny of the females backcrossed with *M. brevipennis* ♂♂ (Table 53F and G).

15.5. HOST AND OVIPOSITION PLANTS

When the diploid species were crossed in cages or test tubes containing *H. lanatus* and *D. caespitosa*, each parent species fed and oviposited on its own host plant. In such cages or test tubes the triploid biotype of *M. fairmairei* fed and oviposited mainly on *H. lanatus* and rarely on *D. caespitosa*. Larvae and adult hybrid progeny of crosses between the bisexual species always were

observed to feed and oviposit on *D. caespitosa* when *H. lanatus* was present. However, *H. lanatus* was acceptable as food and oviposition plant when *D. caespitosa* was absent. Progeny of the female hybrid backcrossed with *M. fairmairei* fed and oviposited mainly on *H. lanatus*, although *D. caespitosa* was available.

These data strongly suggest that feeding and oviposition mechanism are expressed by dominance of genomic dosage effect. Thus, one genome of *M. brevipennis* is dominant over a single genome of *M. fairmairei*, but recessive to a double genome of *M. fairmairei*.

16. GENERAL DISCUSSION AND CONCLUSIONS

The bisexual species

1. Terminology. The term 'sibling species' has been used in the present study for the two bisexual species *M. fairmairei* (2n) and *M. brevipennis* (2n).

This term is widely used in studies of closely related species and in the sense of MAYR (1971) it is defined as 'morphologically similar or identical natural populations that are reproductively isolated'.

A similar definition for this term is given by DOBZHANSKY (1972) ('pairs or groups of species that are morphologically indistinguishable or distinguishable with difficulty').

However, there are several scientists who have objections to this term. For example STEYSKAL (1972) considered that this term is unfortunate because it is a perversion of the meaning of the word sibling and he proposed the term 'aphanic species' which was defined as a term referring to nothing but the non-apparent nature of the difference between species to which it may be applied. Two years later MCCAFFERTY and CHANDLER (1974) agreed with STEYSKAL, but they suggested, instead, the term 'symmorphic' for morphologically indistinguishable and 'allomorphic' for distinguishable species, regardless of phylogenetic relation. In addition, they suggested that the term 'related species' should be restricted to its phylogenetic sense.

Simultaneously GOCHFELD (1974) objected to MCCAFFERTY and CHANDLER's suggestion (1974) that the term 'sibling species' should be redefined for use in the phylogenetic sense, as 'that would be truly confusing and would have no appreciable advantage over the term 'sister species' and 'sister group' currently in use'.

Having defined *M. fairmairei* and *M. brevipennis* as 'sibling species', KONTKANEN (1953) noted that 'which species are to be considered as sibling species and which again are not closely related enough to be classed as such is largely a matter of opinion.'

It is not clear to what extent KONTKANEN (1953) attached a phylogenetic and morphological meaning to the word 'sibling species'. Because it confuses indirectly the morphological with the phylogenetic relationship, two aspects of two or more species which have been found not necessarily related, I prefer not to use this term. Terminology referring to the morphological correspondence between two species should be distinguished from that referring to phylogenetic relations, and therefore, in this particular case I consider the terms proposed by MCCAFFERTY and CHANDLER as the most appropriate ones.

Concerning the phylogenetic relationship of two or more species the term 'related species' is sufficient. The degree of relationship of the species, of course, can not be predicted, but can be experimentally analysed. Such an experimental

analysis can be based on biological studies, for example by estimating the degree of reproductive isolation between populations both in the field and in the laboratory.

Another means of such a classification could be based on the model of genetic differentiation proposed by AYALA (1975). However, he himself concluded that the use of electrophoresis as a criterion underestimates the degree of genetic differentiation between populations, since not all amino acid substitutions in proteins are detectable by this method.

It is interesting to attempt to place *M. fairmairei* and *M. brevipennis* at any of the five decreasing levels of evolutionary divergence found in the *Drosophila willistoni* group (AYALA 1975). Since the first and the last are evidently not applicable, three will be considered:

a. If *M. fairmairei* and *M. brevipennis* are to be considered as 'sibling species' they would be: 'closely related species, distinguishable morphologically mainly by some slight but reliable differences in the male genitalia. They have largely overlapping geographic distributions, share their food resources and are quite similar also in other ecological attributes. Yet we estimate that, on the average, about 58.1 electrophoretically detectable allelic substitutions for every 100 loci have occurred in each pair of siblings since their divergence from a common ancestor'. Moreover such species are genetically quite different and they have completed their second stage of geographic speciation.

b. If *M. fairmairei* and *M. brevipennis* were 'semispecies' they should be 'populations in the second stage of geographic speciation. Populations which had become genetically differentiated while geographically isolated have come into geographic contact'. In addition hybridization between such populations is excluded.

c. If *M. fairmairei* and *M. brevipennis* were 'subspecies' they would be 'populations in the first stage of the process of geographic speciation. They are pairs of allopatric populations, which have diverged from each other to the point where some hybrid progenies are sterile; natural selection would favour the development of complete reproductive isolation between them if they were to become sympatric'.

As it can be seen that *M. fairmairei* and *M. brevipennis* are not in accord with any of these three criteria proposed for *D. willistoni*, it seems appropriate to define these two bisexual species as a pair of 'closely related, slightly allomorphic' species.

2. Speciation. In the present study it has been demonstrated that the bisexual *M. fairmairei* (2n) and *M. brevipennis* are: (a) Morphologically distinguishable by slight but reliable differences in the male genitalia, and possess the same number of chromosomes and sex determination mechanism in males as well as in females. (b) Partly sympatric, with a large overlapping distribution which in *M. fairmairei* extends further south and in *M. brevipennis* further north in Europe. Both species show throughout their area of distribution some differences in respect to habitats and host plants. As yet *M. fairmairei* has a wider distri-

bution than *M. brevipennis*, in spite of the more restricted distribution of its host plant (*H. lanatus*) compared with that of *M. brevipennis* (*i.e.* of *D. caespitosa*). In addition, *M. fairmairei* is assumed to be less mobile than *M. brevipennis*. This consideration is based on the relative abundance of the long winged form, which favours migration or dispersal. (c) Diapause and reproductive physiology of the two species are different, probably because of the more northern range of *M. brevipennis*. Also *M. brevipennis* seems to be strictly monophagous, while *M. fairmairei* appears to be able to develop on a larger number of grasses.

Sympatric speciation is not the most probable mode of evolution of these two species. In general, this mode of speciation concerns also phytophagous species and is correlated with strong premating isolation, but this has not been demonstrated either under laboratory or field conditions. If the two species had such an isolation mechanism within their area of sympatry, their allopatric populations (for example the Greek one of *M. fairmairei* and the Dutch population of *M. brevipennis*) might be expected to have hybridized more readily than their sympatric Dutch populations. Such a phenomenon 'termed character displacement' (see GRAND 1975, MAYR 1971, ROSS 1973) appeared experimentally not to occur between *M. fairmairei* and *M. brevipennis* (chapter 15).

Allopatric speciation is the most probable process in *M. brevipennis* and *M. fairmairei*. Although more lines of speciation can be conceived for these two species, I favour the hypothesis that *M. brevipennis* (a more northern distributed species) originated from an isolated population of *M. fairmairei* which split from the original stock and became allopatric after a glaciation. *D. caespitosa*, the new host of this population as interpreted by HULTEN (1952) already had a worldwide distribution before the Pleistocene glaciation, and remnants of this range survived in several different isolated places. Also the fact that this plant has a more northern area of distribution than *H. lanatus*, favours this hypothesis. After the glacial period *M. fairmairei* presumably spread again to the north thus becoming widely sympatric with *M. brevipennis* which in the meantime had acquired a certain degree of isolation by adopting a different host plant, adapting to lower temperatures, longer photoperiods and in general to habitats other than its ancestor. Its degree of speciation has not reached completion either because (a) a limit of time is required for speciation, or (b) the marked differences between the host plants provide a good isolating substrate even within their area of sympatry. However, when these two plants are gowing syntopically the two species of Delphacidae come in contact and evidently hybridize. The result of this hybridization is the triploid pseudogamous biotype, as has been shown in the laboratory.

Such process of speciation also had been suggested for closely related species of the salamanders *Ambystoma* (UZZELL, 1964) and of the winter stoneflies, *Allocapnia* (ROSS, 1974).

The unisexual biotype

1. Terminology. Up to now we called the pseudogamous triploid *M. fair-*

mairei a 'biotype'. This was done to postpone dealing with the nomenclatorial problem, since nomenclature is inadequate for parthenogenetic species, especially of pseudogamically reproducing organisms. As a first example of this problem I refer to the discussion reported by WHITE (1973, p. 701): 'MAYR (1957, 1963) has discussed the general problem of morphological discontinuities between biotypes of asexual organisms. He concludes that 'the existing types are the survivors among a great number of produced forms, that the surviving types are clustered around a limited number of adaptive peaks, and that ecological factors have given the former continuum a taxonomic structure'. In the great majority of cases that have been investigated, morphologically distinct parthenogenetic biotypes are also chromosomally distinct either in ploidy or in respect of chromosomal rearrangements. They are hence *cytotypes* and we may solve the nomenclatural problems by simply referring to them as 'the triploid biotype', 'the 15-chromosome form', and so forth. A word of warning is in order here, however. From an originally diploid continuum, triploid biotypes may have evolved on several occasions, in different geographic areas. To speak of *the* triploid biotype in such cases would be wrong'.

UZZELL (1964) referring to the same problem, suggests that the two triploid salamanders he studied are distinct species because they are distinct populations between which no gene flow is possible.

SCHULTZ (1969), concerning the hybrid origin of the gynogenetic fishes of the *Poeciliopsis* complex, stated that 'I do not consider these fish to be species nor their designations to be species names, the punctuation is not in conflict with the rules of nomenclature'.

Since the triploid female of *M. fairmairei* most probably is also a hybrid between the two bisexual species I prefer the terminology proposed by SCHULTZ. Therefore, this female hybrid should be called *M.2 fairmairei-brevipennis* (pseudog).

2. Pseudogamy, parthenogenesis, polyploidy. In spite of the fact that BLACK and OMAN (1947) reported parthenogenesis in the leafhopper *Agallia quadripunctata*, WHITE (1973) considers thelytoky absent in Auchenorrhyncha. The reason he suggests is that this group of insects is very mobile and adaptive to a wide variety of environments in contrast to non-mobile scale insects where thelytoky is common. There is, however, evidence that thelythoky is not absent in Auchenorrhyncha, especially in Typhlocybidae where already LE QUESNE (1972) and CLARIDGE (1976) reported cases of a pronounced excess of females. LE QUESNE (1972) ascribes this to sampling problems but CLARIDGE (personal communication) has already considered the possibility of pseudogamy. In addition, pseudogamous females of small insects often do not provide morphological characters distinct from their bisexual 'host species', except in the case of the ptinid beetle *Ptinus mobilis* (MOORE, WOODROFFE and SANDERSON, 1956).

The fact that all well known pseudogamous 'species' or 'biotypes' or 'forms' among invertebrates (CHRISTENSEN and O'CONNOR 1958, BENAZZI-LENTATI

1966, NARBEL-HOFSTETTER 1961, MOORE, WOODROFFE and SANDERSON 1956, DROSOPOULOS 1976) and vertebrates (HUBBS and HUBBS 1932, SCHULTZ 1967, MACGREGOR and UZZELL 1964) are closely related to a complex of two or more closely related bisexual species suggests that such forms are products of hybridization. Most of the above authors have suggested a hybrid origin of their organisms, especially in vertebrates. Exceptionally, SANDERSON (1960) suggested that such a 'form' of *Ptinus* is derived from its bisexual counterpart. However, her suggestion should be reconsidered since the *Ptinus* complex is very similar to the *Muellerianella* complex.

Since 'pseudogamy' or 'gynogenesis' is an 'apomictic parthenogenesis' (SUOMALAINEN 1962) it is worth-while to consider briefly the evolution of this type of parthenogenesis. As far as I am aware, allopolyploidy is strongly suggested for apomictic parthenogenesis by HUBBS and HUBBS (1932), DAREVSKY and KULIKOVA (1964), UZZELL (1964), LOWE and WRIGHT (1966), MASLIN (1968), SCHULTZ (1969), less strongly by PIJNACKER (1964) and ASTAUROV (1969), while SUOMALAINEN (1969), WHITE (1963) and ASTAUROV (1959), suggested that at least the different degrees of polyploidy have arisen from hybridization between bisexual and different, predominantly parthenogenetic races. On the other hand, CHRISTENSEN (1960) and PENNOCK (1965) suggested that larger-scaled investigations on both diploids and triploids are needed to test whether triploidy arose within a single species, or resulted from hybridization between two species. In contrast to these authors, PEACOCK (1952) and NARBEL-HOFSTETTER (1961) held that parthenogenesis induced by hybridization is very rare if not absent in the animal kingdom.

Above references indicate that there is an extensive literature on the role of apomictic parthenogenesis in evolution in invertebrates, vertebrates and plants. However, although for several all-female vertebrate species it is apparent that hybridization, pseudogamy, parthenogenesis and polyploidy are all correlated, this has not previously been demonstrated in insects which have unisexual and polyploid 'species'. The present investigation has shown that they do. In addition, HASKINS, HASKINS and HEWITT (1960) noted that pseudogamy takes place rather late in the evolution of a 'species' and this is relevant in this context.

Recently, WOODROFFE (1968), UZZELL (1969) and MCKAY (1971) have obtained evidence that some triploid pseudogamous organisms no longer compete for sperm, but have 'freed' themselves from their bisexual 'hosts', becoming strictly parthenogenetic. Selection might have been the cause, as was shown by CARSON (1967) to be possible artificially in *Drosophila*. Therefore, it is not impossible that the same may occur in the triploid *M.2 fairmairei-brevipennis*, or has already occurred in some as yet undetected populations.

Bisexuality-unisexuality

CHRISTENSEN (1960) has proposed the term 'obligatory co-existence' to describe the situation in which sperm from a bisexual species is necessary for the normal development of the egg of an unisexual triploid 'species'.

Attempts were made to further investigate this phenomenon in *Muellerianella*, but since the two females are morphologically indistinguishable, we could not procure the proper material for this study. However, it is clear that there is a balance between the bisexual population and at least a large part of the unisexual one, and that the populations occupy the same oviposition site and food plant in the field. This phenomenon is not rare in other leafhoppers, since ROSS (1957) and LE QUESNE (1972) reported that the same ecological niche might be occupied by several species of these phytophagous insects. In my opinion such coexistence is possible in the field since the ecological niche is usually sufficient for two or more species (HARPER with CLATWORTHY, MCNAUGHTON and SAGAR, 1961), or other mechanisms prevent overcrowded situations. However, in the laboratory colonies coexistence of the bisexual and unisexual populations was not possible for more than 1–3 generations (DROSOPOULOS 1977).

The exact mechanisms for the coexistence, geographical distribution and population balance of the bisexual-unisexual *Muellerianella* conglomerations is at present being studied in Wageningen by my colleague C. BOOY. His results may elucidate these important biosystematic problems.

17. SUMMARY

The genus *Muellerianella* comprises the species: *M. fairmairei*, *M. brevipennis*, *M. relicta* and one pseudogamous all-female biotype *M. fairmairei* (3n). The bisexual species *M. fairmairei* and *M. brevipennis* as well as the unisexual *M. fairmairei* (3n) were investigated from a biosystematic point of view. The males of the two bisexual species are morphologically distinct but their females, the female hybrids of both way-crossings between the two bisexual species and the unisexual biotype are morphologically indistinguishable.

The bisexual species *M. fairmairei* and *M. brevipennis* are diploid (2n = 28) and their sex determination system is XY. F1 hybrids also have 28 chromosomes. The pseudogamous unisexual biotype is triploid (3n = 41) reproducing apomictically, but it requires sperm derived from the males of the two bisexual species to initiate embryogenesis (pseudogamy).

The host plants of the *Muellerianella* complex are *Holcus lanatus* or *H. mollis* for the bisexual and unisexual *M. fairmairei*, and *Deschampsia caespitosa* for *M. brevipennis*.

The distributions of the two species and their host plants overlap widely in West and Central Europe. *M. fairmairei* and its host plant are distributed more to the south, while *M. brevipennis* and its host extend more to the north. There is evidence that the unisexual *M. fairmairei* (3n) occurs in the overlapping area of the two bisexual species, but is absent from the peripheral areas where one of the other species is also absent.

In the area of Leersum-Langbroek (prov. of Utrecht) in Holland both species are common, and their respective host plants grow in reasonable numbers. In a few localities where the two hosts are closely intermixed both delphacid species occur syntopically.

Samples of the two species were taken bij the sweep-net and suction methods. *M. fairmairei* is more frequent in wet biotopes of noncultivated meadows in West Europe. *M. brevipennis* is more frequent in the north of Europe and is a stenotopic species typical of fresh biotopes of wooded areas.

In Northern Europe both species are univoltine while in West and Central Europe *M. fairmairei* has two distinct generations in contrast to *M. brevipennis* which has an incomplete second generation. However, there is no important seasonal isolation between the two species. In Southern Europe *M. fairmairei* is probably polyvoltine.

Populations of *M. fairmairei* from regions where *M. brevipennis* does not occur (S. Greece, S. France?, Ireland) have a sex ratio of 1 : 1, while populations occurring sympatrically with *M. brevipennis* (England, France, Holland) have a high proportion of females, comprising a mixture of diploid and triploid individuals. In Holland *M. brevipennis* has a sex ratio of 1 : 1, while populations of this species in Finland have a high proportion of females.

Both diploid species maintained a 1:1 sex ratio in the laboratory rearings.

Crossings between triploid females and males of *M. fairmairei* resulted in absolute all-female triploid progenies. Diploid and triploid females of *M. fairmairei* coexist in Holland. In one biotope (in Leersum), the proportion of the two female biotypes of *M. fairmairei* was 1:1, while in others triploid females were more numerous than the diploid ones. It is not clear, whether both female populations of *M. fairmairei* occupy exactly the same ecological niche.

Regarding the wing form of the two bisexual species and the unisexual biotope, long photoperiod (L:D = 18:6) favors the development of the long wings, while short photoperiod (L:D = 10:14) completely suppresses it. Under long photoperiod the macropterous form of *M. brevipennis* was more common than that of *M. fairmairei*. Under long photoperiod, when the larval density was increased *M. fairmairei* (2n) had proportionately more macropterous adults than *M. fairmairei* (3n).

Summer and winter eggs of the two species were parasitized by *Anagrus sp.* However, eggs of the second generation were more frequently parasitized (up to 40%) that those of the first.

The higher population densities of *M. fairmairei* than of *M. brevipennis* in the field were interpreted from laboratory observations by the fact that *M. fairmairei* has higher egg production than *M. brevipennis*. The triploid females of *M. fairmairei* are assumed to be more prolific than the diploid ones. Also, the rate of egg production of *M. fairmairei* was higher than that of *M. brevipennis*. Females of *M. fairmairei* mated once produced a few unfertile eggs at the end of their oviposition period, in contrast to *M. brevipennis* which always produced fertile eggs. Males of *M. fairmairei* appeared to have greater longevity than the females.

During the first generation and in colonies under long photoperiod *M. fairmairei* oviposits in its food plant *H. lanatus*, while during the second generation and in colonies under short photoperiod in *Juncus effusus*. *M. brevipennis* was found to oviposit in the field only in its food plant, namely *D. caespitosa*, but in the laboratory it also oviposited in *J. effusus*. The egg-group size of both species depends upon the oviposition substrate.

Embryonic development of both species is continuous during the first generation and in colonies under long photoperiod, but embryonic diapause (arrest of development before blastokinesis) takes place during the second generation and in laboratory rearings under short photoperiod. The intensity of diapause is higher in *M. brevipennis* as compared to *M. fairmairei*. Continuous rearings of the unisexual and bisexual *M. fairmairei* were possible under short photoperiod.

The rate of larval development of both species under long photoperiod at 20–25 °C was approximately the same. Under short photoperiod the duration of the last instar larva of females is longer than that under long photoperiod. Temperature, humidity and crowding had an influence upon the rate of larval development.

In laboratory experiments, development and reproduction of *M. fairmairei* occurred on several grass species, in contrast to *M. brevipennis* which appeared

to be monophagous. Mature grasses were more suitable as food plants than seedlings.

Some unmated females of *M. fairmairei* (2n) placed in cages containing *H. lanatus* and *D. caespitosa*, and males of *M. brevipennis* ultimately produced a few male and female hybrids. The reciprocal cross resulted in more female hybrids than males. Egg-fertility of these crosses was variable (0–100%) during the course of the oviposition period. Hybrid larvae can develop on both grass species, but they prefer *D. caespitosa* to *H. lanatus*. Males were sterile but females were often fertile and some of them crossed back with *M. fairmairei* produced a triploid pseudogamous biotype very similar to that collected in the field. The all-female progeny of the triploid *M. fairmairei* was greater when it was crossed with *M. fairmairei* males than with *M. brevipennis* males. Hybrids were obtained even when unmated females (2n + 3n) of both species together were caged with males of one species.

In conformity with these results it is proposed that the two bisexual species should be called 'allomorphic-related species' instead of 'sibling species'. Allopatric speciation is considered as the most probable cause of divergence between both species. It is suggested that *M. brevipennis* originated from an isolated population of *M. fairmairei* during a period of glaciation and has survived on *D. caespitosa*.

It is proposed to call the unisexual biotype *M.2 fairmairei-brevipennis*, following the nomenclatorial system of hybrids. In this context, it has been demonstrated that in insects hybridization may lead to unisexuality followed by polyploidy.

In addition to this summary, all the differences found so far between *M. fairmairei* and *M. brevipennis* are tabulated below (Table 54).

TABLE 54. Differences between the two *Muellerianella* species.

Characteristics	*M. fairmairei*		*M. brevipennis*
	Bisexual	Unisexual	
Morphology	♂ different	–	♂ different
Chromosome number	2n = 28	3n = 41	2n = 28
Sex-chromosome (♂)	trivalent	–	heteromorphic bivalent
Range	European-wide	W. & C. Europe	Western Siberian
Food plants in field	*Holcus lanatus* and *H. mollis*		*Deschampsia caespitosa*
Food plants in lab.	oligophagous		monophagous
Oviposition plants			
(long day)	*H. lanatus*		*D. caespitosa*
(short day)	*J. effusus*		*D. caespitosa*
Voltinism			
(in Holland)	bivoltine		partly bivoltine
Wing dimorphism			
(long winged under long photoperiod)	±25%	±15%	±60%
Sex ratio (♂:♀)	1:1	0:1	1:1
Reproduction:			
1. Sexual maturity (♂)	earlier	–	later
2. Sexual maturity (♀)	earlier		later
3. Preoviposition	shorter		longer
4. Fecundity	high	higher	lower
5. Reproduction rate	high	higher	lower
6. Longevity (♂–♀)	♂♂ > ♀♀	–	♂♂ = ♀♀
Diapause			
1. Induction	later		earlier
2. Intensity	ateleo-oligopause		teleo-oligopause
3. Termination	earlier		later
4. Short-day rearing	possible		impossible

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19. SAMENVATTING

Het cicaden-genus *Muellerianella* omvat de soorten: *M. fairmairei*, *M. brevipennis*, *M. relicta* en een zich pseudogaam voortplantend biotype van *M. fairmairei*, dat geheel uit triploide vrouwelijke individuen bestaat. De bisexuele soorten *M. fairmairei* en *M. brevipennis*, als ook de unisexuele *M. fairmairei* (3n) vormden het onderwerp van een biosystematisch onderzoek. Van de twee bisexuele soorten zijn de mannetjes morfologisch te onderscheiden; er is echter geen exterieur verschil tussen de vrouwtjes van beide soorten, de vrouwtjes van het unisexuele biotype en de bij kruisingen geproduceerde vrouwelijke hybriden.

De bisexuele soorten *M. fairmairei* en *M. brevipennis* zijn diploid (2n = 28) en hebben een XY-sexdeterminatie-mechanisme. F1-hybriden hebben eveneens 28 chromosomen. Het pseudogame unisexuele biotype is triploid (3n = 41) en heeft een apomictische wijze van reproduktie waarbij echter sperma van mannetjes van één van beide bisexuele soorten nodig is voor het op gang brengen van de embryogenese (pseudogamie).

De voedselplanten van het *Muellerianella*-komplex zijn *Holcus lanatus* en *H. mollis* voor de bisexuele en unisexuele *M. fairmairei*, en *Deschampsia caespitosa* voor *M. brevipennis*.

De verspreidingsgebieden van beide soorten en hun waardplanten overlappen elkaar over een groot gebied in West en Centraal Europa. Daarbuiten vertonen *M. fairmairei* en waardplant een meer zuidelijke verspreiding, terwijl *M. brevipennis* en waardplant meer noordelijk verspreid zijn. Er zijn aanwijzingen dat de unisexuele *M. fairmairei* (3n) slechts voorkomt in het gebied waar de twee bisexuele soorten elkaar overlappen, en afwezig is in gebieden waar één van de twee soorten ontbreekt. In de omgeving van Leersum en Langbroek (provincie Utrecht) komen beide soorten cicaden en hun waardplanten algemeen voor. Op enkele plaatsen waar beide waardplanten gezamenlijk voorkomen, zijn ook de beide delphacide-soorten in elkaars direkte gezelschap te vinden.

Bemonstering van de twee soorten werd uitgevoerd met sleepnet en zuigapparaat.

M. fairmairei komt voor in vochtige biotopen van ongekultiveerde graslanden en is talrijker op meer zuidelijke breedten in Europa. *M. brevipennis* is een stenotope soort, typisch voor koele, vochtige biotopen in bosachtige gebieden, en is talrijker in Noord Europa.

In Noord Europa zijn beide soorten univoltien. In West en Centraal Europa is *M. fairmairei* bivoltien, terwijl daar *M. brevipennis* slechts een gedeeltelijke tweede generatie heeft. Van belangrijke seizoensisolatie tussen de beide soorten

is echter geen sprake. In Zuid Europa is *M. fairmairei* waarschijnlijk polyvoltien.

Populaties van *M. fairmairei* afkomstig uit gebieden waar *M. brevipennis* niet voorkomt (Zuid Griekenland, Ierland) hebben een sex-ratio van 1:1, terwijl populaties die sympatrisch voorkomen met *M. brevipennis* (Engeland, Frankrijk, Nederland) uit een hoger percentage vrouwtjes bestaan vanwege het gemengd voorkomen van diploïde en triploïde individuen.

Beide diploïde soorten vertoonden in laboratorium-kweken altijd een sexratio van 1:1. Kruisingen tussen mannetjes van *M. fairmairei* en triploïde vrouwtjes resulteerden zonder uitzondering in een geheel vrouwelijk triploïd nageslacht. Diploïde en triploïde vrouwtjes van *M. fairmairei* komen in Nederland in gemengde populaties voor. In één biotoop (Leersum) kwamen de beide typen vrouwtjes van *M. fairmairei* voor in een verhouding van 1:1, terwijl elders de triploïde vrouwtjes talrijker waren dan de diploïde. Het is niet duidelijk of beide typen vrouwtjes van *M. fairmairei* op de gemeenschappelijke voedselplant dezelfde niche bezetten.

Zowel de twee bisexuele soorten als het unisexuele biotype vertonen vleugeldimorfie. Lange dag tijdens de larvale ontwikkeling (L:D = 18:6) stimuleert vorming van volledig ontwikkelde vleugels, terwijl bij korte dag (L:D = 10:14) de dieren uitsluitend korte vleugels ontwikkelen. Onder lange dag omstandigheden was de macroptere vorm van *M. brevipennis* talrijker dan die van *M. fairmairei*. Onder lange dag leidde 'crowding' tijdens de larvale ontwikkeling bij *M. fairmairei* (2n) tot een hoger percentage macroptere adulten dan bij *M. fairmairei* (3n).

Zomer- en wintereieren van de beide soorten werden geparasiteerd door *Anagrus sp.* (Mymaridae), echter de wintereieren in sterkere mate (tot 40%) dan de zomereieren.

Het feit dat in Nederland *M. fairmairei* in het veld hogere populatiedichtheden ontwikkelde dan *M. brevipennis*, werd in verband gebracht met de in het laboratorium waargenomen grotere eiproduktie van de eerste soort. De triploide vrouwtjes van *M. fairmairei* hebben vermoedelijk een hogere reproduktie-kapaciteit dan de diploïde. Tevens had *M. fairmairei* een grotere ovipositiesnelheid. Vrouwtjes van *M. fairmairei* die éénmaal gepaard hadden, produceerden enkele onbevruchte eieren aan het eind van de ovipositieperiode, in tegenstelling tot vrouwtjes van *M. brevipennis* die steeds bevruchte eieren produceerden. Mannetjes van *M. fairmairei* hadden een langere levensduur dan vrouwtjes.

Tijdens de eerste generatie en in de kweek onder lange dag, vond bij *M. fairmairei* ovipositie plaats in de voedselplant *H. lanatus*, daarentegen tijdens de tweede generatie en in de kweek onder korte dag in *Juncus effusus*. In het veld werd bij *M. brevipennis* uitsluitend ovipositie in de voedselplant *D. caespitosa* waargenomen, echter in het laboratorium vond ook ovipositie in *J. effusus* plaats. Beide soorten zetten hun eieren af in groepjes. De grootte van deze ei-groepjes hangt samen met het ovipositie-substraat.

In de eieren van de eerste generatie en in de kweek onder lange dag, verloopt

de embryonale ontwikkeling kontinu, maar in de eieren van de tweede generatie en in de kweek onder korte dag, treedt er embryonale diapauze op (stilstand in de ontwikkeling vóór het blastokinese-stadium). De intensiteit van de diapauze was bij *M. brevipennis* groter dan bij *M. fairmairei*. Het was mogelijk zowel de unisexuele als de bisexuele *M. fairmairei* onder korte dag door te kweken.

De snelheid van larvale ontwikkeling was voor beide soorten in de kweekproeven onder lange-dag kondities bij 20–25°C ongeveer hetzelfde. Onder korte-dag omstandigheden duurde het laatste larvestadium bij vrouwtjes langer dan onder lange dag. Temperatuur, luchtvochtigheid en 'crowding' waren van invloed op de snelheid van larvale ontwikkeling.

In tegenstelling tot *M. brevipennis*, bleek in laboratorium experimenten ontwikkeling en reproduktie van *M. fairmairei* mogelijk op verscheidene grassoorten. Volgroeide grassen waren geschikter als voedselplant dan zaailingen.

Enkele maagdelijke vrouwtjes van *M. fairmairei* (2n) die samen met mannetjes van *M. brevipennis* in een kooi met *H. lanatus* en *D. caespitosa* geplaatst waren, produceerden enkele mannelijke en vrouwelijke hybriden. De reciproke kruising resulteerde in meer hybride vrouwtjes dan mannetjes. Bij beide kruisingen was de ei-fertiliteit variabel (0–100%) in de loop van de ovipositieperiode. Hybride larven bleken zich op beide grassoorten te kunnen ontwikkelen, maar *D. caespitosa* werd geprefereerd boven *H. lanatus*. Hybride mannetjes waren steriel, maar vrouwtjes waren vaak fertiel. Enkele van deze hybride vrouwtjes produceerden bij terugkruising met *M. fairmairei* een triploïd pseudogaam biotype. Bij kruising met *M. fairmairei* mannetjes, produceerde de triploïde *M. fairmairei* meer vrouwelijke nakomelingen dan bij kruising met *M. brevipennis* mannetjes. Hybriden werden zelfs verkregen wanneer vrouwtjes (2n + 3n) van beide soorten samen met mannetjes van één soort in één kooi werden geplaatst.

Voorgesteld wordt de twee bisexuele soorten te betitelen als 'allomorphic-related species' in plaats van 'sibling species'. Allopatrische soortvorming wordt beschouwd als het meest waarschijnlijke proces dat heeft geleid tot de differentiatie van beide soorten. Vermoedelijk stamt *M. brevipennis* af van een populatie van *M. fairmairei* die tijdens een ijstijdperiode geïsoleerd raakte en op *D. caespitosa* heeft overleefd.

Voorgesteld wordt het unisexuele biotype te betitelen als *M. 2 fairmairei-brevipennis*, volgens het nomenclatuur-systeem voor hybriden. Aangetoond is dat bij insekten hybridisatie kan leiden tot unisexualiteit en polyploïdie.

Alle verschillen die tot nu toe gevonden zijn tussen de twee soorten zijn samengevat in Tabel 54.

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