

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

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Abstract

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

Introduction

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

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

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

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

Material and methods

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

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

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

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

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

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

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

By daily inspection of the cages information was

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

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

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

Results

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

Interspecific mating and insemination

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

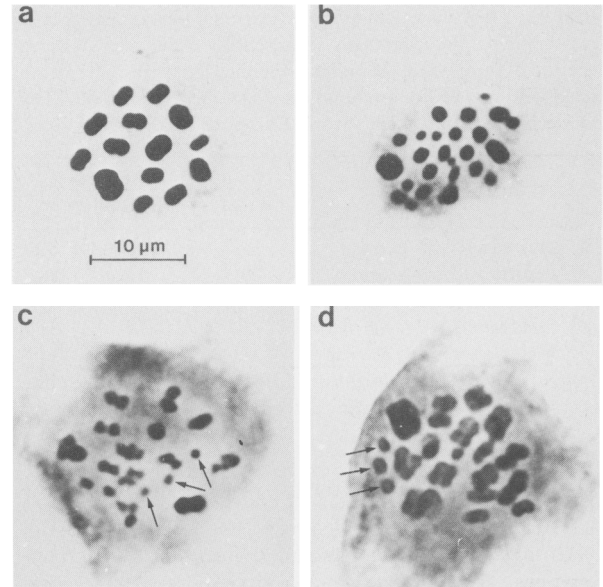


Fig. 1. Meiotic configurations in eggs of normal and aberrant *Muellerianella* females: (a) metaphase plate with 14 bivalents of a normal female (*M. extrusa*), polar view; – (b) metaphase plate in a hybrid female *M. fairmairei* × *M. brevipennis* containing mainly univalents; – (c) the same with a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

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

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

Cross Nr	<i>n</i>	B ₁ (= F ₁ × <i>Mf</i>)			<i>n</i>	B ₂ (= B ₁ × <i>Mf</i>)			<i>n</i>	B ₃ (= B ₂ × <i>Mf</i>)			<i>n</i>	B ₄ (= B ₃ × <i>Mf</i>)		
		♀♀	♂♂	prog/♀		♀♀	♂♂	prog/♀		♀♀	♂♂	prog/♀		♀♀	♂♂	prog/♀
2	9	9	3	1.3	3	59	39	33	5	ca 200	ca 40	10	ca 500	ca 50		
11	15	39	24	4.2												
12	15	96	56	10.1												
14	16	71	29	6.7	30	103	84	6	10	ca 1400	ca 140					
19	10	4	2	0.6	4	61	34	24	10	36	26	6.2	20	370	210	24
21	10	2	0	0.2												
Total	75	221	114	4.5	37	223	157	10.2	25	ca 1660	ca 66	30	ca 1080	ca 36		

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

Cross nr.	F ₁		B ₁ (F ₁ × Mf)		B ₂ (B ₁ × Mf)		B ₃ (B ₂ × Mf)		B ₄ (B ₃ × Mf)	
	tested	fertile	tested	fertile	tested	fertile	tested	fertile	tested	fertile
2	6	0%	3	0%	14	7%	8	37%		
11	10	0%	14	7%						
12	10	0%	18	6%						
14	10	0%	24	0%	41	34%				
19	3	0%	2	0%	18	0%	5	20%	10	90%
21	7	0%								
Total	46	0%	61	3%	73	20%	13	30%	10	90%

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

Genera- tion	14 bivalents (normal)	7-13 bivalents + univalents	0-6 bivalents + univalents
F1	0	10	4
B1	28	38	6
B2	42	6	0

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

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

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

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

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

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

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

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

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

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

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

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

Hybrid females of crosses 22, 23, and 24 were backcrossed with males of *M. fairmairei* and *M. extrusa*. Table 8 shows that the results of the backcrosses vary, but in 4 out of 6 possible backcrosses progeny was obtained. Unfortunately the B₁ generation of cross 23 was lost and could not be further studied. Males and females of the B₁ in cross 24 appeared to be fully fertile and could be crossed inter se.

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

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

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

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

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

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

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

Table 8. Production of adult offspring by female hybrids *M. fairmairei* (*Mf*) × *M. extrusa* (*Me*) after backcrossing with the parent species and results of crosses of animals of the B₁ generation inter se. Total production and progeny per female are given.

Cross	B ₁ (F ₁ × <i>Mf</i>)			B ₁ (F ₁ × <i>Me</i>)			B ₁ × B ₁		
	No. of ♀♀ used	Progeny	Prog/♀	No. of ♀♀ used	Progeny	Prog/♀	No. of ♀♀ used	Progeny	Prog/♀
22	10	0	0	10	0	0	—	—	—
23	10	6	0.6	10	100	10	—	—	—
24	5	60	12	10	140	14	10	ca 650	65

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

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

Sex ratios in progenies of intraspecific crosses

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

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

Deviating sex ratios may also be found when intraspecific crosses are made between populations which are genetically differentiated. Such effects were apparent in crosses between Greek and Dutch

populations of *M. fairmairei* made by Drosopoulos (1977). I found similar effects in crosses between remote populations of both *M. brevipennis* and *M. fairmairei*. Eight pairwise crosses were made between *M. brevipennis* from N. Sweden and from S. France. Although the distance between the populations is about 2600 km, only in one case were significantly more females than males produced.

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

Mating relations of gynogenetic females with the bisexual species

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

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

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

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

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

Discussion

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

The crossability of the species under artificial

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

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

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

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

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

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

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

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

Probably the best way to elucidate the genetic constitution of the triploid *Muellerianella* forms with more certainty, is to study their allozyme patterns and those of their bisexual relatives. The fact that the triploids, which resemble *M. fairmairei*, can also reproduce after insemination by *M. brevipennis* males cannot be used as an argument for the

hybrid origin hypothesis, since this holds true also for *M. extrusa*, which actually is an even better sperm donor, because more offspring is produced in that case. Up till now we have no certain records of triploids in association with *M. extrusa* in the field, but it is possible that they will be found in the future. In that case the gynogenetic forms could maintain themselves by using one out of three sperm donor species in the field. As far as I know, such a complex has not been reported in nature before.

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