

ACOUSTIC COMMUNICATION AND MATING BEHAVIOUR
IN THE MUELLERIANELLA COMPLEX
(HOMOPTERA - DELPHACIDAE)

by

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(With 6 Figures)
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The importance of acoustic communication and mating behavior in maintaining reproductive isolation between sympatric-synchronic species has been stressed in the past (*e.g.* ALEXANDER, 1967; STRUBING, 1966; CLARIDGE & REYNOLDS, 1973). On the other hand there are examples of closely related synchronic species where species-specific differences in mating or acoustic behavior could not be found (BLONDHEIM & SHULOV, 1972), or where there were similarities in acoustical repertoires (SHAW, VARGO & CARLSON, 1974).

In the genus *Muellerianella* which includes three bisexual species and at least four pseudogamous species (DROSOPOULOS, 1976, in prep.; BOOIJ, 1981) originally differences in acoustic communication were assumed to play a major role in speciation. This aspect of acoustic behavior has been studied in detail by BOOIJ (1982). He showed that the male calling songs of *M. brevipennis* differ from those of *M. fairmairei* by the absence of chirps between roll-sequences (Fig. 1A, B; for terminology see BOOIJ, 1982). However, interspecific differences between the calling songs of females are less spectacular (Fig. 1C, D, E). Female calling songs of *M. fairmairei* and *M. 2fairmairei-brevipennis* mostly consist of shorter pulse-series than the songs of *M. brevipennis* females, but no consistent other differences were found. Diploid hybrids produced by both combinations of crossing the two bisexual species emitted calling songs of an intermediate pattern (Fig. 2).

Concluding we may state that there are large differences between the calling songs of males of the two bisexual species. However, among

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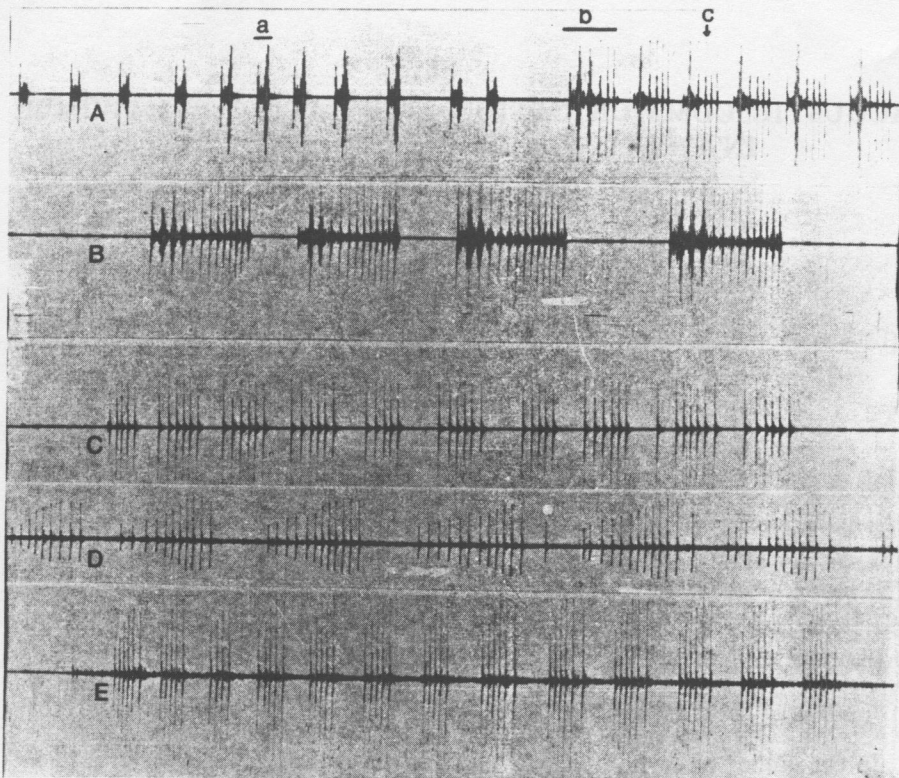


Fig. 1. Calling songs of *M. fairmairei* ♂ (A), *M. brevipennis* ♂ (B), *M. fairmairei* ♀ (C), *M. brevipennis* ♀ (D) and *M. 2fairmairei-brevipennis* ♀ (E). (a) indicates a chirp; (b) a roll; (c) a pulse. (The recordings and oscillograms were made by P. de VRIJER and C. BOOIJ).

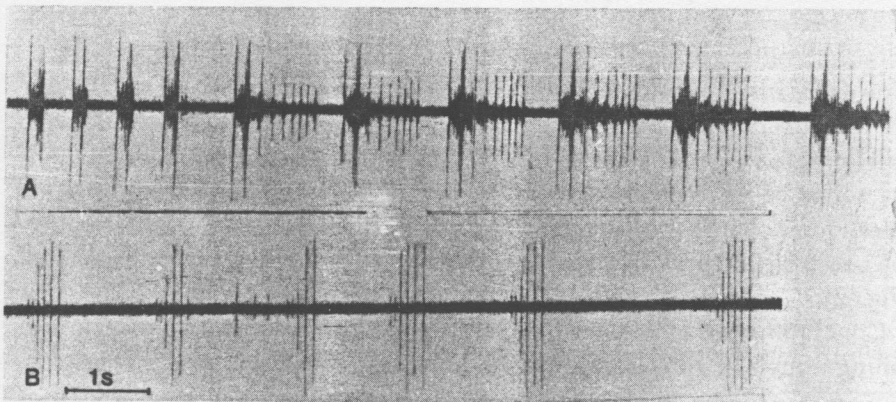


Fig. 2. Calling songs of a male (A) and a female (B) hybrid produced by crossing females of *M. fairmairei* with males of *M. brevipennis*.

females of the bisexual and unisexual species and the hybrids there are no sharp differences and if we measure the variability in the number of pulse-series (BOOIJ, 1982) the situation is obviously complicated.

In addition extensive studies on morphology, ecology, behavior, zoogeographic distribution, ecophysiology, cytogenetics and several interspecific crosses were made (DROSOPoulos, 1976, 1977, 1978, 1983, in prep.; BOOIJ, 1981, 1982). The data from these studies, which in the sense of MAYR (1971) form the study of "isolating mechanisms", show that the main isolation mechanism between the bisexual species is food-plant preference and that other biological differences are of secondary importance, thus, under field and laboratory conditions none of the bisexual species can breed on the food-plant of another. We conclude therefore that the bisexual species are primarily kept separated by food-plant preference, though other differences, *e.g.* in the sounds emitted by the males, may be of secondary value. However, there are places where food-plants are mixed and there the bisexual species may come into contact with each other (DROSOPoulos, 1977). In these places pseudogamous species, which rely for sperm on the males of bisexual species (DROSOPoulos, 1976) are present coexisting with one bisexual species, while outside of the sympatric area the pseudogamous species are absent. In the laboratory it has been demonstrated that the two bisexual species can be crossed; the males of the diploid F_1 are all sterile, some females are fertile. When some of these fertile females were backcrossed with males of *M. fairmairei* the pseudogamous triploid "species" *M. 2fairmairei-brevipennis* was produced (DROSOPoulos, 1978). With respect to this rare association of bisexual-unisexual species it was of interest to investigate how divergent differences in acoustic communication and/or mating behavior had to be in order to prevent hybridization and thereby to lessen mating of bisexual males with their pseudogamous "reproductive parasite".

Methods and material

Many types of sounds are emitted by males and females of delphacids (OSSIANILSSON, 1949). In this paper only the pair-forming (ALEXANDER, 1967) or calling signals are considered.

Observations on intraspecific mating behavior were made as follows: last instar larvae were collected in the field and were reared individually in test-tubes. Each test-tube contained two stems of either *Holcus lanatus*, the food-plant of *M. fairmairei* or *Deschampsia caespitosa*, the food-plant of *M. brevipennis*. For each test-tube the time of adult moult, the sex and wing form of the reared insect was noted. The checks were made at intervals of 12 hours. The time of first mating of males and females was observed as follows: each day after their adult moults, a male was transferred into the living tube of a female. The pair

was observed for a period of four hours and then separated. In this way the onset of sexual maturity of males and females was determined. Furthermore, preoviposition, oviposition and post-oviposition periods, egg production, rate of reproduction, egg fertility and longevity of each individual were recorded.

Interspecific mating behavior also was studied in test-tubes containing one stem of *H. lanatus* in contact with one stem of *D. caespitosa*.

The experimental insects were from diploid populations of *M. fairmairei* originating from Holland and Greece, a diploid population of *M. brevipennis* from Holland, diploid hybrids of their F_1 and the triploid pseudogamous "species" *M. 2fairmairei-brevipennis* from Holland.

Experiments were made simultaneously for all species at temperatures of 20-25°C and under long day conditions (L:D = 18:6).

Statistical analysis. The mean values and standard deviations (S.D.) are given for each set of observations. Comparison of regression lines (in fact between two regression coefficients) was made using the methods of SOKAL & ROHLF (1969). The levels of significance of differences are indicated with asterisks as follows: * $0.05 > P > 0.01$, ** $0.01 > P > 0.001$, *** $P \leq 0.001$.

TABLE 1. Days before first mating of females of the species of *Muellerianella*

	N° ♀♀ observed	Mean ± S.D.	Range
<i>M. fairmairei</i>	11	3.27 ± 1.85	2-8
<i>M. 2fairmairei-brevipennis</i>	12	3.17 ± 0.94	2-5
<i>M. brevipennis</i>	23	4.91 ± 1.00	3-7

Results

Sexual maturity.

Motile sperm is present in the testes of the last larval instars of both *M. fairmairei* and *M. brevipennis*. Males of *M. fairmairei* however can start mating during the first day following emergence, whereas males of *M. brevipennis* wait until the second day.

In females of the three species mature eggs were never observed before the fourth day of adulthood, but mating in these species may start before egg maturation (Table 1). Thus females of *M. fairmairei* and *M. 2fairmairei-brevipennis* started mating during the second day following emergence, while females of *M. brevipennis* did so during the third day. The number of days before first mating does not differ significantly between *M. fairmairei* and *M. 2fairmairei-brevipennis* ($t = 0.16$, $P > 0.05$), but in *M. brevipennis* it is significantly different from both *M. 2fairmairei-brevipennis* ($t = 5.08^{***}$) and *M. fairmairei* ($t = 2.75^{**}$).

Pre-copulatory behavior.

Pre-copulatory behavior of the sexes of both the bisexual and the pseudogamous species was easily observed in isolated sexes over a long period (2-3 weeks). Then, males and females may emit signals during hours. Whenever males were introduced in the cage with the isolated females, they responded quickly to the calling signals of the females by alternately emitting their special type of signals, moving rapidly along the stem. Males first localized the females acoustically and when they were at 1-3 cm optical proximity from the female, that continued to perform abdomen vibrations, they would start courtship followed by copulation. It is likely that optical proximity is crucial for finding a female, since males failed to contact females when they moved along the other side of the stem. When there were more females on one stem or its leaves, males responded to the signals emitted by all females. Whenever the male came in close optical proximity to a given female it started courting songs and did not call back any more to the calling sounds of other females. The courted female would also stop calling.

Males of the two species exhibited a similar pattern of courtship, consisting of courtship songs with simultaneous rapid raising of their fore wings, which simultaneous acts increase in frequency until just before copulation. It was observed however, that this frequency was higher in *M. fairmairei* than in *M. brevipennis*. The duration of the courtship was variable and not different between species. In all observations copulation occurred when the females stopped their abdominal vibrations.

In test-tubes, where most of the observations were made, remarkable differences were found between the two bisexual species.

1. *Female starts calling, male responds.* In single pairs of male *M. fairmairei* and female *M. fairmairei* or *M. 2fairmairei-brevipennis*, calling songs emitted by the female were followed by pair forming which always led to copulation within a few minutes. In *M. brevipennis* calling songs of the females (especially virgin females) were followed by pair formation as well, but not always by copulation. Then the males would remain behind the females for a long time, courting them and attempting from time to time to copulate with the females which kept refusing them.

2. *Male starts calling, female responds.* Females of both species may take the initiative in pair-forming and doing so respond to a calling male. These females usually silently approached the males from behind until they were in close proximity. Then they started to emit signals or touched the male with their fore-legs. The male would respond by moving 1-2 cm

forewards and, after reversing his direction and coming around behind the female, would start courtship. Females of *M. fairmairei* and *M. 2fairmairei-brevipennis* would always copulate, while in *M. brevipennis* again this was not the case, even though in *M. brevipennis* females initiating pair forming were more frequent than in the two other species combinations. Thus, females of *M. fairmairei* and *M. 2fairmairei-brevipennis* that call for a male or respond to a calling male are always willing to copulate, while in *M. brevipennis* there seems to be a threshold to overcome between readiness to be courted and to copulate, especially in virgin females but undependingly of their age. It might be that *M. brevipennis* females require more acoustical stimulation by the males in order to overcome this threshold.

Copulation.

Copulation in both species occurred in a similar manner. Males intensify their courtship until females slightly rise their abdomen. Then the males rapidly turn about 180°, raise their last abdominal segments and with prolonged and spread parameres lead their aedeagus along the ovipositor into the female genital organ. Males may copulate either from the left or the right side. During copulation both sexes remain immobile.

Attempts to copulate may fail several times, as was observed frequently even with females ready to oviposit, recognizable from their swollen abdomens. Whenever the males had failed several times to copulate, the females refused to mate with the same male. Females of all species, which refused to copulate, did so in a similar manner: that is by raising their fore wings, by raising or moving their abdomen, by moving away or even by kicking the male. However, a few times males still succeeded in copulating with such females which then gave up their rejecting behavior.

Frequency of copulation.

There are interspecific differences in the frequency of copulation. Males of *M. fairmairei* may recopulate with a new partner or start a renewed courtship with the female they had just copulated with within a few minutes. It was noticed that one male copulated in a period of four hours with three virgin females (after each copulation the mated female was replaced by another virgin female). Males of *M. brevipennis* never renewed courtship with a female they had just copulated with, even if she remained in close proximity, but occasionally they might renew courtship or accept another virgin female within one observation period. After

copulation the females generally refused to copulate again. A few females however, of either species, copulated twice during a period of observation. Females of all species may copulate several times during their lifespan. A second copulation may occur before oviposition begins or is performed after the onset of it. There is no relation between the frequency of copulation and egg-fertility, which remains constantly 100% during the whole oviposition period of each female, even if she copulated only once. Thus, a once mated female can produce a full complement of fertile eggs (DROSOPoulos, 1977). During their lifespan, females of *M. fairmairei*

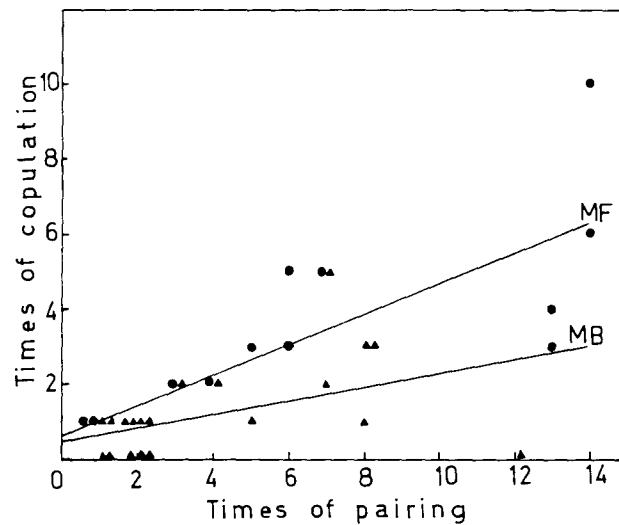


Fig. 3. Relation between times of pairing and times of copulation. Regression lines: ●, M.F., (*M. fairmairei*) $y = 0.69 + 0.400x$, $r = 0.771^{***}$; ▲, M.B., (*M. brevipennis*) $y = 0.49 + 0.181x$, $r = 0.447$.

show a tendency to copulate more frequently than *M. brevipennis*. Thus, whenever males were placed into tubes containing females which already had copulated (Times of pairing, Fig. 3), *M. fairmairei* females appeared to copulate significantly more frequently than *M. brevipennis* females ($F = 8.06^{**}$ df. 1 and 32). When a couple failed to copulate this could not be ascribed to unwillingness of the male, since males of either species, kept in separation for a few hours only, are always willing to copulate. The frequency of copulation could not be related to the age of either the female or the male, nor to the duration of previous copulations, the time interval after the previous copulation or to egg production.

TABLE 2. Duration of copulation (in minutes) of unmated females (A), of once mated females (B) and of more than two times mated females (C)

Number of observations	Mean \pm S.D.	Range
A. <i>M. fairmairei</i> ♀♀ \times ♂♂ (Dutch colony)		
23	18.96 \pm 6.87	9-43
<i>M. fairmairei</i> ♀♀ (Dutch colony) \times <i>M. fairmairei</i> ♂♂ (Greek colony)		
6	17.50 \pm 1.64	15-20
<i>M. fairmairei</i> ♀♀ \times ♂♂ (Greek colony)		
17	12.53 \pm 4.77	4-34
<i>M. fairmairei</i> ♀♀ (Greek colony) \times <i>M. fairmairei</i> ♂♂ (Dutch colony)		
6	8.50 \pm 5.24	4-17
<i>M. 2fairmairei-brevipennis</i> ♀♀ \times <i>M. fairmairei</i> ♂♂ (Greek colony)		
16	17.13 \pm 3.53	11-25
<i>M. brevipennis</i> ♀♀ \times ♂♂ (Dutch colony)		
38	32.97 \pm 14.29	9-68
B. <i>M. fairmairei</i> ♀♀ \times ♂♂ (Dutch colony)		
13	33.31 \pm 17.75	9-84
<i>M. brevipennis</i> ♀♀ \times ♂♂ (Dutch colony)		
14	40.71 \pm 21.13	16-83
C. <i>M. fairmairei</i> ♀♀ \times ♂♂ (Dutch colony)		
36	30.28 \pm 10.80	14-61
<i>M. brevipennis</i> ♀♀ \times ♂♂ (Dutch colony)		
11	41.00 \pm 20.50	14-73

Duration of copulation.

The duration of copulation was measured in colonies of *M. fairmairei* originating from a Greek population (Latitude °N = 38.40) and a Dutch one (Latitude °N = 52.45). As is shown in Table 2 the duration of copulation was very variable, yet pairs of virgin females and males of *M. fairmairei* originating from Holland copulated significantly longer than pairs of this species originating from Greece ($t = 3.49^{***}$), and significantly shorter than females of *M. brevipennis* ($t = 5.14^{***}$). No significant difference in duration of copulation was found between Dutch *M. fairmairei* and *M. 2fairmairei-brevipennis* ($t = 1.09$, $P > 0.05$). The second copulation showed a tendency to last longer than the first. This difference was somewhat more pronounced in *M. fairmairei* than in *M. brevipennis*, but in neither case it was statistically significant.

The copulations following the second one were not significantly longer than the second ($t = 0.58$, $P > 0.05$ for *M. fairmairei* and $t = 0.08$, $P > 0.05$ for *M. brevipennis*). In the second and following copulations there is no longer a significant difference in duration of the copulation between the Dutch population of *M. fairmairei* and *M. brevipennis*.

The duration of copulation is somehow controlled by the females, because when in *M. fairmairei* Dutch females copulated with Greek or Dutch males the duration of the copulation was not significantly different ($t = 0.92$, $P > 0.05$). The same was true for Greek females copulating with either Greek or Dutch males ($t = 1.66$, $P > 0.05$, Table 2). It was indeed observed that the copulation was terminated by the female, since at a certain moment the female rose her abdomen and a few seconds afterwards the pair separated. In cases where the males still remained in copula, the females tried to displace them with their hind legs or other active movements.

A factor influencing the duration of copulation appeared to be the time interval between copulations (Figs 4, 5). Especially in *M. fairmairei* the duration of the copulation increased significantly when the time intervals between two copulations increased.

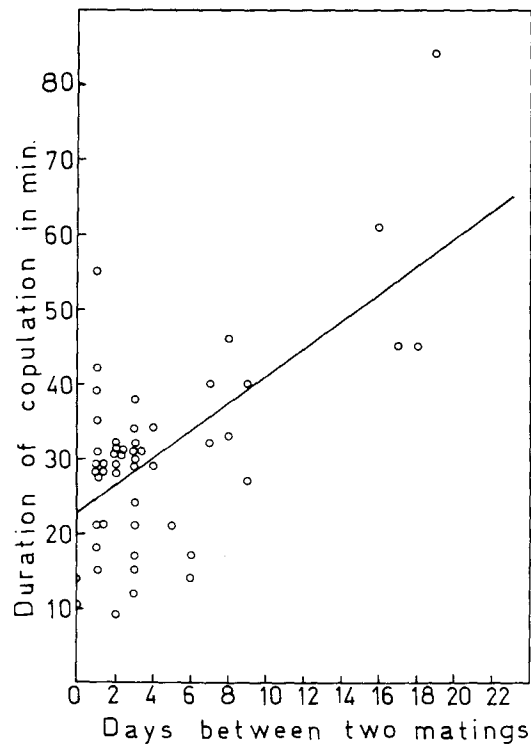


Fig. 4. *M. fairmairei*: Relation between days between two matings and duration of copulation ($y = 22.79 + 1.845x$, $r = 0.629^{***}$).

Post-copulatory behavior.

After copulation, males of *M. fairmairei* moved away, while males of *M. brevipennis* usually remained immobile behind the females. In general, males of *M. fairmairei* started mating behavior with another female in considerably shorter time than *M. brevipennis*. Females of all species may remain immobile, but in case they were ready for oviposition they were often observed to search for oviposition sites and could start ovipositing within 5-10 minutes after copulation.

Competition.

Experiments were carried out in test-tubes by caging one individual of one sex with more than one of the other. In no case did any of the excess females caged with one male disturb a pair in copula. The same was observed when several males of *M. brevipennis* were caged with one

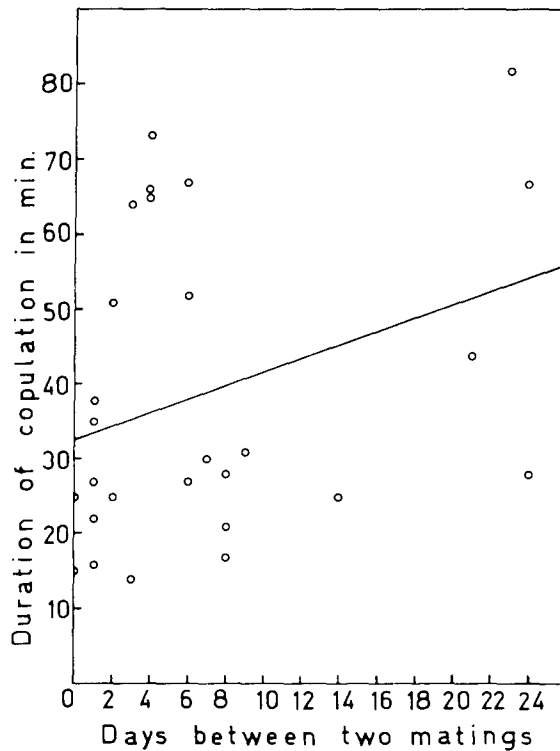


Fig. 5. *M. brevipennis*: Relation between days between two matings and duration of copulation ($y = 32.61 + 0.913x$, $r = 0.334$, $P > 0.05$).

female. However, when two males of *M. fairmairei* were caged with one female competition between the males occurred. Thus when a virgin female was introduced into a test-tube having two males (which had emerged on the same date, but happened to be different in colour and were easily distinguishable), only one male (the darker) copulated, even when the other had already started courtship with the female. During this copulation the light male continued his courtship activities and pushed the copulating male violently. During the copulation and even afterwards the non-copulating male was actively refused by the female, while the male in copula took no action against it. The refusal of the female in copula was performed in the same way as when not being in copula. The same phenomenon was observed five more times when other females, one per day, were introduced to the test-tube with these two males. Competition between these two males did not occur after the dark male had copulated within two hours with two different females. After that period, when a third female was introduced into the test-tube, it copulated with the light male without being disturbed by the dark one. These data suggest that there is a struggle for mating between the males of *M. fairmairei*.

TABLE 3. Interspecific mating behavior between *M. fairmairei* (*M.f.*) and *M. brevipennis* (*M.b.*) and between pseudogamous females (3n) *M. 2fairmairei-brevipennis* (*M. 2f-b.*) with males of *M.f.* and *M.b.* during three days of observation (3-4 hours per day)

Number of crossed ♀ × ♂	Number of courtships	Number of copulations	Duration of copulations in min.
8 ♀♀ <i>M.f.</i> × 8 ♂♂ <i>M.b.</i>	21	2	0.5, 5
11 ♀♀ <i>M.b.</i> × 11 ♂♂ <i>M.f.</i>	15	1	3
12 ♀♀ <i>M. 2f-b.</i> × 12 ♂♂ <i>M.f.</i>	12	12	11-25
10 ♀♀ <i>M. 2f-b.</i> × 10 ♂♂ <i>M.b.</i>	26	3	0.5, 4, 19

Pair crossings. Once copulated females were not crossed again.

Interspecific mating behavior.

All combinations of crossings between the two bisexual species and between each of the bisexual species and the pseudogamous triploid "species" *M. 2fairmairei-brevipennis* were made. Hybrid males and females of the F₁ can copulate with each other and with the parental species. Observations on interspecific mating behavior were made as follows: virgin females, 6-10 days old, originating from the same popula-

TABLE 4. Crossings between *M. fairmairei* (*M.f.*), *M. brevipennis* (*M.b.*) and *M. 2fairmairei-brevipennis* (*M.2f-b.*) originating from populations from Holland (H) and Greece (G)

Number of crossings ♀♀ × ♂♂	No ♀♀ with sperm in spermatheca	Egg-production (fertility %)	Adult progeny
A. 1. <i>M.f.</i> (G) 10 ♀♀ × <i>M.b.</i> (H) 10 ♂♂	3		
2. <i>M.b.</i> (H) 8 ♀♀ × <i>M.f.</i> (G) 8 ♂♂	2		
3. <i>M.b.</i> (H) 8 ♀♀ × <i>M.f.</i> (H) 8 ♂♂	2		
4. { <i>M.2f-b.</i> (H) 6 ♀♀ + <i>M.b.</i> (H) 6 ♀♀} × <i>M.b.</i> (H) 6 ♂♂	3 <i>M.2f-b.</i> 6 <i>M.b.</i>		
5. { <i>M.f.</i> (H) 6 ♀♀ + <i>M.b.</i> (H) 6 ♀♀} × <i>M.b.</i> (H) 6 ♂♂	3 <i>M.f.</i> 6 <i>M.b.</i>		
6. { <i>M.b.</i> (H) 6 ♀♀ + <i>M.f.</i> (H) 6 ♀♀} × <i>M.f.</i> (H) 6 ♂♂	3 <i>M.b.</i> 6 <i>M.f.</i>		
B. 1. { <i>M.f.</i> (H) + <i>M.2f-b.</i> (H)} 9 ♀♀ × <i>M.b.</i> (H) 9 ♂♂	7	1544 (38.5%)	
2. <i>M.b.</i> (H) 9 ♀♀ × <i>M.f.</i> (H) 9 ♂♂	2	1668 (6.8%)	
C. 1. <i>M.f.</i> (G) 40 ♀♀ × <i>M.b.</i> (H) 44 ♂♂			44
2. <i>M.f.</i> (H) 60 ♀♀ × <i>M.b.</i> (H) 66 ♂♂			105
3. <i>M.b.</i> (H) 30 ♀♀ × <i>M.f.</i> (G) 33 ♂♂			26
4. <i>M.b.</i> (H) 42 ♀♀ × <i>M.f.</i> (H) 42 ♂♂			294
5. <i>M.2f-b.</i> (H) 12 ♀♀ × <i>M.b.</i> (H) 12 ♂♂			166
6. <i>M.2f-b.</i> (H) 5 ♀♀ × <i>M.f.</i> (G) 5 ♂♂	5		1531
7. <i>M.f.</i> (H) 4 ♀♀ × <i>M.f.</i> (G) 4 ♂♂	4		246

A. interspecific crossings made for testing successful copulation of females within a period of one month after adult moult. B. interspecific pair - crossings for testing egg-fertility. C. interspecific crosses for testing adult progeny. (C. 6,7 pair-crossings).

tions used in intraspecific experiments, were placed together with males of the other species and observed for 3-4 hours per day. Data, partly presented in Table 3, revealed the following: 1. All females of all species come in contact with males of the other species acoustically in the same way, both quantitatively and qualitatively, as in intraspecific crosses. 2. Females were not observed to refuse interspecific courtship. In fact they always accepted the male. 3. However, many efforts of the males to copulate were unsuccessful and if they were successful they resulted in very short copulations (0.5-5 minutes). The cross between female *M. 2fairmairei-brevipennis* and male *M. fairmairei* is not distinguishable from an intraspecific *M. fairmairei* cross. Each pairing was followed by copulation of normal duration. 4. Males and females of *M. fairmairei* showed more activity in trying to find a partner than *M. brevipennis* as is also seen in intraspecific crosses.

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In order to investigate further the consequences of interspecific mating behavior more crossings were made. Of these crossings either the spermatheca of the female was dissected in order to test whether the female had been successfully inseminated (Table 4,A.), or the egg fertility during the oviposition period was followed at intervals of five days (Table A., B.; Fig. 6), or the adult progeny was measured (Table 4,C.). The females of which the egg fertility or the adult progeny were measured were later dissected as well in order to check whether their spermatheca contained active sperm. In addition, crossings between allopatric (greek-dutch) or sympatric (dutch) populations of the species were made.

The results of the crossings made for testing successful copulation revealed the following: 1. A few females may copulate successfully with males of the other species. However, large quantities of sperm were never observed in the spermatheca of these females in contrast with females of the same species as the males when presented simultaneously with the interspecific female (Table 4, A.). Thus males of one species do not adequately inseminate the females of the other species. 2. In general, more females of *M. fairmairei* and *M. 2fairmairei-brevipennis* successfully copulated interspecifically than females of *M. brevipennis* (Table 4, A., B.,C.). 3. There was no difference in the number of mated females of *M. brevipennis* when crossed with sympatric or allopatric males of *M. fairmairei* (Table 4, A. 2-3). 4. Obviously some females of one species may mate even when they were caged with males and females of the other species (Table 4, A. 4-6).

Egg-fertility in interspecific crosses was not constant, but it fluctuated rapidly during the oviposition period (Fig. 6). This phenomenon demonstrated that interspecific inseminations, when they occurred, were not complete. Interspecific copulations of *M. fairmairei* and *M. 2fairmairei-brevipennis* females can be more than one (2-4) as indicated by the course of the egg-fertility during the oviposition period. This phenomenon was not present in crosses between females of *M. brevipennis* with males of *M. fairmairei*, resulting in a lower egg-fertility than that in the reciprocal crosses (Table 4, B.). But then also in intraspecific crosses *M. brevipennis* females copulate less frequently than females of *M. fairmairei*.

Hybrids could be obtained by all combinations of interspecific crosses. The number of produced progeny was variable, but comparatively much smaller than that produced by intraspecific crosses. Evidently that was due to the low egg-fertility and to the large embryonic mortality. Larval mortality did not occur (DROSOPoulos, 1977). The number of hybrids produced was not different in interspecific crosses between either sym-

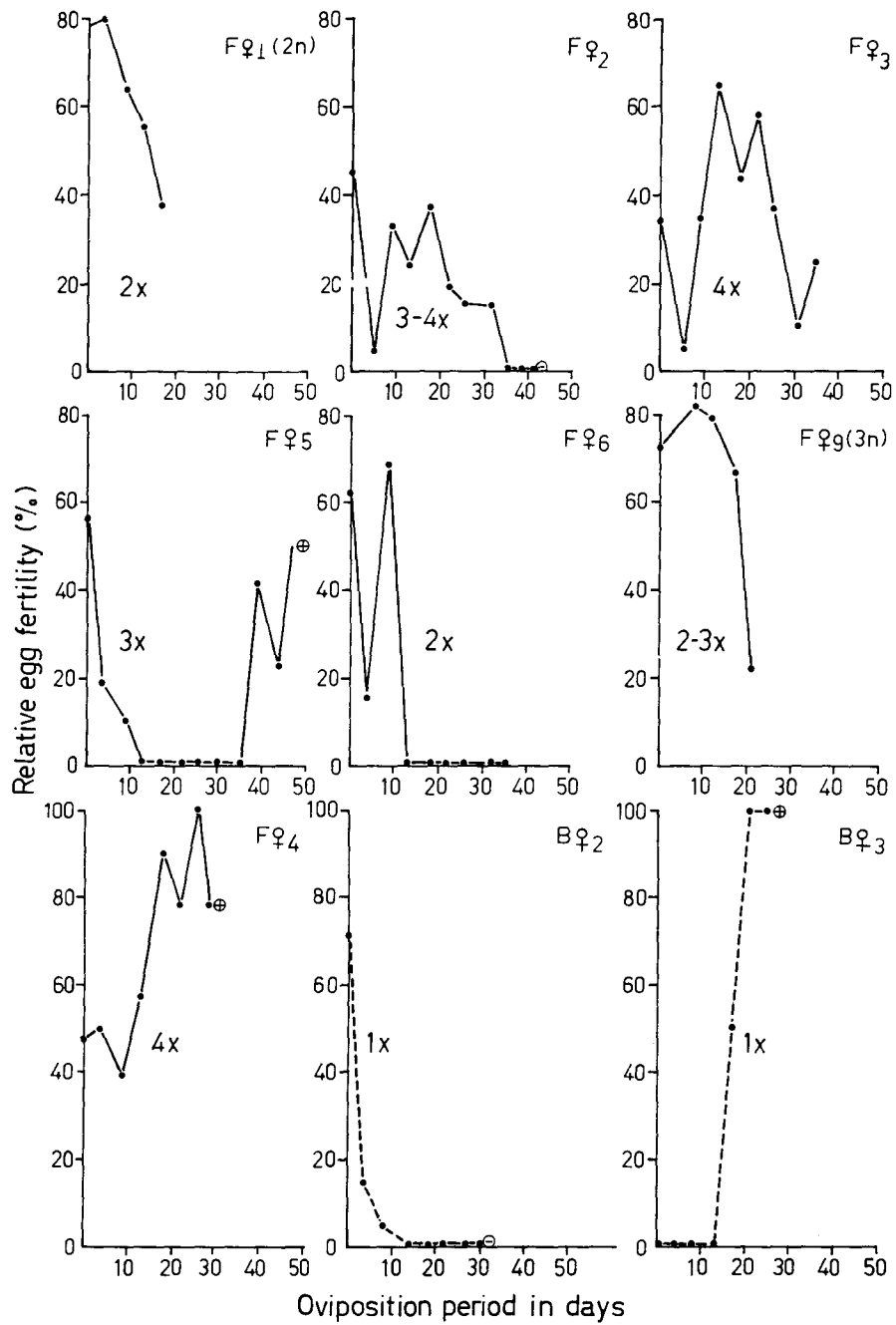


Fig. 6. The fertility of eggs deposited by each female of *M. fairmairei* (solid line) and *M. brevipennis* (dashed line) during its oviposition period, when each female was reared in a test tube together with a male of the other species. The symbols (⊕, ⊖) indicate whether females had or had no sperm in their spermathecae, respectively; x indicates times of possibly successful copulations.

patric or allopatric populations of the two bisexual species (Table 4, C. 1-4). Pseudogamous and *M. fairmairei* females could copulate readily with allopatric males and produce large progeny (Table 4, C. 6-7). On the average pseudogamous females produced larger progeny than females of *M. fairmairei* when they were crossed with males of *M. brevipennis* (Table 4, C. 2, 5), but also in intraspecific crosses they have a higher reproduction than *M. fairmairei*.

All hybrid females back-crossed with males of *M. fairmairei* or *M. brevipennis* had more sperm in their spermatheca than the interspecifically crossed females (DROSOPoulos, 1978). In addition, the few adults produced by crossing the pseudogamous females of hybrid origin with males of *M. brevipennis* produced large (normal) progeny when they were "back-crossed" with males of *M. fairmairei*, but again low progeny with *M. brevipennis* males (apomictic reproduction) (DROSOPoulos, 1977). These data suggest that existing differences in the male genitalia of the two species (DROSOPoulos, 1977) contribute largely in preventing interspecific crosses. Indeed, hybrid males (F_1), possessing intermediate characters in the structures of their genitalia, could copulate more easily with their parental species and pseudogamous females than males of the parental species in interspecific crosses. The same seems to hold for the females of hybrid origin, but female genitalia have not been studied so far.

Discussion

The data presented in this paper in combination with other reports (DROSOPoulos, 1975, 1976, 1977, 1978) allow us to make some reasonable conclusions regarding the evolutionary relations between the two bisexual species of *Muellerianella* as well as the evolutionary relationships between the two bisexual species and the pseudogamous triploid "species".

Regarding the two bisexual *Muellerianella* species, as stated before, food-plant preference acts as the main isolation mechanism. In the second place morphological differences in the male and probably also in the female genitalia contribute greatly to prevent hybridization. Although bioacoustic differences were found, they appeared to be insufficient to prevent bioacoustic interspecific understanding. This is not surprising since it was already known that virgin females of one species mated when caged with males and females of the other species (DROSOPoulos, 1978). Furthermore, interspecific understanding was also observed in two other

delphacid species in spite of bioacoustic differences between them (STRUBING & HASSE, 1975).

Other differences in mating behavior found between *M. fairmairei* and *M. brevipennis* such as the onset of sexual maturity, the form of pre-copulatory behavior, duration and frequency of copulation are more probably due to differences in environmental influences rather than that they are the result of reproductive isolation. These differences are in conformity with other biological differences reported previously (DROSPOULOS, 1977). Indeed, *M. brevipennis* occurs in more northern habitats and is adapted to a longer photoperiod and lower temperatures, whereas *M. fairmairei* is more distributed to the South (DROSPOULOS, 1977; BOOIJ, 1981). These northern conditions have established a stronger egg-diapause (regarding initiation, duration and termination), lower rates of development and reproduction and shorter seasonality in *M. brevipennis* than in *M. fairmairei*. In Greece the latter breeds almost during the whole year (DROSPOULOS, 1982). Thus environmental conditions have established analogous physiological and behavioral requirements which probably are associated with the place where speciation occurred. It was assumed (DROSPOULOS, 1977) that *M. brevipennis* originated from an isolated population of *M. fairmairei* which split from the original stock and became allopatric by glaciation. 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 of its ancestors. According to this hypothesis it is likely, that *M. brevipennis* developed behavioral differentiation during its isolation, what is supported by the fact that it did not show differences in crossings either with sympatric or allopatric populations of *M. fairmairei*. Furthermore, sympatric speciation is correlated with strong pre-mating isolation. Conclusively, existing differences in acoustic communication and mating behavior between the two bisexual species of *Muellerianella* are associated with ecophysiological adaptations and do not function as isolating mechanisms between these two species. These conclusions are supported also by recent work on other arthropods (WHITESELL & WALKER, 1978; JANSSON, 1979a, b; KILIAS, ALACHIOTIS & PELECANOS, 1980).

Regarding the pseudogamous triploid "species" *M. 2fairmairei-brevipennis* which in Holland coexists with *M. fairmairei*, it is behaviorally not distinguishable from females of *M. fairmairei*. This "species" can be

identified only by chromosome number and its gynogenetic mode of reproduction. Although it contains one genome of *M. brevipennis*, no difficulties in preventing matings with *M. fairmairei* males occur. Indeed, this "species" is competing reproductively for males produced by females of another species. However, because of the ability of males to copulate several times during their lifespan, there is probably no struggle for sperm requirements between the females of the two coexisting species. Unfortunately, there were no experiments performed which might test whether males of *M. fairmairei* originating from Holland (where the natural ratio of males to females is 1:3) possess a different mating frequency from males originating from Greece, where the sex-ratio in field populations is 1:1. However, during three years of laboratory observations no peculiar phenomena were observed which might suggest that there are any capacity differences between males of *M. fairmairei* from different populations. Thus, Greek males mated as easily with diploid and triploid females from Holland, despite some small differences in calling songs. Matings with *M. brevipennis* were also possible but were not as successful as those with *M. fairmairei*.

Regarding interspecific matings, it is likely that mechanical barriers to copulation (*e.g.*, differences in structure of the genitalia) are more efficient than courtship differences. What was observed during the experimental interspecific crosses was, that some females could not copulate with a given male, but could occasionally with another male. This might be a reasonable explanation why experimental synthesis of the pseudogamous triploid *M. 2fairmairei-brevipennis* was performed only twice out of 12 trials. However, in the field it never failed to be collected from habitats where the parental species coexist. Thus, the field conditions must be more favourable for its creation than the laboratory, due to the high frequency of random hybridizations that are not prevented in dense mixed populations of *M. fairmairei* and *M. brevipennis*. This implies a greater chance under field conditions for the establishment of a favourable gene assortment of chromosomes contributed by the two parental species which are producing the pseudogamous "species" through hybridization. In associations of bisexual-unisexual species of the salamander *Ambystoma* and the fish *Poeciliopsis*, similar to that of *Muellerianella*, each of the bisexual species, evidently, is "contaminated" by a triploid pseudogamous "species" containing two genomes of the bisexual species with which it coexists (UZZELL, 1964; SCHULTZ, 1969). The absence, so far, of a triploid *M. 2brevipennis-fairmairei* might be due to incomplete investigations in discovering this organism.

Recently another bisexual species *M. extrusa* has been found to coexist in Greece with another pseudogamous "species" *M. 2extrusa-fairmairei* (DROSPOULOS, 1983, in prep.). This pseudogamous species is evidently of hybrid origin between the bisexual species *M. fairmairei* and *M. extrusa*, species which morphologically differ less than *M. fairmairei* and *M. brevipennis*. Indeed, preliminary crossing experiments between these pseudogamous females with males of *M. fairmairei* (which in Greece is a "pure" bisexual species) were made more readily and produced larger progeny than crosses between *M. 2fairmairei-brevipennis* and males of *M. brevipennis*.

All evidence reported here in combination with other, first published elsewhere, suggest that several isolation mechanisms are functioning between the not yet fully genetically differentiated bisexual species of *Muellerianella*. However, none of them is strong enough to prevent hybridization and pseudogamy acts as the final isolation mechanism since the apomictical reproduction of the triploid pseudogamous "species" prevents a further gene flow from the one parental species to the other (DROSPOULOS, in prep.).

Summary

Some data on acoustic communication and mating behavior of two biparentally reproducing species and the clonally reproducing pseudogamous "species" of the genus *Muellerianella* are reported. Although bioacoustic differences were found in the calling songs between the species, these did not prevent pairforming. Also, differences in mating behavior, such as pre-copulation behavior, courtship activities, frequency and duration of copulation were not sufficient to prevent successful hybridization between both the two biparentally reproducing species and between each of these two species and the pseudogamous "species".

The data reported here are related to other biological differences reported previously. According to these data there is some evidence that differences in acoustic communication and mating behavior between the two species are established by ecological influences which in turn have established analogous physiological requirements. These differences are rather weak isolating mechanisms. Regarding the behavioral relation of the pseudogamous species with males of the two parental species it was found that these females behave exactly as the females of *M. fairmairei* with which they coexist in the field.

In interspecific crosses mechanical barriers to copulation are more efficient than courtship differences.

Finally it is assumed that pseudogamy is a strong isolation mechanism between the not yet fully genetically differentiated bisexual species of *Muellerianella*.

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