

THE IMPORTANCE OF ZOOGEOGRAPHIC AND ECOLOGICAL INVESTIGATIONS IN STUDYING SPECIATION IN CLOSELY RELATED SPECIES AND THEIR PSEUDOGAMOUS ASSOCIATIONS.

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In the previous Congress of 1981 the delphacid fauna of Greece was compared to that of the adjacent countries (DROSOPOULOS, 1983a). In the meantime the Greek planthopper fauna was further investigated, so that now the ecology and distribution of many species is adequately known (DROSOPOULOS, ASCHE and HOCH. in press a). There are certain areas in Greece very rich in species. An exciting example of such an area is the Prespa Lakes area (N.W. Greece), where about half of the number of species occurring in Greece (112) were found (DROSOPOULOS, ASCHE and HOCH. in press b). Apart from the faunistic aspect of this family, special attention was paid to the investigation of the ecology and distribution of some species complexes with apparent unbalanced sex-ratios. In particular the *Muellerianella* and *Ribautodelphax* complexes were studied extensively on populations collected from many localities in western Europe. However, Greece possesses a special geographic position with respect to these species, since the southern boundaries of their distribution areas are in this country.

For the *Muellerianella* complex it was found, that one species *M. fairmairei* had a high proportion of females (75-100%) in Holland and central Europe, while in Greece and some other mediterranean areas it displaces a normal sex ratio (1:1) (DROSOPOULOS, 1976, 1977). On the contrary, the closely related species *M. brevipennis* always has a normal sex ratio of 1:1 in central Europe and also in Greece, as was found recently. Outside of the borders of Greece these two species are widely sympatric (Fig. 1). A third species *M. extruda* was found later in north-western Greece, mainly on the grass *Molinia caerulea* but also on other grass species (BOOIJ, 1982; DROSOPOULOS, 1983). In that area *M. extruda* is sympatric with *M. fairmairei* (Fig. 2). In contrast to

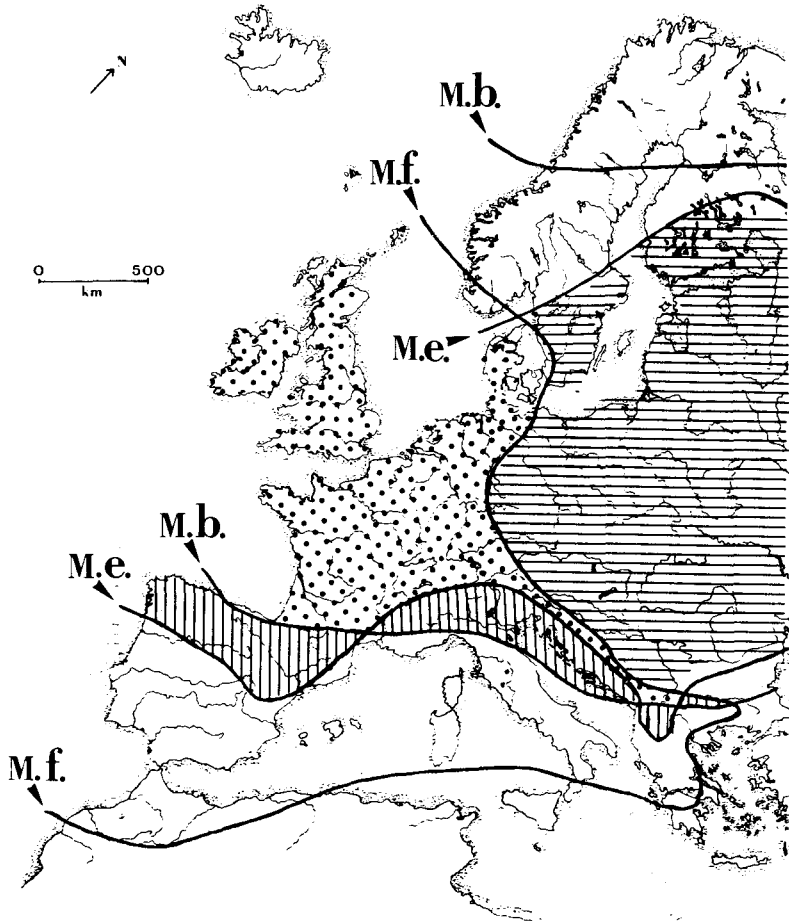


Fig. 1. Distribution of *Muellerianella fairmairei* (M.f.), *M. brevipennis* (M.b.) and *M. extrusa* (M.e.) in western Europe. Regions with solid circles indicate the sympatric area of the three species; regions with vertical lines indicate the sympatric area of *M. fairmairei* with one of the other two species; regions with horizontal lines indicate the sympatric area of *M. extrusa* and *M. brevipennis*.

the situation of *M. fairmairei* - *M. brevipennis* mentioned before, in this case *M. fairmairei* had a normal sex ratio and *M. extrusa* a high proportion (90-100%) of females.

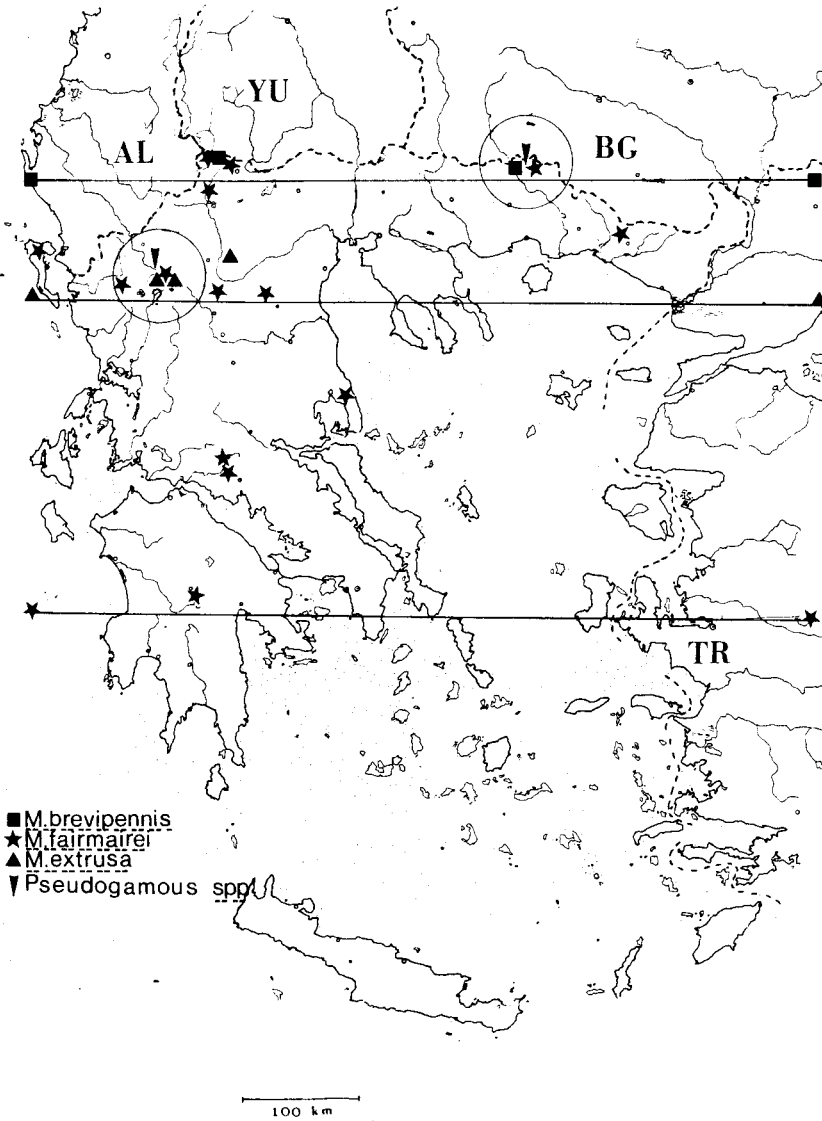


Fig. 2. Distribution of the *Muellerianella* species within the area of Greece. In circles the areas where pseudogamous triploid females were found until now. *M. extrusa* indicated with a question mark refers to a population feeding on *Carex divulsa*.

By studying several aspects of the biology of these species complexes (Table 1), it is now well known that these high proportions of females are due

TABLE 1
Differences between two *Muellerianella* species

| Characteristics | <i>M. fairmairei</i> | | <i>M. brevipennis</i> |
|------------------------------------------------------------|--------------------------------------------|----------------------------|-------------------------------------|
| | Bisexual | Unisexual | bisexual |
| 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 | <i>Holcus lanatus</i> and <i>H. mollis</i> | | <i>Deschampia caespitosa</i> |
| Food plants in lab | oligophagous | | monophagous |
| Oviposition plants (long day) | <i>H. lanatus</i> | | <i>D. caespitosa</i> |
| (short day) | <i>J. effusus</i> | | <i>D. caespitosa</i> |
| Habitats | common in wet natural meadows | | stenotopic in wooded places |
| Phenology (in Holland) | bivoltine | bivoltine | partly bivoltine |
| (in Greece) | polyvoltine | - | univoltine |
| 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 period | | 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 |
| Acoustic communication (signals) | | ♂ different ♀♀ the same | ♂♂ different ♀♀ little different |
| Mating behavior: | | | |
| 1. Frequency of copulation | | higher | lower |
| 2. Duration of copulation | | shorter | longer |
| 3. Interspec. crossings | | ♀♀ easier | ♀♀ difficult |

to the fact that two morphologically undistinguishable types of females coexist. One of these two types are diploid normal females, producing females and males in equal proportion. The other type of females are triploid and pseudogamous (not parthenogenetic), reproducing again triploid pseudogamous females, but only after copulation with males produced by the normal diploid females, since copulations with sterile hybrids are not effective (DROSOPOULOS, 1976, 1977, 1978). Sperm therefore is necessary to initiate embryogenesis but it does not contribute hereditary material to the egg nucleus of the pseudogamous females. This enigmatic and paradoxical phenomenon in biology is difficult to detect in invertebrates, since pseudogamous females usually are not different morphologically from the normal females they are coexisting with. Only tedious caryological analysis of the egg nucleus reveals the presence of these organisms (Fig. 3). Thus, although there has been very little evidence for finding pseudogamy in other delphacid complexes, again triploid pseudogamous females were found to coexist with a diploid species of the genus *Ribautodelphax* (DEN BIEMAN, 1981). In addition to this, a triploid parthenogenetic species of the genus *Delphacodes* was recently discovered near the Prespa Lake (N.W. Greece) (COBBEN, DE VRIJER, DEN BIEMAN, personal communications). There is finally strong evidence that pseudogamy might be the case in other Auchenorrhyncha species complexes where already species consisting of many females and very few males have been recorded (e.g. Typhlocybrids).

In other organisms pseudogamy (or gynogenesis) has also been found, both among invertebrates (e.g. fresh water triclads, BENAZZI and LENTATI, 1966; the genus *Lumbricillus* (Oligochaeta), CHRISTENSEN and O'CONNOR, 1958; ptinid beetle, MOORE, WOODROFF and SANDERSON, 1956) and in vertebrates (e.g. fishes, SCHULTZ and KALLMAN, 1968; SCHULTZ, 1969; salamanders, MACGREGOR and UZZELL, 1964). These findings suggest that pseudogamy is not associated with a particular group of organisms, but is widely spread among various groups of animals. In addition there are indications that in several cases of pseudogamy the phenomenon was abusively interpreted as embryonic or larval male mortality or as an unbalanced sex determining mechanism etc.

The studies on *Muellerianella* and *Ribautodelphax* have the advantage over other similar cases, that there are detailed studies on many aspects of the biology and ecology of both the unisexual and the bisexual species. Concerning the *Muellerianella* complex there are several reports covering this topic (DROSOPOULOS, 1976, 1977, 1978, 1983, in press b; BOOIJ, 1981, 1982a, 1982b). The main differences between the species studied are shown in Table 1. For the *Ribautodelphax* complex studies are in process (DEN BIEMAN, 1981, personal communications). These biological differences between the species of

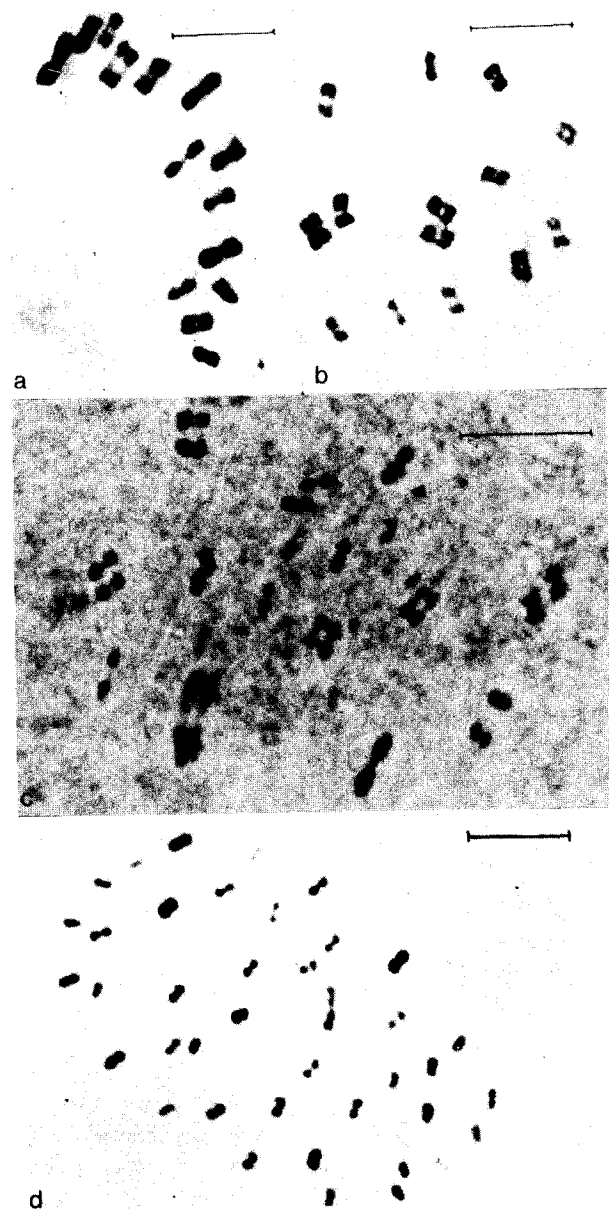


Fig. 3. The chromosome complements at metaphase I in the nucleus of ovarian eggs of *M. fairmairei* (a), *M. brevipennis* (b), hybrid between *M. fairmairei* ♀ X *M. brevipennis* ♂ (c), and a triploid pseudogamous «species» (d) which is coexisting with *M. fairmairei* (*M. 2fairmairei-brevipennis*). Each bar represents 10 μ m.

these complexes, especially those concerning their distribution and ecology, allow us to investigate the origin of pseudogamy and its role in speciation. As mentioned, Greece possesses an important geographic position where the borderlines of the distribution of these species overlap. The distribution borderlines of the three bisexual species of *Muellerianella* are shown in Fig. 2, and their food plants are presented in Table 2 according to the data resulting from six

TABLE 2
Food plants of the known *Muellerianella* species within the area of Europe and Greece.

| (Bisexual species) | Europe | Greece |
|--------------------------------------------------------------------------|-------------------------------|----------------------|
| <i>M. brevipennis</i> | <i>Deschampsia caespitosa</i> | <i>D. caespitosa</i> |
| <i>M. fairmairei</i> | <i>Holcus lanatus</i> | <i>H. lanatus</i> |
| | <i>H. mollis</i> | |
| <i>M. extrusa</i> | <i>Molinia caerulea</i> | <i>M. caerulea</i> |
| <i>M. extrusa</i> (forms) | various grass spp. | <i>Carex divulsa</i> |
| (Pseudogamous species) | | |
| <i>M. 2 fairmairei-brevipennis</i> | <i>Holcus lanatus</i> | |
| | <i>H. mollis</i> | <i>H. mollis</i> |
| <i>M. 2 extrusa-fairmairei</i> | various grass spp. | <i>Carex divulsa</i> |
| <i>M. 2 brevipennis-fairmairei</i> or <i>M. 2 brevipennis-extrusa</i> | <i>D. caespitosa</i> | |

years of investigations. Triploid pseudogamous species occur only in those areas where two bisexual species are closely sympatric. That is pronounced in Miliotades (600m), in Epirus, where *M. fairmairei* and *M. extrusa* occur syntopically (DROSOPOULOS, 1983). Close to this area on the mountain of Vourinos (1200 m), where *M. extrusa* was found on *Molinia caerulea* and where no other *Muellerianella* species occurs, pseudogamous females were not found. The second area, where triploids were found was in the Rhodopi mountain range, where *M. fairmairei* and *M. brevipennis* are sympatric. In that area triploids were collected in one locality on *Holcus mollis* and in the other on *H. lanatus*, the food plants of *M. fairmairei*. It must be mentioned here that in the former locality where *M. brevipennis* occurred in high population densities on *Deschampsia caespitosa*, males of *M. fairmairei* could not be found. It is possible, however, that males would be found if more intensive collections were made, as the author experienced from other collecting sites in Europe. In the second locality, males and a mixture of pseudogamous and bisexual females were found (DEN BIEMAN, personal communication).

These new data are in conformity with previous ones from western Europe. Then the hybrid origin of pseudogamy was supported. In further studies of the *Muellerianella* complex BOOIJ (1981) also supported hybrid origin. He did not, however, exclude the possibility that pseudogamy could arise by spontaneous formation of a diploid egg nucleus which could establish a triploid female after fertilization with a haploid sperm nucleus. On the other hand there are several theories in literature which support the spontaneous origin in general of parthenogenesis or theletoky (e.g. GUELLAR, 1974). Another suggestion is, that theletoky does not occur in Auchenorrhyncha since this group of insects is very mobile and adaptive to a wide variety of environments, in contrast to non-mobile scale insects where theletoky is common (WHITE, 1973). Finally SUOMALAINEN (1969) considered parthenogenesis more common in organisms adapted to cold climates. The above examples from literature emphasize the importance of theories on the origin of parthenogenesis. Focussing on the case of pseudogamy (which is also a way of apomictic reproduction) found in invertebrates, we have the following arguments against the spontaneous origin of pseudogamy:

- a. The spontaneous production of a diploid egg nucleus which can establish a triploid female is not impossible, but can not explain how pseudogamic reproduction arises (incomplete pairing of chromosomes in F_1 hybrids and pseudobivalents in the pseudogamous triploid egg nucleus).
- b. If pseudogamous females are an adaptation to cold climates, they should not be found in low altitudes in Greece (Miliotades, 600 m).
- c. Of course theletoky cannot be prevented in Auchenorrhyncha because they are mobile and adaptive to a wide variety of environments.
- d. Pseudogamy was never reported in populations of one isolated species or in monotypic genera.

On the other hand, the hybrid origin of pseudogamy is supported in vertebrates with reliable arguments (SCHULTZ, 1969; UZZEL, 1964), but not in invertebrates. However the case of *Muellerianella* and probably that of *Ribautodelphax* suggests that pseudogamy is not a natural event or an error, but that it has a role and a function in speciation of closely or very closely sympatric species which are differentiating from each other on different host plants. The whole situation was experimentally proved by the synthesis of pseudogamous *Muellerianella* females (DROSOPOULOS, 1978). Unfortunately the synthesis could not be repeated so far, either by the author or by BOOIJ (1982a). Indeed, DROSOPOULOS (1978) demonstrated how a diploid nucleus with incomplete pairing of its chromosomes will give rise to the formation of pseudobivalents (Fig. 3).

In addition ecological and biogeographic studies showed, that natural hybridization is possible, since there are many localities where the specific food

plants of each bisexual species are growing in a mixture. There, as was shown in similar laboratory food plant associations, these species come in contact with each other. And since there is no strong reproductive isolation between the species, they produce hybrids (DROSOPOULOS, in press b). The F_1 female hybrids range from fully fertile till sterile and can copulate again with the parental males, thus producing triploid pseudogamous females. Naturally, if pseudogamy was not established, there would be continuous gene flows between the closely related species, that would interfere with speciation. This is avoided by the triploid pseudogamous females which, once produced, lead the gene flow between the parental species into another canal or dead track.

Therefore, pseudogamy is always associated with species complexes containing very closely related species, some of which are difficult to distinguish morphologically, especially the females.

Conclusively existing data support the theory that pseudogamy is a product of hybridization between closely related species and functions as a strong isolation mechanism within the sympatric area of these species.

Περίληψη

Μερικά πρόσθετα οικολογικά και ζωογεωγραφικά δεδομένα από τον Έλλαδικό χώρο, όπου απαντώνται οι όριακοί πληθυσμοί από μερικά σύμπλοκα «συγγενών», και «ψευδογαμικών» ειδών φυτοτέτιγων Ευρωπαϊκής ή Ευρωσιβηρικής εξάπλωσης, συνεισφέρουν στη γνώση της προέλευσης και λειτουργίας του παράδοξου φαινομένου της «ψευδογαμίας».

Με βάση τα δεδομένα αυτά υποστηρίζεται ή θεωρία του συγγραφέα ότι η «ψευδογαμία» είναι προϊόν υβριδισμού μεταξύ συγγενών ειδών και λειτουργεί σαν ένας πολύ καλός «μηχανισμός απομόνωσης» αποτρέποντας έτσι «γονιδιακή ροή» μεταξύ των ειδών αυτών, τα όποια όπως έχει πειραματικά αποδειχθεί διασταυρώνονται πολύ εύκολα.

Résumé

Quelques données additionnelles sur l'écologie et la zoogéographie provenant de la Grèce, où se trouvent les limites sud de quelques complexes «voisins» et «pseudogamiques» des espèces de cicadelles d'origine européenne et eurosibérienne, contribuent à la connaissance de l'origine et de la fonction du phénomène paradoxal de la «pseudogamie».

Sur la base de ces données l'auteur soutient la théorie que la «pseudogamie» est un produit d'hybridation entre espèces voisines et fonctionne comme un très bon «mécanisme d'isolement» qui empêche le «flux génique» entre ces espèces, lesquelles se croisent très facilement, comme on a déjà constaté expérimentalement.

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