between density-dependent, frequency-dependent, and soft selection as elaborated by Wallace (1975). Substantative support for Gromko's critique may be found in DeBenedictis (1977c).

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LABORATORY SYNTHESIS OF A PSEUDOGAMOUS TRIPLOID "SPECIES" OF THE GENUS *MUELLERIANELLA* (HOMOPTERA, DELPHACIDAE)

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Triploid pseudogamous biotypes or "species" are those which are closely related to two or more biparentally reproducing species. The males are necessary to provide sperm for activiation of development but they do not contribute hereditary material

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to the egg nucleus of the pseudogamous form. Examples are known both among vertebrates (Schultz and Kallman, 1968; Schultz, 1969; Macgregor and Uzzell, 1964) and invertebrates (Moore et al., 1956) including the leafhopper *Muellerianella-2-fairmairei-brevipennis* (Drosopoulos, 1976).

Previous ecological, morphological, cytological, and biochemical studies indicated a hybrid origin of naturally occurring pseudogamous triploid forms. There are no reports, however, of successful exper-
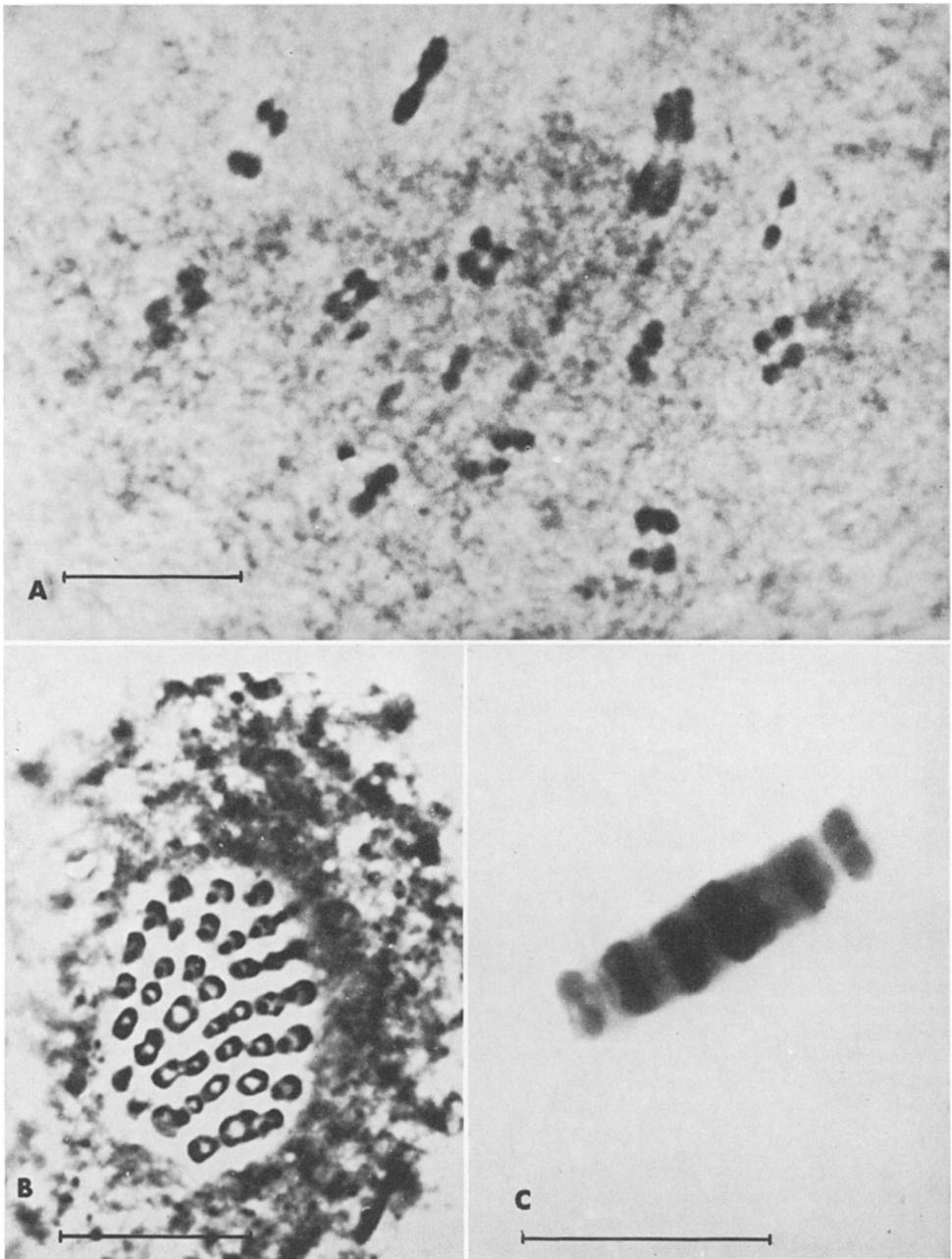
FIG. 1. The chromosome complements of the hybrids between diploid females of *M. fairmairei* and diploid males of *M. brevipennis*: A, *M.-2-fairmairei-brevipennis* polar view and phase contrast; B, same in side view: C, chromosomes in the nucleus of a semi-mature egg. Each bar represents 10 μm.

imental production of any of them. Insects are prob- ably more suitable than other groups for experimen- tal resynthesis of pseudogamous triploids, since they can be reared easily and in large numbers. It should be mentioned here that synthesis of a diploid hybri- dogenetic "species" has been reported by Schultz (1973). This paper documents the successful experi- mental production of triploid pseudogamous "spe- cies," *M.-2-fairmairei-brevipennis*.

MATERIAL AND METHODS

The material used in this investigation comprises colonies of Dutch and Greek populations of the bi- parentally reproducing species of *M. fairmairei* (Per- ris, 1857) ($2n = 13AII + XY$), a Dutch colony of the also biparentally reproducing species of *M. brevi- pennis* (Boheman, 1847) ($2n = 13AII + XY$) and a Dutch colony of the pseudogamous "species" *M.-2-fairmairei-brevipennis* (Drosopoulos, 1976) ($3n = 38 + XXX$).

Crossing experiments were made in cages contain- ing *Deschampsia caespitosa* (the food and oviposition plant of *M. brevipennis*) and *Holcus lanatus* (the food and oviposition plant of *M. fairmairei* and *M.-2-fairmairei-brevipennis*) under long photoperiod (LD = 18:6) at 20–28 C. Matings were made be- tween virgin females and mature males.

In all crossings described here hybrids were stud- ied cytologically by squashing semiripe eggs or testes (Drosopoulos, 1977). No morphological differences between the females of *Muellerianella* including the female hybrids have been found. However, the males and the hybrid males provide good morpho- logical differences in their caudal margin and phallus (Drosopoulos, 1977).

CROSSING EXPERIMENTS

When diploid *M. fairmairei* females from a stock derived in Greece were crossed with *M. brevipennis* males of Dutch origin, nine pairs produced six males and six females over a period of three months. The hybrid females were backcrossed with six males of *M. fairmairei* of the same Greek colony. After a pe- riod of two weeks, two of these backcrossed hybrid females were examined. Motile sperm were observed in their spermathecae. Somatic metaphases in follicle cells consistently had the diploid number of chro- mosomes ($2n = 28$). Their egg nuclei usually con- tained metaphase I with a mixture of amphitelic univalents and closely apposed pairs of homologous chromosomes, that were amphitelically oriented and apparently lacking chiasmata (Fig. 1a). In some eggs, 28 univalents were observed. In the six hybrid males the testes ranged from normal to abnormal or undeveloped. In about 100 hybrid males obtained during a period of three years, mature sperm was never observed. Cytological studies of normally formed hybrid testes gave no evidence of meiosis beyond the first metaphase. In those plates that could be analyzed, 28 univalents were observed without exception. Over a period of four months only 18 B₁ females were obtained, nine of which were back-

crossed to *M. fairmairei* males. These B₁ females were reared for two more generations and consis- tently produced numerous all-female progeny. In the same four months unmated females of the B₁ prog- eny failed to reproduce. Examination of five females of the B₁ and nine of the females of the next gener- ation showed that all were triploid and that their meiotic behavior was identical to that of the wild pseudogamous triploid "species" of *M.-2-fairmairei- brevipennis* (Fig. 1b and 1c).

From the reciprocal mating, *M. brevipennis* ♀♀ × *M. fairmairei* ♂♂ (Dutch colonies), nine pairs produced 21 females and seven males. Ten hybrid females were backcrossed with *M. brevipennis* and seven to *M. fairmairei* (Dutch colonies). Examina- tion of females, five and two of each from the re- spective backcrosses, made after one month, re- vealed that all had mated, but only two had formed eggs. The other five had either undeveloped or partly developed ovaries or they contained morphologically abnormal eggs. Meiotic behavior was similar to that of the female hybrids of the reciprocal cross. Since no larvae appeared during a period of three months, the stems of *H. lanatus* and *D. caespitosa* were checked for unhatched eggs. Those hybrids that had been backcrossed with *M. brevipennis* had ovipos- ited 67 eggs. Three alive and two of the 64 dead eggs exhibited early embryogenesis. Similar results were obtained with hybrids backcrossed with *M. fair- mairei* males. Thus, two out of 87 eggs had under- gone embryo rotation. Under the same conditions, embryogenesis in both bisexual species and the trip- loid pseudogamous "species" lasts two weeks (Dro- sopoulos, 1977). Undoubtedly all embryonic devel- opment in F₁ and B₁ hybrids was greatly prolonged.

Laboratory crosses between *M.-2-fairmairei-brev- ipennis* and males of *M. fairmairei* resulted in more all-female progeny than the crosses with males of *M. brevipennis*.

DISCUSSION

Ecological evidence of hybrid origin.—The two biparentally reproducing and related species *M. fair- mairei* and *M. brevipennis* are readily crossed in the laboratory, even when females of one species were caged with both sexes of the other. Regular associ- ations of these species and the pseudogamous "spe- cies" *M.-2-fairmairei-brevipennis* have often been observed in the field in Holland where their respec- tive host grasses grow syntopically. However, hybrid males were not observed in the field. Kuntze (1937) reported a similar association of the two then known closely related species in western Germany. At lo- calities in Holland where *M. fairmairei* is found, the pseudogamous "species" is always present feeding on *H. lanatus* and apparently relying on *M. fairmairei* as its "host species."

Attempts in the laboratory to rear either bipar- entally reproducing species on the host plant of the other failed. It is therefore likely that the main "iso- lating mechanism" between the two species is host plant preference. Moreover, there is no "seasonal

isolation" between the two biparentally reproducing species and the pseudogamous "species" (Drosopoulos, 1977). Where these plants grow together, the "isolating mechanism" may fail and hybridization between the biparentally reproducing species occur.

The geographic distribution of the two biparentally reproducing species is not known precisely, but in Europe *M. fairmairei* extends farther south than *M. brevipennis*, and *M. brevipennis* farther north than *M. fairmairei*. The range of the triploid "species" appears to lie in the widely overlapping region of the parental species in central and western Europe. Populations of *M. fairmairei* from England, Holland, and central France contain *M.-2-fairmairei-brevipennis* females, while in populations from southern France and southern Greece only diploids have been found. All three Dutch populations of *M. brevipennis* studied were diploid.

The fecundity and population densities of *M.-2-fairmairei-brevipennis* are higher than those of *M. fairmairei* or *M. brevipennis*, suggesting that it has "hybrid vigor" (Drosopoulos, 1977).

Cytogenetic documentation of hybrid origin.—The main features of the hybrid theory are touching a wide subject. In this part of the discussion I have considered mainly the reports of Guellar (1974) and Cimino (1972) as the most appropriate for the present study. Guellar reviewing the hybrid and spontaneous origin of parthenogenesis stated: "To be sure, existing facts lend some support to both although overall evidence presented here leans toward the spontaneous side." This statement was supported by the fact that backcrosses of diploid parthenospecies failed to form viable or fertile allotriploid parthenospecies. On the other hand, Cimino proposed several possibilities of resynthesizing triploid unisexuals in *Poeciliopsis* arisen from a hybridogenetic "species." The difference in my investigation is that, so far, I have not any indication that the F_1 hybrids could be parthenospecies, nor is there found any other form of parthenospecies than that of *M.-2-fairmairei-brevipennis*, both in the field and in laboratory colonies. Moreover, all F_1 hybrid progenies obtained so far had sterile males and females ranged from sterile up to fertile.

The results from the mentioned crosses indicate that sperm of *M. fairmairei* fused with a diploid egg nucleus of the F_1 diploid hybrid female. It is likely that such a nucleus formed due to incomplete pairing, resulting in replacement of the two meiotic divisions by a single equational one. Moreover, examination of numerous eggs of the biparentally reproducing species failed to reveal any unreduced egg nuclei. Consequently, triploids within F_1 were never found and both diploid species maintained their 1:1 sex-ratio for the two to three years they were kept in the laboratory. Suggestions similar to this have been given for the origin of the triploid salamanders *Ambystoma platineum* and *A. tremblayi* (Uzzell and Goldblatt, 1967). Apparently in this investigation absence of reduction is a consequence of hybridity.

Embryonic development in eggs laid by the backcrossed F_1 depends upon a favorable gene assortment of the chromosomes contributed by the two parental species. Such gene assortment appeared to be provided only by the egg nucleus comprising a maternal genome of *M. fairmairei* and a paternal genome of *M. brevipennis* with the following fertilization of *M. fairmairei*. The resulting pseudogamous females *M.-2-fairmairei-brevipennis* having already passed through the assortment obstacle, is fully fertile and viable unhampered by addition of a second genome of *M. fairmairei*.

Questionable remain the results of some other crosses between females of *M. brevipennis* with males of *M. fairmairei*. These crosses produced numerous females, while the reciprocal crosses produced a lower number of offspring and a 1:1 sex-ratio (Drosopoulos, 1977). Schultz (1966) reported an unbalanced sex-determining mechanism in crosses between two related fishes of the genus *Poeciliopsis*. Thus, it may also be that the sex-determining mechanism in *M. brevipennis* is stronger than in *M. fairmairei*. Whenever these numerous hybrid females were backcrossed either with *M. fairmairei* or *M. brevipennis* males, a true B_1 never resulted. In addition, it has been reported that feeding and oviposition mechanisms are expressed by dominance of genomic dosage effect. It appeared that one genome of *M. brevipennis* is dominant over a single genome of *M. fairmairei* but recessive to a double genome of *M. fairmairei* (Drosopoulos, 1977).

Whether the pseudogamous triploid should be called a "species" is a matter of opinion. In a previous paper (Drosopoulos, 1977) the nomenclatorial system of hybrids is proposed, thus I call this clonally reproducing females *M.-2-fairmairei-brevipennis*.

The suggestion of Haskins, et al. (1960) "that pseudogamy has taken place rather recently in time in the evolution of the species," is a relevant remark in this context. Complete parthenogenesis might be the next step of the evolutionary process of pseudogamy, since 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 biparentally reproducing "hosts."

SUMMARY

The wild *M.-2-fairmairei-brevipennis* appeared to have two genomes of *M. fairmairei* and one of *M. brevipennis*. In order to confirm this hypothesis reciprocal crosses involving different populations were made between the two biparentally reproducing species; these were followed by a cytological study of the hybrids.

The only successful process of crosses which resulted in the synthesis of the triploid pseudogamous species was as follows: hybrid progeny produced by crossing the diploid species, *Muellerianella fairmairei* ♀♀ × *M. brevipennis* ♂♂. Backcrosses of the F_1 females to *M. fairmairei* produced only a few prog-

eny, of which all were triploid females. Backcross of these females to *M. fairmairei* produced numerous matromorphous progeny very similar to the wild pseudogamous triploid "species" of *M.-2-fairmairei-brevipennis*.

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SELECTION FOR AMYLASE ALLOZYMES IN *D. MELANOGASTER*: A WORD OF CAUTION

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Recently, Hickey (1978) reported the results of laboratory selection experiments on the amylase locus of *Drosophila melanogaster*. These results, supporting the findings of DeJong and Scharloo (1976), were based on experiments in which heterozygotes for two amylase "alleles" were placed on different media types and gene frequencies were monitored over a period of several generations. One allele, *Amy 1-c*, was characterized by a single band on electrophoretic gels and relatively low enzyme activity while the other "allele," *Amy 4,6,* was characterized by two

bands and high activity. The findings in the experiments were that on a starch containing medium, the high activity *Amy 4,6* "allele" was at a selective advantage. The conclusions reached were that environmental factors could have a specific effect on allozyme alleles and that selection had been demonstrated at a specific enzyme locus. Quoting, "Therefore, we have isolated an environmental variable which affects a specific enzyme locus *in vivo*. . . ." These conclusions must be approached with caution!