Biochemical *Systematics and Ecology*, Vol. 13, No. 3. pp 239–250, 1985.
Printed in Great Britain.

0305–1978/85 $3.00 + 0.00

A New Species of *Muscari* Subgenus *Leopoldia* from the Iberian Peninsula

M. RUIZ REJÓN, **L. PASCUAL, C.** RUIZ REJÓN, **B.** VALDÉS* and **J. L.** OLIVER†

Departamento de Genética, Facultad de Ciencias. Universidad de Granada, Spain;
"Departamento de Botánica, Facultad de Biologia, Universidad de Sevilla, Spain;
†Departamento de Genética, **Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, Madrid 34, Spain**

Key Word Index—*Muscari comosum; Muscari matritensis* **sp. nova;** ***Leopoldia; Muscari;*** **Liliaceae; cytogenetic analysis; electrophoretic analysis; morphological analysis; breeding systems; speciation process.**

Abstract-The biological analysis of several populations confirms the existence in the Iberian Peninsula of two species of *Muscari* subgenus *Leopoldia: M. comosum* and *M. matritensis* (the latter described as new). Karyologically, they differ in at least a translocation rearrangement, in the presence of heterochromatin, which is lacking in *M. matritensis,* and in some chromosomal characters. At thegenic level, the distance between *M. comosum* and *M. matritensis* is high (0.378). Morphologically, they show some differences in floral structure which are correlated with different reproductive systems, allogamy in *M. comosum* and autogamy in *M. matritensis.* The possible evolutionary relationships between these taxa is discussed

Introduction

Understanding the dynamics of the speciation process is of central importance to the study of evolution. It is of particular interest to know what changes (physiological, developmental, behavioural, ecological, chromosomal) are directly associated with evolution or reproductive isolation, how rapidly and under what circumstances (allopatry, sympatry, etc.) such changes occur, and the relative importance of selection and stochastic factors in producing these changes.

A widely accepted model of geographic speciation [I, 2] suggests that new species arise in isolated populations through the gradual accumulation of genetic differences. However, it has become increasingly clear that speciation events do not always conform to this pattern [3–5]. Alternative models of speciation have been developed to explain observed relationships among closely related species. These include models of sympatric and quantum speciation. for example. In this latter model, the chromosome rearrangements, or polyploidy, can play important roles as rapid reproductive isolation mechanisms. In these cases the genetic distances and the degree of morphological differentiation between related species are very low [6–13].

Muscari Miller subgenus ***Leopoldia*** (Parl.) Zahar or ***Leopoldia*** Parl. (refs [14,15], respectively) is a critical biological complex in which the delimitation of taxa is, to a certain extent, arbitrary. The geographical distribution of the subgenus iscentred in the Mediterranean region and extends from the Canary Islands to Afghanistan and from central Germany and southern U.S.S.R. to north Africa. Although many species have been described for this subgenus, most of them probably do not deserve taxonomic recognition. According to Bentzer [16], this group includes *ca.* 20 species, six of which have been reported for the European flora [14]. Five of these *(M.cycladicum,* ***M. gussonei, M. spreitzenhoferi, M.*** *weissi* and ***M. tenuiflorum)*** occur in the central and eastern Mediterranean region in 'phrygana' communities (dwarf scrub communities) [I 7] or in dry places, where most have a rather reduced geographical area. In contrast, ***M. comosum*** (L.) Miller, the only species of this subgenus so far reported from the western Mediterranean, has a wider ecological tolerance and grows in dry grasslands and cultivated ground over most of the Mediterranean area.

(Received 16 March 1984)

Although all these species have $2n=18$ chromosomes, they show important differences at the chromosomal level [I 8–26]. Thus, whilst *M. gussonei,* endemic to Sicily, has the most symmetrical karyotype, with an idiogrammatic formula of 10L+8S, *M. cycladicum, M. spreitzenhoferi, M. weissi* and *M. tenuiflorum* have a more asymmetrical karyotype: 4L+6M+8S. *M. comosum* has the most asymmetrical karyotype: 2L+2M + 14S. It seems clear, therefore, that chromosome rearrangements have played a very significant role in the speciation of the group.

In the course of an analysis of the chromosome and gene variability of *M. comosum* from the Iberian Peninsula and neighbouring regions, involving three Spanish populations from 'phrygana' communities, the existence of another biological species with a more symmetrical karyotype than *M. comosum* has been detected. In this paper, some genetical, morphological and biological differences between these two groups are reported. The role that the chromosome rearrangements may have played in the differentiation of these two biological entities is also discussed.

Results

Bulbs, flowers and immature seeds have been collected in 25 populations of *Leopoldia* from the Iberian Peninsula and neighbouring countries (see Table 1). As *Muscarican* multiply vegetatively, the bulbs, flowers and seeds were usually collected more than 1 m apart.

Cytogenetic Analysis

Plants with the standard karyotype of M. comosum. The standard diploid karyotype of *M. comosum* has been detected in 22 populations (all analysed except DOR, CAP and ARG, see Table 1). Two stable chromosomal polymorphisms for a pericentric inversion and a duplication (or segment) are present in all these populations on the second pair of chromosomes, as previously reported for this species [18, 25, 27]. In a previous paper [28] we have quantified and discussed the rearrangement frequencies of seven Spanish populations compared with those from the Aegean Islands [27]. Another paper is being prepared to cover the remaining populations analysed here.

The standard karyotype of *M. comosum* (Fig. 1 A) includes one long pair of telocentric chromosomes (first pair), one pair of intermediate size metacentric, submetacentric or subtelocentric chromosomes, due to the above-mentioned polymorphisms (second pair) and seven pairs of small metacentric chromosomes (third to ninth pairs). The nucleolar organizer is located on a small bivalent (Fig. 2). In the interphase nuclei of somatic and germinal tissues there are two or more large heterochromatic chromocentres (Fig. 4). With *C*-banding, the long arm of the first pair of chromosomes shows four or five heterochromatic bands (see Fig. 5 and ref. [29]). This is also observed in slides without C-banding, if pretreatment is performed in cold (oxyquinoline at 0–4°).

Plants with the 'phrygana' karyotype. A different karyotype has been found in three Spanish populations (La Cabrera, Arganda and Dornajo, see Table 1) all from 'phrygana' communities. According to Bentzer [22] this karyotype is named 'phrygana'. Whereas in Arganda and Dornajo all the plants studied have the 'phrygana' karyotype, in the population from La Cabrera some individuals [37] have the 'phrygana' karyotype and others [37] the standard karyotype of *M. comosum.* To differentiate these plants, the former bulbs are grouped as a CAP population and the latter as a LAC population (see Table 1).

The 'phrygana' bulbs from La Cabrera and Arganda (two neighbouring populations from central Spain) have a karyotype with $2n=18$ chromosomes including two pairs of long subtelocentric chromosomes (first and second pairs), three pairs of intermediate size, two (third and fifth pairs) metacentric and one (fourth pair) submetacentric chromosomes, and four pairs of small metacentric chromosomes (fifth to ninth pairs) (Fig. 1 B). The 'phrygana' bulbs from Dornajo (the highest populations studied from the Sierra Nevada at *ca.* 2000 m) have a similar karyotype, but the third pair of chromosomes is submetacentric (Fig. 1 C). In both cases, the nucleolar organizer is located in the long arm of one of the two longest pairs (Fig. 3). No chromocentres were observed in the interphase

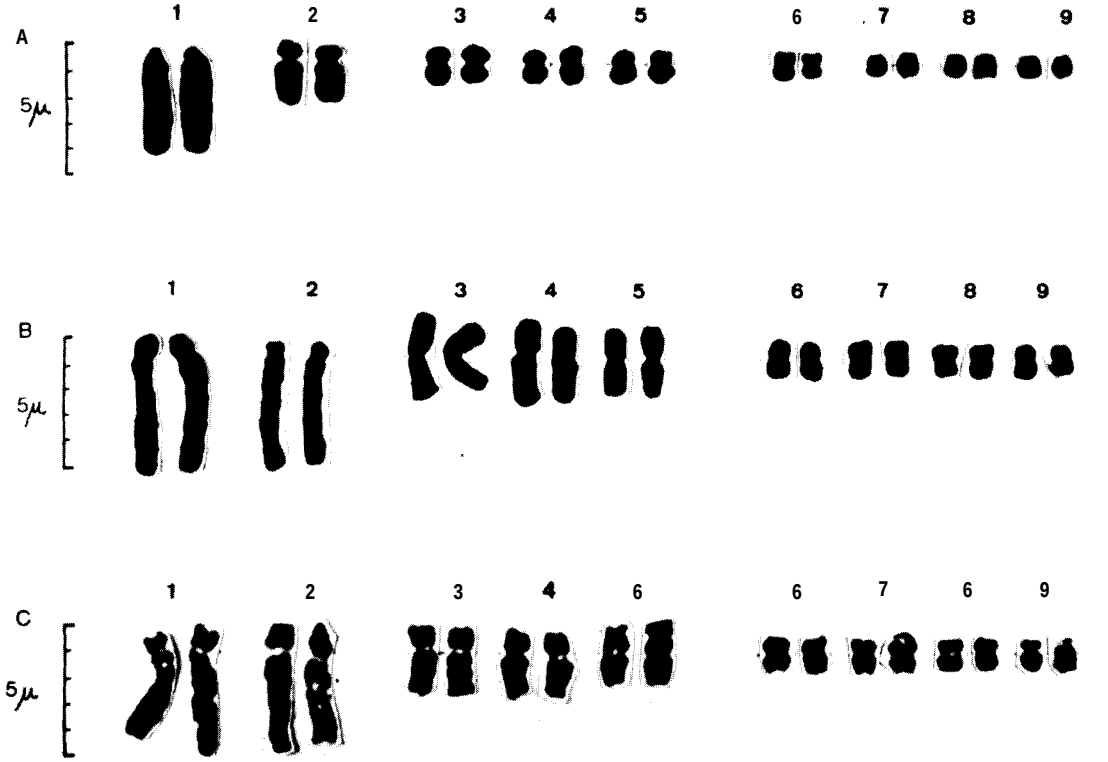

FIG 1. KARYOTYPES OF M. *COMOSUM* **(A) AND 'PHRYGANA' SPECIES (B AND C). In 'phrygana'. the third paw of chromosomes is metacentric in La Cabrera and Arganda populations (B) and submetacentric in Dornajo (C).**

FIG 2 DIPLOTENE CELL OF M. *COMOSUM. One small* bivalent is associated with the nucleolus.

FIG. 3. DIPLOTENE CELL OF 'PHRYGANA'. One long bivalent is associated with the nucleolus.

FIG. 4. INTERPHASE NUCLEI OF *M. COMOSUM* SHOWING TWO OR MORE BIG HETEROCHROMATIC CHROMOCENTRES

FIG. 5. MITOTIC METAPHASECELLOFM. COMOSUMAFTER *C*-BANDING TREATMENT. The long arms of the first pair of chromosomes display five heterochromatic bands

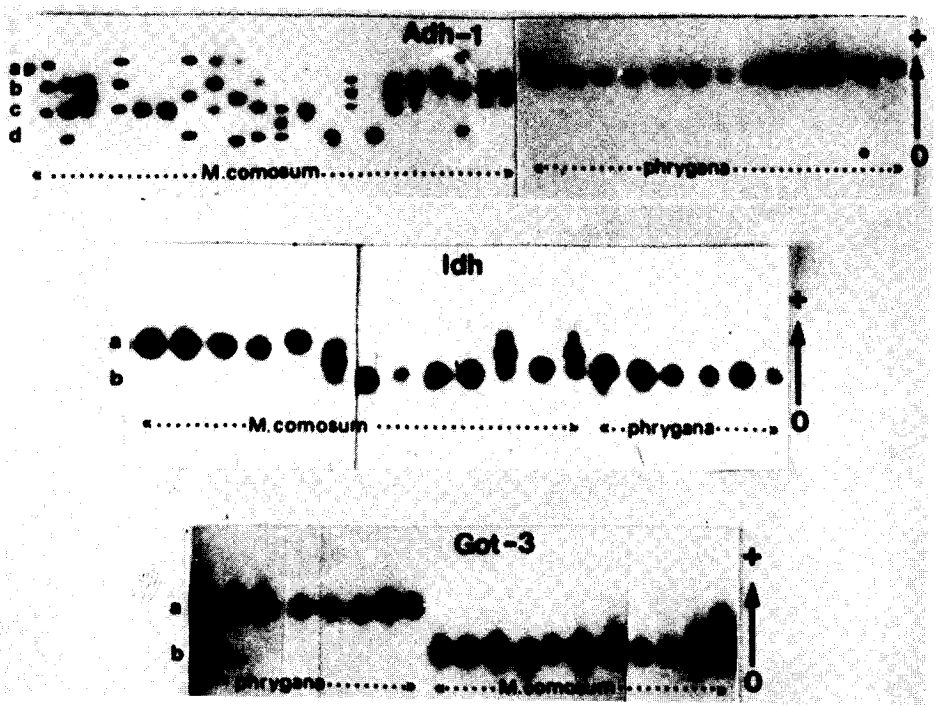

FIG. 8. ISOZYME DIFFERENCES BETWEEN *M. COMOSUM* AND 'PHRYGANA'.

TABLE 1. ALLELIC FREQUENCIES FOR FIVE LOCI *MUSCARI COMOSUM* AND 'PHRYGANA' SPECIES. Locus 1 represents the fastest, anodally-migrating locus for a system. Allele a represents the fastest, anodally-migrating allele. *N* represents the number of analysed individuals in each population

	Adh-1*					Idh			Got-1			Got-2			Got-3		
Population	*N*	a	b	c	d	*N*	a	b	*N*	a	b	*N*	a	b	*N*	a	b
1. ROM	47	0.09	0.18	0.40	0.33	47	0.15	0.85	47	0.06	0.94	47	1.00	0.00	47	0.06	0.94
2. RAG	37	0.07	0.22	0.35	0.36	33	0.08	0.92	39	0.07	0.93	39	0.97	0.03	39	0.01	0.99
3. DUR	46	0.09	0.15	0.46	0.30	45	0.17	0.83	48	0.10	0.90	50	0.98	0.02	50	0.06	0.94
4. AVI	21	0.12	0.20	0.48	0.20	21	0.07	0.93	22	0.12	0.88	21	0.98	0.02	21	0.07	0.93
5. LLU	23	0.13	0.28	0.26	0.33	23	0.02	0.98	23	0.30	0.70	23	1.00	0.00	23	0.00	1.00
6. VAL	18	0.08	0.39	0.25	0.28	18	0.00	1.00	18	0.08	0.92	18	0.97	0.03	18	0.00	1.00
7. GAR	94	0.15	0.21	0.47	0.17	22	0.16	0.84	94	0.07	0.93	95	0.98	0.02	95	0.08	0.92
8. LAC	37	0.04	0.35	0.40	0.20	20	0.10	0.90	37	0.05	0.95	37	0.96	0.04	37	0.00	1.00
9. CAP†	37	0.00	1.00	0.00	0.00	24	0.00	1.00	21	0.00	1.00	21	1.00	0.00	21	1.00	0.00
10. ARG†	80	0.00	1.00	0.00	0.00	25	0.00	1.00	43	0.00	1.00	43	1.00	0.00	43	1.00	0.00
11. BNS	24	0.10	0.10	0.44	0.36	11	0.05	0.95	18	0.08	0.92	18	0.92	0.08	18	0.03	0.97
12. PAD	42	0.05	0.15	0.54	0.26	21	0.12	0.88	38	0.17	0.94	38	1.00	0.00	38	0.04	0.96
13. COZ	68	0.04	0.25	0.40	0.31	24	0.32	0.68	68	0.07	0.93	68	1.00	0.00	68	0.01	0.99
14. CAN	48	0.09	0.15	0.55	0.21	92	0.17	0.83	54	0.05	0.95	54	0.97	0.03	54	0.06	0.94
15. HIG	42	0.10	0.14	0.52	0.24	107	0.12	0.88	42	0.06	0.94	42	1.00	0.00	42	0.04	0.96
16. SIL	129	0.11	0.17	0.42	0.30	25	0.18	0.82	128	0.10	0.90	128	0.99	0.01	128	0.02	0.98
17. SAB	29	0.09	0.22	0.48	0.21	101	0.10	0.90	30	0.08	0.92	30	0.97	0.03	30	0.00	1.00
18. DOR†	23	0.00	1.00	0.00	0.00	40	0.00	1.00	23	0.00	1.00	23	1.00	0.00	23	0.87	0.13
19. COL	67	0.02	0.26	0.48	0.24	18	0.11	0.89	74	0.24	0.76	74	0.98	0.02	74	0.02	0.98
20. POA	41	0.08	0.04	0.60	0.28	40	0.08	0.92	38	0.00	1.00	40	1.00	0.00	42	0.03	0.97
21. POB	41	0.01	0.09	0.50	0.40	43	0.24	0.76	45	0.08	0.92	45	0.98	0.02	45	0.00	1.00
22. POC	24	0.06	0.17	0.58	0.19	18	0.06	0.94	26	0.04	0.96	18	1.00	0.00	26	0.00	1.00
23. POD	32	0.00	0.00	0.38	0.62	7	0.30	0.70	32	0.00	1.00	32	1.00	0.00	32	0.03	0.97
24. MOY	19	0.05	0.10	0.35	0.50	7	0.28	0.72	15	0.00	1.00	16	1.00	0.00	16	0.00	1.00
25. SUR	23	0.11	0.07	0.56	0.26	22	0.05	0.95	23	0.00	1.00	23	1.00	0.00	23	0.02	0.98

*There is another locus of ADH(Adh-2), only expressed in pollen grains, not included in the present paper.

†Populations belonging to the 'phrygana' species.

Geographic localities of *Muscari* here analysed. Collecting sites: (1) Rome (Italy); (2) Ragusa (Sicily); (3) Durance (France); (4) Avignon (France); (5) LLuvi (Mallorca); (6) Valldemosa (Mallorca); (7) Gargantilla (Spain); (8) La Cabrera (Spain); (9) La Cabrera—'phrygana' (Spain); (10) Arganda (Spain); (11) Baños (Spain); (12) Padul (Spain); (13) Cozvijar (Spain); (14) Canales (Spain); (15) Higuera (Spain); (16) Silleta (Spain); (17) Sabinas (Spain); (18) Dornajo (Spain); (19) Colmenar (Spain); (20) Portugal—A; (21) Portugal—B; (22) Portugal—C; (23) Portugal—D; (24) Moya (Canary Islands); (25) Santa Ursula (Canary Islands).

nuclei of these plants, nor do they display any *C*-banding pattern.

Comparison of the karyotypes. To further analyse the chromosome differences, a detailed comparison of three karyotypes has been produced: the standard *M. comosum* type, the 'phrygana' type from Dornajo and the 'phrygana' type from La Cabrera and Arganda. Measurements of the relative contribution to the karyotype (*C*) and of the arm ratio (*r*) of each pair of chromosomes have been made of 15 metaphase plates of different plants of each type (45 cells analysed in total). For the standard *M. comosum* karyotype the cells analysed were homozygous for the second pair of chromosomes (i.e. ++ plants, see ref. [28]); for the 'phrygana' type from La Cabrera and Arganda, seven of the studied cells were taken from La Cabrera plants and eight from Arganda plants; for the 'phrygana' type from Dornajo, the cells were derived from different plants of this population.

A discriminant analysis for the three types of cells was made using 18 characters for each cell ($r_2, \ldots r_9; C_1, C_2, \ldots C_9$) by using a BMDP 7M program. From this analysis it can be deduced that there is no overlap between the cells of the three karyotypes (see Tables 2 and 3 and Fig. 6).

TABLE 2. CLASSIFICATION MATRIX

Group	Per cent correct	Number of cases classified into group		
		Comosum	*D.*	*P.*
Comosum, *C.*	100	15	0	0
Dornajo, *D.*	100	0	15	0
Arg.-La *C.*, *P.*	100	0	0	15
Total	100	15	15	15

TABLE 3. SUMMARY OF DISCRIMINANT ANALYSIS

Step number	Variable		*F*-Value to enter or remove
	Entered	Removed	
1	*r*3	—	113.6126
2	*C*1	—	110.0589
3	*r*2	—	28.3531
4	*C*4	—	18.6927
5	*C*6	—	5.7905

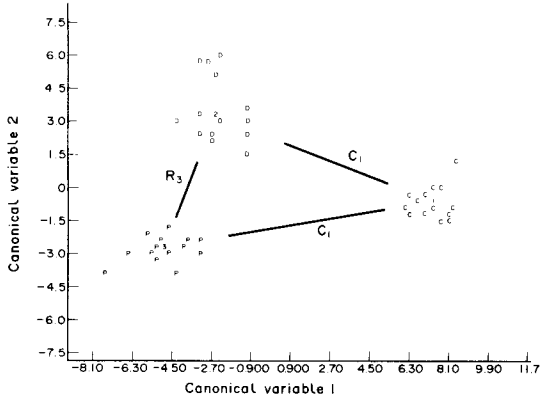

FIG. 6. MULTIVARIATE DISCRIMINANT ANALYSIS BETWEEN CELLS OF: 1,*C* = *M. COMOSUM* CELLS; 2,*D* = 'PHRYGANA' FROM DORNAJO AND 3,*P* = 'PHRYGANA' FROM LA CABRERA AND ARGANDA. C_1 = Relative contribution of the first pair of chromosomes. R_3 = Arm ratio of the third pair of chromosomes.

Electrophoretic Analysis

Table 1 lists the allelic frequencies for five loci in the 25 populations. Table 4 shows the mean values of alleles per locus, the percentage of polymorphic loci and the mean observed heterozygosity in *M. comosum* and in 'phrygana' populations. Table 5 shows the averaged genetic identity (*I*) and distance (*D*) values among *M. comosum* and 'phrygana' populations, computed according to Nei [30]. Furthermore, by means of the program BIOSYS-1 [31], a Nei [30] identity matrix was subjected to UPGMA clustering to obtain the cluster shown in Fig. 7. The isozyme differences that exist between *M. comosum* and 'phrygana' are shown graphically in Fig. 8.

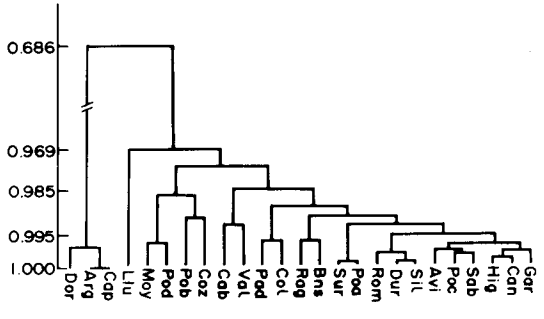

FIG. 7. UPGMA CLUSTERING BASED ON NEI'S GENETIC IDENTITY BETWEEN POPULATIONS OF *M. COMOSUM* AND 'PHRYGANA'. La Cabrera (Cap), Arganda (Arg) and Dornajo (Dor) populations belong to the 'phrygana' species; the remaining localities to *M. comosum*.

TABLE 4. MEAN NUMBER OF ALLELES PER LOCUS, PER CENT LOCI POLYMORPHIC AND HETEROZYGOSITY MEAN PER SPECIES FOR *M. COMOSUM* AND 'PHRYGANA'

	Mean alleles per locus	Per cent loci polymorphic	Heterozygosity mean
M. comosum	2.18	56.36	0.219
'Phrygana'	1.06	6.66	0.015

TABLE 5. ESTIMATES OF *I* (BELOW THE DIAGONAL) AND *D* IN *M. COMOSUM* AND 'PHRYGANA' SPECIES ACCORDING TO REF. [30]

	M. comosum	'Phrygana'
M. comosum	—	0.378
'Phrygana'	0.686	—

Morphological Analysis

A comparative morphological analysis between living plants from the Arganda population (with 'phrygana' karyotype) and from a neighbouring population from Gargantilla (with the standard karyotype of *M. comosum*) has been made (see Table 6).

The following points must be stressed. First, the morphological characters of plants from Dornajo, also with 'phrygana' karyotype, agree with those of plants from Arganda. Secondly, the morphological characters of these 'phrygana' plants differ from those of the species of central and eastern Mediterranean species with the 'phrygana' karyotype. Thirdly, *M. comosum* is rather polymorphic from the morphological point of view, and in some populations the fertile flowers have a cylindrical and yellowish perigon, as in the plants from Arganda. Specimens are deposited in the Herbarium of Sevilla (Spain).

Breeding Systems

The plants from Gargantilla (with the standard karyotype of *M. comosum*) have a very marked protandry: the dehiscence of anthers is very precocious in relation to the stigma receptivity such that the anthers begin to dehisce in the young fertile flowers whilst the perianth is still closed. However, the flowers open immediately (leaving an opening of *ca.* 3 mm) and, since the dehiscing anthers are placed near the aperture, they are presumably very conspicuous to insect visitors, with the white pollen and the white or cream perianth teeth contrasting with the brown background of perianth. Subsequently, the flowers grow to *ca.* 7.5 mm (at least in the population studied). At this stage the perianth begins to fade and the style, which was very short when the flower opened, has grown such that the receptive stigma is located near the perianth opening. As the perianth ages it shortens so that the stigma becomes extruded by up to *ca.* 1 mm in the population studied.

Thus, the characters of *M. comosum* flowers favour cross-pollination. Knoll [32] made experimental studies on the attraction of insects, such as bees and flies, to the inflorescence of *M. comosum*. According to Knoll, the colour of sterile flowers functions as a primary visual stimulus to insects, which are attracted by the

TABLE 6. MORPHOLOGICAL DIFFERENCES BETWEEN PLANTS FROM GARGANTILLA, WITH STANDARD *M. COMOSUM* KARYOTYPE, AND PLANTS FROM ARGANDA, WITH 'PHRYGANA' KARYOTYPE

Character	Plants from Gargantilla	Plants from Arganda
Leaf width	11–15 mm	9–10 mm
Fertile flowers	Obconical	Cylindrical
Perianth length of fertile flowers at anthesis	5–7.5 mm	6.5–7.5 mm
Maximum width of perianth	4.5–5.5 mm	3.5–4.6 mm
Colour of adult fertile flowers	Brown	Yellowish
Colour of the perianth teeth	White or cream	Yellow
Perianth opening	*ca.* 2 mm	*ca.* 1.5 mm
Anthers position	Reaching the aperture of perianth	Never reaching the aperture of perianth
Anther size	0.9–1 mm	1.3–1.7 mm

colour of the sterile flowers to the apex of inflorescence and, subsequently, move towards the base. Since the flowering sequence in *Muscari* is acropetal and since each flower is protandrous, one may expect visitors to transfer pollen from flowers with a non-receptive stigma to others with a receptive stigma in the same inflorescence. As each insect visits a number of plants within a short period of time [32], it is likely that at least some pollen can be transferred from one plant to another. In fact, in natural populations of *M. comosum* progeny, analyses [33, 34] have confirmed that there is a certain level of cross-pollination.

The plants from Arganda (with 'phrygana' karyotype) have a different floral biology. The flowers open very late, almost when they have reached maximum size. At this time, the opening of the perianth is small (*ca.* 1.5 mm) and the dehiscent anthers never reach the aperture. Furthermore, the yellow perianth teeth are scarcely conspicuous against the yellow-brown background of the upper part of the perianth. As the flowers begin to fade the perianth closes and the receptive stigma is located between the dehiscent anthers. Finally, the perianth collapses and the stigma becomes extruded but it is already dry. Thus, the floral biology of plants with the 'phrygana' karyotype favours self-pollination. The existence of self-pollination in these plants is confirmed by means of progeny analyses of esterases [34] in which 'phrygana' plants are polymorphic.

Consequently, the plants with the 'phrygana' karyotype from Arganda present a derived situation, with respect to the breeding system, in which the dehiscence of anthers is delayed until the stigma is receptive. These plants have a reproductive system which differs from that of all previously studied species of this group [16] and it is obviously different from the breeding system of *M. comosum*.

Finally, there is also a prezygotic temporal reproductive isolating mechanism between these two types of plants. In the mixed population from La Cabrera, the plants with the standard *M. comosum* karyotype begin to flower 15–20 days before those with the 'phrygana' karyotype. Furthermore, the plants with the 'phrygana' karyotype from Arganda and Dornajo were also found to flower some days later than the plants with *M. comosum* karyotype from the neighbouring localities.

Discussion

Karyologically, the plants with the standard *M. comosum* karyotype differ from those with the 'phrygana' karyotype in at least one translocation rearrangement, since the nucleolar organizer has changed its position from a small to a large pair of chromosomes (see Figs 2 and 3). Furthermore, they differ in the presence of heterochromatin in *M. comosum* which is lacking in the 'phrygana' karyotype (see Figs 4 and 5). The multivariate discriminant analysis we have carried out shows that there is no overlap between the cells of *M. comosum* and those of the 'phrygana' type (from La Cabrera, Arganda and Dornajo): they differ in the contribution of the first pair of chromosomes (see Tables 2 and 3 and Fig. 6). The plants from Dornajo differ from those from La Cabrera and Arganda in the arm ratio of the third pair of chromosomes due, probably, to a fixed pericentric inversion on this pair. The different contribution of the first pair of chromosomes of the standard *M. comosum* type is probably due to the presence of some heterochromatic bands in its long arm (see Fig. 5), which are absent in the 'phrygana' karyotype, or (and perhaps in addition) to unequal translocations. *M. comosum* has an assymmetrical karyotype (almost a bimodal karyotype in the sense of Stebbins [35]), with two sharply distinctive sizes of chromosomes. The origin of bimodal karyotypes has been explained in two different ways. Darlington [36] suggested that they are derived from symmetrical karyotypes of polyploid origin, with the small chromosomes produced by differential loss of chromosome segments. However, according to the Levitzky principle of increasing asymmetry [35] bimodal karyotypes could also result from unequal translocations. In the first case it would be expected that the resulting species contain many duplicate loci. In the second, some gene duplication is also possible if unequal translocations cause duplications which are then fixed by means of inbreeding [9, 37]. In *M. comosum* there are two duplicate genes which show fixed heterozygosity and lack of divergence in duplicate gene expression,

which are characteristics of regional chromosome duplication phenomena [38]. Consequently, it is possible that unequal translocations may have played an important part in the chromosome evolution of *M. comosum*.

At the genic level, the distance between the standard *M. comosum* and the 'phrygana' plants is high (0.378), using the measures developed by Nei [30]. Furthermore, when the mean number of alleles per locus, degree of heterozygosity and proportion of polymorphic loci in the two kinds of plant are compared, the low polymorphism of 'phrygana' stands out. This is possibly due to its breeding system (with autogamy) and/or to the fact that this species seems to be an endemic taxon confined to some populations from the Iberian Peninsula and, therefore, with a population size which is presumably smaller than that of the cosmopolitan *M. comosum*. Both types of plant are clearly apart in the cluster that we have constructed using data of isozymes (Fig. 7).

In contrast, the morphological differences between the two types of plant are sparse and are directly derived from the different breeding systems; allogamy in the standard *M. comosum* and autogamy in the 'phrygana' plants. However, the biological differences are sufficient to justify separation of the plants with the 'phrygana' karyotype from La Cabrera (Madrid), Arganda (Madrid) and Dornajo (Granada) from those with the standard *M. comosum* karyotype at specific level. The new species (see below) is named *M. matritensis*. The two species show a prezygotic temporal isolating mechanism since *M. comosum* flowers before *M. matritensis,* and cytogenetic and electrophoretic analyses confirm the lack of hybrid plants in any of the populations studied, including that from La Cabrera where both taxa are sympatric.

Conclusions

Muscari matritensis: M. Ruiz Rejón, L. Pascual, C. Ruiz Rejón, B. Valdés and J. L. Oliver, *sp. nova*.

M. comosum: primo adspecto maximo simile, sed perigonio cylindrico flavido dentibus flavis, staminibus brevioribus. Typus, SEV asservatur.

As far as the relations between the two species are concerned, it seems clear that the distinctive karyotype of *M. comosum* must have originated from a karyotype similar to the 'phrygana' karyotype present in most taxa of the subgenus *Leopoldia,* which is also rather similar to the karyotypes present in the remaining subgenera of *Muscari*. Furthermore, *M. comosum* can be considered as one of the most advanced taxa of this subgenus, both by its specialized morphology and by its very efficient reproductive biology [16]. The possibility of derivation of *M. comosum* and *M. matritensis* by chromosome rearrangement is supported by studies on other taxa, e.g. *Coreopsis* [39], *Clarkia* [8], *Gaura* [10], *Stephanomeria* [7] and *Lycopersicon* [13]. However, in these cases, there is a very high degree of genetic identity in the species pairs which is not the case in *M. comosum* and *M. matritensis*. Consequently, if *M. comosum* did arise by rapid speciation from *M. matritensis,* the speciation events must have occurred long enough ago to allow for a high genetic differentiation of the kind discussed, for example, for *Clarkia franciscana* and *C. rubicunda* [40].

It is also possible that *M. comosum* may be derivative from another species with the 'phrygana' karyotype, which is now extinct or only distributed today in the central or eastern Mediterranean. To establish the true origin of *M. comosum,* a complete genetical analysis of *Muscari* subgenus Leopoldia should be performed.

Experimental

Cytogenetics. Mitotic analysis was carried out on root tips squashed in acetic acid–orcein. *C*-Banding, according to ref. [29], and meiotic behaviour of the PMCs was analysed by staining young anthers in acetocarmine.

Electrophoresis. Roots, flowers (petals, anthers, pollen grains and ovaries) and immature seeds were employed. Crude extracts, obtained after crushing the above-mentioned material, were analysed by horizontal starch gel electrophoresis. Alcohol dehydrogenase (ADH), glutamate–oxaloacetate transaminase (GOT) and isocitrate dehydrogenase (IDH) were resolved on 12% starch gels. The gel buffer was Tris-citrate (pH 8.4) and the electrode buffer LiOH–Borate (pH 8.1) [41].

Genetic measurements. Genetic variability measures, computation of genetic distances and identity, and UPGMA analysis were performed with the program BIOSYS-1 [31] on a DEC(VAX-11) computer. Multivariate tests utilizing stepwise discriminate function analysis were performed on an IBM 375/158 computer using the BMDP7M program (University of California, Los Angeles). Both programs were run at the Computation Center of the Universidad Autónoma de Madrid.

Acknowledgements—The authors are indebted to Drs. D. L. Swofford and R. B. Selander for supplying the BIOSYS-1 program and to Drs. Battaglia, C. Marceno, M. A. Cardona, J. de Barros Neves, J. Ortega and the Société Botanique de Vaucluse (France) for supplying bulbs of *Muscari*. We are grateful to Dr. P. Gibbs, D. López, L. Pérez and L. J. García for assistance in preparation of the manuscript.

References

1. Mayr, E. (1963) *Animal Species and Evolution,* p. 493. Harvard University Press, Cambridge.
2. Dobzhansky, T. (1970) *Genetics of Evolutionary Process,* p. 351. Columbia University Press, New York.
3. Grant, V. (1971) *Plant Speciation,* p. 114. Columbia University Press, Cambridge.
4. Ayala, F. J. (1975) *Evol. Biol.* **8,** 1.
5. White, M. J. D. (1978) *Modes of Speciation,* p. 169. W. H. Freeman, San Francisco.
6. Gottlieb, L. D. (1972) *Madrono* **21,** 463.
7. Gottlieb, L. D. (1973) *Am. J. Botany* **60,** 545.
8. Gottlieb, L. D. (1974) *Evolution* **28,** 244.
9. Gottlieb, L. D. (1976) *Molecular Evolution* (Ayala, F. J., ed.), p. 123. Sinauer.
10. Gottlieb, L. D. and Pilz, G. (1976) *Syst. Botany* **1,** 181.
11. Nevo, E. and Shaw, C. R. (1972) *Biochem. Genet.* **7,** 235.
12. Nevo, E., Kim, Y. J., Shaw, C. R. and Thaeler, C. S., Jr. (1974) *Evolution* **28,** 1.
13. Rick, C. M., Kesicki, E., Fobes, J. F. and Holle, M. (1976) *Theoret. Appl. Genet.* **47,** 55.
14. Tutin, T. G. *et al.* (eds) (1980) *Flora Europaea,* Vol. 5, p. 47. Cambridge University Press, London.
15. Garbari, F. and Greuter, W. (1970) *Taxon* **19,** 329.
16. Bentzer, B. (1973) *Bot. Notiser* **126,** 69.
17. Greuter, W. (1972) *Taxonomy, Phytogeography and Evolution* (Valentine, D. H., ed.) p. 165. Academic Press, London.
18. Garbari, F. (1966) *Caryologia* **19,** 419.
19. Garbari, F. (1968) *Giorn. Bot. Italiano* **102,** 87.
20. Garbari, F. (1969) *Giorn. Bot. Italiano* **103,** 1.
21. Garbari, F. (1973) *Webbia* **28,** 57.
22. Bentzer, B. (1969) *Bot. Notiser.* **122,** 457.
23. Bentzer, B. (1972) *Bot. Notiser.* **125,** 180.
24. Bentzer, B. (1972) *Bot. Notiser* **125,** 329.
25. Bentzer, B. (1972) *Bot. Notiser.* **125,** 406.
26. Bentzer, B. (1974) *Bot. Notiser.* **127,** 69.
27. Bentzer, B. and Ellmer, M. (1975) *Hereditas* **81,** 127.
28. Ruiz Rejón, M. and Oliver, J. L. (1981) *Heredity* **47,** 403.
29. Bentzer, B. and Lanström, T. (1975) *Hereditas* **80,** 219.
30. Nei, M. (1972) *Am. Nat.* **106,** 283.
31. Swofford, D. L. and Selander, R. B. (1981) *J. Heredity* **72,** 281.
32. Knoll, F. (1921) *Abh. Zool.-Bot. Ges. Wien* **12,** 1.
33. Ruiz Rejón, M., Pérez, L., Pascual, L., Ruiz Rejón, C., López, D. and Oliver, J. L. (1982) *The Isozyme Bull.* **15,** 136.
34. Pascual, L. (1983) Ph.D. thesis, Universidad de Granada, Spain.
35. Stebbins, G. L. (1971) *Chromosomal Evolution in Higher Plants,* p. 96. Edward Arnold, London.
36. Darlington, C. D. (1963) *Chromosome Botany and the Origin of Cultivated Plants*, p. 119. Hafner, New York; George Allen & Unwin, London.
37. Gottlieb, L. D. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71,** 1816.
38. Oliver, J. L., Martínez-Zapater, J. M., Pascual, L., Enríquez, A. M., Ruiz Rejón, C. and Ruiz Rejón, M. (1983) *Isozymes: Current Topics in Biological and Medical Research* (Rattazzi, M. C., Scandalios, J. G. and Whitt, G. S., eds) Vol. 10, p. 341. Alan R. Liss, New York.
39. Crawford, D. J. and Smith, E. B. (1982) *Evolution* **36,** 379.
40. Gottlieb, L. D. (1973) *Evolution* **27,** 205.
41. Selander, R. K., Smith, M. H., Yang, S. Y., Johnson, W. E. and Gentry, J. B. (1971) *Univ. Texas Publ.* **7103,** 49.