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Chromosome Studies in Species and Hybrids of *Petrorhagia* sect. *Kohlrauschia* (*Caryophyllaceae*)

By

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Abstract: Cytogenetic investigations have been made in the four *Petrorhagia* species and hybrids of the section *Kohlrauschia*. The three diploid species show close similarities in chromosome number, size and morphology, with the exception of *P. velutina*, where one pair of metacentric chromosomes is represented by a pair of telocentrics. Meiotic studies in hybrids indicate close genomic homology between the diploid species and also between the two floral forms of *P. prolifera*. The tetraploid *P. nanteuilii* behaves as an allotetraploid forming only bivalents at meiosis and results suggest that *P. velutina* and *P. prolifera* are the diploid progenitors of this species. Since meiosis in diploid and triploid hybrids results in extensive intergenomic pairing it is concluded that the natural tetraploid has a bivalent promoting mechanism that prevents pairing between the genomes of its diploid progenitors.

The section *Kohlrauschia* (KUNTH) BALL & HEYWOOD of the genus *Petrorhagia* (SER.) LINK (*Caryophyllaceae*) comprises four annual, self-compatible species. Breeding systems, hybridization and geographical distribution have been recently investigated in this group (THOMAS & MURRAY 1981). One of the species, *P. prolifera* (L.) BALL & HEYWOOD, consists of two distinct floral forms which appear to be reproductively isolated and which will be classified as separate species in a taxonomic revision of the section (THOMAS in prep.). Breeding systems range from facultative xenogamy in *P. glumacea* (BORY & CHAUB.) BALL & HEYWOOD and large flowered *P. prolifera* to autogamy in *P. velutina* (GUSS.) BALL & HEYWOOD.

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Previous reports on the cytology of the section *Kohlrauschia* have been mainly confined to counts of chromosome number (BLACKBURN 1933, FAVARGER 1946, BÖCHER & al. 1953, LÖVE & LÖVE 1961, BALL & HEYWOOD 1962, GADELLA & KLIPHUIS 1970, HOLUB & al. 1972). However, BÖCHER & al. (1953) have recorded the presence of a distinctive karyotype in *P. velutina*. The tetraploid member of the section, *P. nanteuilii* (BURNAT) BALL & HEYWOOD was considered to be a cytological race of the diploid *P. prolifera* by BLACKBURN (1933) and BÖCHER & al. (1953). BALL & HEYWOOD (1962), on the other hand, have suggested that *P. nanteuilii* is an allotetraploid derived from the diploid species *P. prolifera* and *P. velutina*.

This study deals with chromosome numbers, karyotypes and meiotic behaviour in the species and artificially produced hybrids of the section *Kohlrauschia*.

Materials and Methods

The plants used in this study were obtained from seed of naturally growing populations or from botanic gardens. Details of the original location and code for each population studied are given in Table 1. Voucher specimens are deposited at the British Museum (Natural History), London (BM).

For cytological examination of somatic chromosomes, actively growing root tips were pre-treated with a saturated solution of para-dichlorobenzene for 18-20 hours at 4 °C, fixed in 1:3 acetic alcohol for 24 hours and stained in Feulgen for 2 hours after 9 minutes hydrolysis in NHCl at 60 °C.

Meiosis was studied in pollen mother cells of immature flowers that had been fixed in a modified Carnoy's fluid (absolute ethanol, chloroform, acetic acid 6:3:1) and squashed in lactopropionic orcein.

For the synthesis of artificial tetraploids an 0.5% aqueous solution of colchicine, stabilized with the addition of 1% agar, was pipetted onto the stem apex of young seedlings. Successful induction of polyploidy was indicated by the production of dark green, swollen leaves a few weeks later. Confirmation of polyploidy was obtained by subsequent meiotic analysis.

Hybrids were produced and their pollen viability tested using previously described techniques (THOMAS & MURRAY 1981).

Results

Chromosome Numbers and Karyotypes. All chromosomes counts for the species of the section *Kohlrauschia* are given in Table 1. Where possible, counts of chromosome number were made from populations drawn from a wide area of the species range. The results of this investigation confirm earlier counts and show that the base number for the section *Kohlrauschia* is $x = 15$.

Table 1. Details of the origin, location and chromosome number of populations of species in *Petrorhagia* sect. *Kohlrauschia* used in the present investigation and their codes (G = garden origin, W = wild origin)

Population number	Accession number (RBG, Kew)[1]	Locality Data	Chromosome number $2n$
P. prolifera			
1	000 69 12718	Jocketa, Vogtland, GDR (DDR), Germany (W)	30
2	000 69 12702	Near Nikita, Crimea, Ucrainian SSR, U.S.S.R. (W)	30
3	000 69 13719	Ain, Switzerland (G)	30
5	173 71 01586	5 km E. of Blagaj, Mostar, Hercegovina, Yugoslavia (W)	30
6	173 71 01590	3 km N. of Kărnare (betw. Klisura and Sopot), Bulgaria (W)	30
7	070 70 00618	Tuscany (Toscana), Italy (G)	30
9	644 75 10009	Budapest, Hungary (G)	30
10	647 75 10012	Sully-sur-Loire, France (G)	30
11	173 71 01592	7 km E. of Petrovac, Montenegro, Yugoslavia (W)	30
12	606 74 10622	Koulaura, Corfu, Greece (W)	30
14	046 70 00046	Besancon, France (G)	30
15	173 71 01591	4 km N. of Klis, S. W. Croatia, Yugoslavia (W)	30
16	173 71 01589	1 km S. of Kalokastron, E. Macedonia, Greece (W)	30
17	173 71 01593	6 km N. of Čepelare, Bulgaria (W)	30
18	173 71 01587	8 km N. of Kastoria, Greece (W)	30
19	713 76 70258	St. Martin-la-Riviere, Vienne, France (W)	30
40	710 76 10203	Aveny, Doubs, France (W)	30
105	605 74 10483	3 km N. Paradhisia, Greece (W)	30
P. glumacea			
32	605 74 10407	4 km N. of Sparta, Greece (W)	30
33	605 74 10427	3 km W. of Geraki, Greece (W)	30
P. velutina			
22	000 69 12496	Turkey (W)	30
24	000 69 17496	Apoilka, Andros Is., Greece (W)	30
25	605 74 10537	5 km N. of Lalas, Greece (W)	30
27	603 74 10145	3 km S. of Vroukes, Crete, Greece (W)	30
28	604 74 10344	1 km E. of Aetos, Evvia Is., Greece (W)	30
29	328 70 03144	E of Kyrenia, Cyprus (W)	30
30	605 74 10479	8 km E of Fialta, Greece (W)	30
P. nanteuilii			
20	724 76 10404	2 km S of Foinbellida, Spain (W)	60
34	000 69 12777	N. W. of Tafira Alta, Gran Canaria, Canarian Islands (W)	60
35	244 69 02165	Botanic Garden, Lisbon, Portugal (G)	60
36	645 75 10010	La Carolina, Spain (W)	60
37	727 66 10439	Biarritz, France (W)	60
61	773 76 11026	Ain Leuh, Middle Atlas, Morroco (W)	60

[1] RBG, Kew. Accession number of the Royal Botanic Garden, Kew, for location of seed collections and herbarium specimens.

The karyotypes of *P. glumacea* and *P. prolifera* appear to be very similar and consist of 15 pairs of metacentric chromosomes of similar size, approximately 1 μm long (Fig. 1 *a*, *b*). The karyotype of *P. velutina* differs from the other two diploids only by one pair of chromosomes which are telocentric and approximately 0.5 μm long (Fig. 1 *c*). *P. nanteuilii* has 29 pairs of metacentric chromosomes similar in size to those present in the diploids and one pair of telocentric chromosomes 0.5 μm long (Fig. 1 *d*). The chromosomes of an artificial triploid hybrid *P. prolifera* × *P. nanteuilii* are illustrated in Fig. 1 *e*.

Meiotic Behaviour in the Species. The meiotic behaviour of the species was investigated as a prelude to a study of the meiotic behavior of the artificially produced hybrids, and the results are shown in Table 2. *P. velutina*, *P. glumacea* and *P. prolifera* (Fig. 1 *f*) form 15 bivalents with one chiasma per bivalent, although bivalents with two chiasmata are occasionally formed in the population of large flowered *P. prolifera*. Chiasma frequency is very similar in the three species. The tetraploid species, *P. nanteuilii* is exclusively bivalent-forming. Because of the large number of bivalents assembled at metaphase 1, it was not possible to unambiguously estimate chiasma frequency; however, bivalents with a single chiasma were frequently formed (Table 2).

Meiotic Behaviour in Interspecific Hybrids. Details of the meiotic analysis of the hybrids are given in Table 3. In the three sets of hybrids *P. prolifera* × *P. glumacea* meiosis was characterized by regular bivalent formation. Hybrids between small-flowered *P. prolifera* and *P. glumacea* exhibited failure of pairing in one, and occasionally two pairs of chromosomes in a small percentage of PMCs (14.7% in 5 × 32 and 0.9% in 3 × 32). Pollen fertility was low in both sets of these hybrids, showing a mean of 5.1% (range 0.6-9.9%) in 5 × 32 and 14.3% (range 4.7-25.1%) in 3 × 32. Hybrids between large-flowered *P. prolifera* and *P. glumacea* (105 × 32) on the other hand, had a mean pollen fertility of 74.1% (range: 68-85%).

Triploid hybrids *P. prolifera* × *P. nanteuilii* showed trivalents, bivalents and univalents at meiosis (Fig. 1 *h*) and the percentage of multivalents varied between plants (29.7-53.0%) (Table 3). Because of the large number of chromosomes present, it was not possible to accurately determine the pairing behavior of the single telocentric chromosome donated from *P. nanteuilii*. Micronuclei were conspicuous in the early stages of tetrad formation, pollen development was subsequently disturbed, and all plants were male sterile. Ovule fertility was similarly affected as backcrosses to either parent failed when the hybrid was used as the female parent.

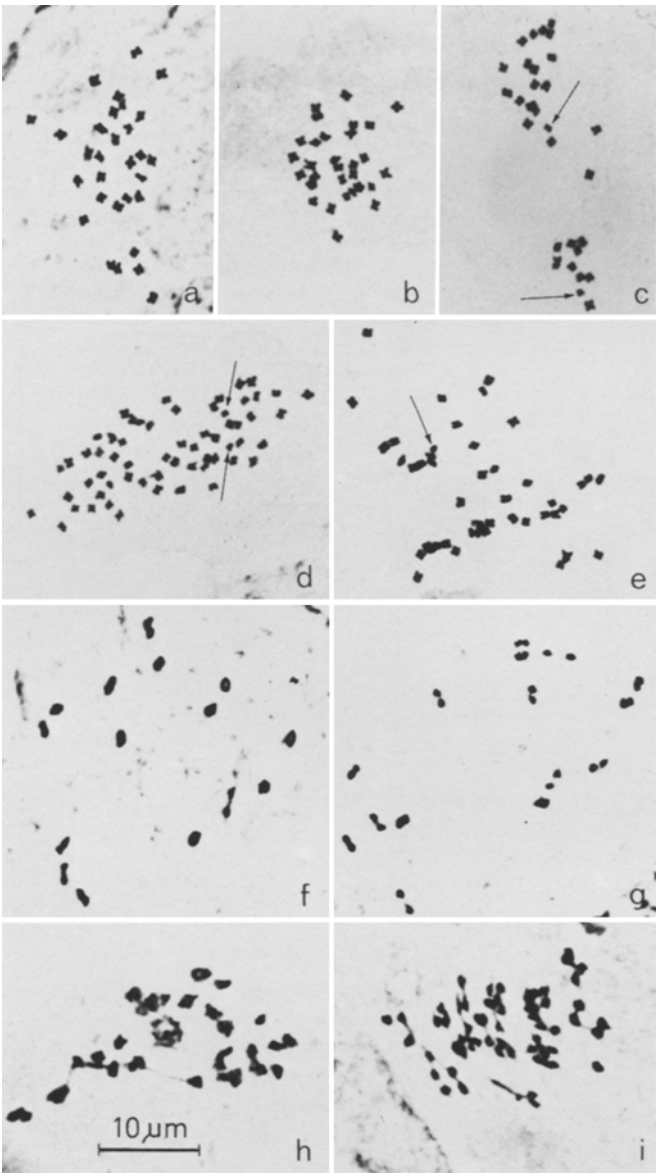

Fig. 1. Mitotic and meiotic chromosomes of the species of *Petrorhagia*, sect. *Kohlrauschia*. *a* mitotic metaphase in *P. glumacea*; *b* mitotic metaphase in *P. prolifera*; *c* mitotic metaphase in *P. velutina*, note telocentric pair (arrows); *d* mitotic metaphase in *P. nanteuilii*, note telocentric pair (arrows); *e* mitotic metaphase in the triploid hybrid *P. prolifera* × *P. nanteuilii*, note single telocentric chromosome (arrow); *f* meiotic metaphase I in *P. prolifera* (small-flowered); *g* meiotic metaphase I in a hybrid between the large and small-flowered races of *P. prolifera*; *h* meiotic metaphase I in the triploid hybrid *P. prolifera* = *P. nanteuilii*; *i* meiotic metaphase I in a colchicine induced tetraploid of *P. velutina*

Table 2. Chromosome configurations at metaphase 1 and chiasma frequencies in species of *Petrorhagia* sect. *Kohlrauschia*. Values are means of N cells/plant, population numbers are given in brackets

Plant No.	No. of Cells (N)	Chromosome association	$\bar{x}$ chiasma/cell
P. prolifera			
(18) 1	20	15 II	15.3
2	20	15 II	15.5
3	20	15 II	15.0
4	11	15 II	15.1
5	6	15 II	15.0
6	20	15 II	15.1
7	18	15 II	15.0
			$\bar{x}$ 15.1
(3) 1	20	15 II	—
2	9	15 II	15.0
3	11	15 II	15.0
4	3	15 II	—
			$\bar{x}$ 15.0
P. velutina			
(29) 1	20	15 II	15.0
2	6	15 II	15.0
			$\bar{x}$ 15.0
P. glumacea			
(32) 1	20	15 II	15.0
2	12	15 II	15.0
3	20	15 II	15.0
4	20	15 II	15.0
5	20	15 II	15.0
			$\bar{x}$ 15.0
P. nanteuilii			
(35) 1	20	30 II	—
2	20	30 II	—
3	20	30 II	—
4	5	30 II	—
5	9	30 II	—

Seed viability was very low in the progeny of the cross *P. glumacea* × *P. nanteuilii* (32 × 25) as a result of seed incompatability and consequently only one triploid hybrid was available for meiotic analysis. Chromosomes associated as trivalents, bivalents and univalents (Table 3) and no fertile pollen was produced.

Meiotic Behaviour in Intraspecific Hybrids. In hybrids between small-flowered and large-flowered *P. prolifera* (3 × 18), the chromosomes showed regular pairing behaviour and appeared as rods with a

Table 3. Chromosome configurations at metaphase 1 in synthesized *Petrorhagia* hybrids. Values are means of N cells/plants, range in brackets

Plant No.	No. of cells (N)	Chromosome association I	II	III	% Multivalents
P. prolifera × *P. glumacea*					
3 × 32					
1	20	0	15.0 (15)	0	0
2	20	0.2	14.9 (14–15)	0	0
3	20	0	15.0 (15)	0	0
4	9	0	15.0 (15)	0	0
5	16	0	15.0 (15)	0	0
6	16	0	15.0 (15)	0	0
32 × 5					
1	20	0.5	14.75 (14–15)	0	0
2	20	0.6	14.70 (14–15)	0	0
3	20	0.1	14.95 (14–15)	0	0
105 × 32					
1	20	0	15.0	0	0
2	17	0	15.0	0	0
3	7	0	15.0	0	0
4	20	0	15.0	0	0
5	7	0	15.0	0	0
P. prolifera × *P. nanteuilii*					
5 × 35					
1	20	9.30	9.30	5.75 (2–10)	35.7
2	20	8.65	8.65	6.35 (4–8)	42.3
3	20	7.25	7.25	7.65 (5–9)	51.3
4	20	7.05	7.05	7.95 (5–9)	53.0
5	20	10.55	10.55	4.45 (1–9)	29.7
P. glumacea × *P. nanteuilii*					
32 × 35					
1	8	8.50	8.50	6.50 (3–9)	43.0
P. prolifera (small flowered) × *P. prolifera* (large flowered)					
3 × 18					
1	20	0	15	0	0
2	20	0	15	0	0
3	20	0	15	0	0
4	20	0	15	0	0

single chiasma (Fig. 1*g*, Table 3). Failure of pairing between one pair of chromosomes was recorded in 3% of PMCs. Pollen fertility was low with a mean of 14% and range of 1-37%. F_2, and backcross progeny produced from these hybrids showed an increased pollen fertility. F_2 progeny showed a mean of 54.7% (range 7.3-96.0%) and backcross progeny, obtained by backcrossing the F_1, hybrids to the large-flowered parent (18), had a mean of 48% (range 14.3-97.0%).

Table 4. Chromosome configurations at metaphase 1 in synthesized autotetraploids of *P. prolifera* and *P. velutina*. Values are the means of 20 cells/plant, range in brackets

Plant No.	Chromosome association II	IV	% Multivalents	'Minimum' $\bar{x}$ chiasma/cell
P. prolifera				
1	16.8	6.60 (3-9)	44.0	36.6
2	16.3	6.85 (2 9)	47.1	36.8
				$\bar{x}$ 36.7
P. velutina				
1	13.5	8.3 (6-11)	54.6	38.3
2	11.4	9.3 (7-12)	62.0	39.3
3	16.8	6.9 (5-10)	46.0	36.9
4	13.4	8.3 (3-10)	54.6	38.3
				$\bar{x}$ 38.2

Meiotic Behaviour in Synthesized Tetraploids. The frequency of the chromosome associations in synthesized tetraploids of *P. prolifera* and *P. velutina* are given in Table 4. The synthesized tetraploid of *P. prolifera* shows bivalent and quadrivalent formation at meiosis; trivalents and univalents were not observed. The percentage of chromosomes associated as quadrivalents ranged from 44-47%. It was not possible to estimate total chiasma frequency per cell on account of the small size of the quadrivalents. However, as it can be assumed that at least three chiasmata per quadrivalent and at least one per bivalent were present, it is possible to calculate a 'minimum' chiasma frequency. The mean value obtained was 36.7 per cell, or 0.61 per chromosome. This represents an increase of 0.11 over the diploid value of 0.5 per chromosome. Pollen fertility was high with a mean of 81.4% (range 80.5-82.1%).

Chromosome behaviour in synthesized autotetraploids of *P. velutina*, illustrated in Fig. 1*i*, was very similar; chiasma frequency showed an increase of 0.13 per chromosome over the diploid value and pollen fertility was also high (mean 91.3%, range 85-96%).

Discussion

Morphological variation in the largely symmetrical karyotypes of the four species is limited to the presence of one pair of telocentric chromosomes in *P. velutina* and *P. nanteuilii*. The origin and stability of telocentric chromosomes has been the subject of considerable controversy and it is only recently that they have been accepted as a permanent feature of some organisms (JOHN & LEWIS 1968, JONES 1970).

Two schemes can be envisaged for the origin of the telocentric chromosomes in *Petrorhagia*. Firstly, they may be derived from metacentric chromosomes as a result of pericentric inversion or centric fission. As the telocentrics are only half the length of the metacentrics it seems unlikely that a pericentric inversion could be responsible for such a change. Starting with a karyotype of 30 metacentrics, centric fission of two metacentric homologues and the subsequent loss of two of the four telocentrics so formed would account for the karyotype of *P. velutina*. There are a number of convincing instances involving the production of stable telocentrics by centric fission of metacentric chromosomes in natural populations, for example in *Nigella doerfleri* (STRID 1968). However in all these cases, derived telocentrics are retained within the chromosome complement.

An alternative explanation is based on the idea that the original karyotype of *P. velutina* was entirely telocentric with $2n = 30$. Polyploidy, followed by successive centric fusion would culminate in the evolution of the basic 'diploid' *Petrorhagia* karyotype. Incomplete fusion coupled with the loss of two telocentrics would result in the *P. velutina* karyotype. A similar mechanism has been proposed to account for the existence of diploid and tetraploid species with the same chromosome numbers in the genus *Cymbispatha* (JONES 1977). Recent work in the genus *Silene* lends further support to the idea of cryptic polyploidy outlined above. Most *Silene* species have a diploid number of $2n = 24$ and the base number of the genus has been thought to be $x = 12$. However MIEGE & GREUTER (1973) have reported a diploid number of $2n = 12$ for *Silene vulgaris* which raises fundamental doubts about the apparent diploid status of the $2n = 24$ plants.

Unfortunately, the homologies between the telocentric and metacentric chromosomes could not be determined in artificial hybrids as *P. velutina* is unable to hybridize with the other three species. In addition, in artificially produced triploid hybrids the telocentric chromosomes donated by *P. nanteuilii* could not be readily distinguished at meiosis.

As the chromosome changes in this instance are not accompanied by changes in breeding system or ploidy level, it is difficult to determine the direction of chromosome evolution. If the telocentric pair are

derived from metacentrics as outlined above, it is not easy to explain why natural selection has favoured an individual which has lost a whole chromosome. The alternative scheme postulating successive centric fusion is more acceptable since the chromosome loss would be buffered by the polyploid nature of the chromosome complement.

The presence of a single pair of telocentric chromosomes in tetraploid *P. nanteuilii* suggests an allopolyploid origin for this species. As the telocentric pair are similar in morphology to those in *P. velutina*, it seems highly probable that the latter is a putative diploid parent. However, the possibility remains that the loss of two chromosome arms could have occurred after autopolyploidy. The meiotic behaviour of the tetraploid is typical of an allopolyploid in that it is exclusively bivalent forming. However, our observations on chromosome pairing in interspecific hybrids of these species casts doubt on the validity of calling *P. nanteuilii* an allotetraploid. In the diploid hybrids *P. glumacea* × *P. prolifera*, pairing behavior indicates that no major structural rearrangements have taken place during the evolutionary divergence of these two species, although minor differences must be present as indicated by the low fertility of some of these hybrids. Similarly, in crosses between *P. nanteuilii* with *P. prolifera* and *P. glumacea* there is extensive chromosome pairing with the formation of trivalents being a common event. In both cases up to nine trivalents are formed in some cells which indicates that there is considerable homology between the constituent genomes of the tetraploid and also between them and the genomes of the two diploid species. Synthetic autotetraploids of *P. velutina* and *P. prolifera* showed significant increases in chiasma frequency/chromosome over the diploid values and formed quadrivalents at fairly high frequencies. Therefore, the chromosomes of these *Petrorhagia* species are not too small to allow quadrivalent formation. Thus we can conclude that *P. nanteuilii* is genomically autoploid although it probably is of hybrid origin. This raises the question as to why does this species not form multivalents at meiosis when synthetic tetraploids and artificial triploid hybrids show extensive multivalent formation. In *P. nanteuilii* it may simply be that there are not sufficient chiasmata to permit multivalent formation since the chiasma frequency is seldom greater than one per bivalent. There is no need therefore, to postulate that genetic diploidising mechanisms such as those found in wheat (RILEY & CHAPMAN 1954), *Avena strigosa* (LADIZINSKY 1973), some *Festuca* species (JAUHAR 1975) and a number of other genera. Although this may be true, a further problem arises when the pairing behaviour of the triploids is examined, since they show high frequencies of trivalents. Clearly homologous pairing is occurring in the

triploid hybrids but not in the tetraploid. VIDA (1970) found a very similar situation in tetraploid *Asplenium rutamuraria* and in triploid hybrids produced from the cross with the diploid subspecies *dolomiticum*. In addition, SIEBER & MURRAY (1980) report high frequencies of trivalents in autotriploid *Alopecurus bulbosus* but low frequencies of multivalents in the autotetraploids of the same species. We propose that *P. nanteuilii* contains a recessive bivalent-promoting gene(s) and on crossing with diploid species such as *P. prolifera* and *P. glumacea* which have the dominant, wild type allele, the triploid progeny will be heterozygous and therefore exhibit multivalent formation.

It would seem reasonable to suggest that *P. nanteuilii* has arisen from the diploid species *P. prolifera* and *P. velutina* by hybridization and chromosome doubling. All these species are sympatric in W. Europe and it is envisaged that hybridization occurred before the evolution of the breeding barrier that isolates *P. velutina*. It is unlikely that *P. glumacea* has donated one of the genomes in *P. nanteuilii* in view of their extremely disjunct distribution (THOMAS & MURRAY 1981). It is also relevant to note that BALL & HEYWOOD (1962) considered the morphology of *P. nanteuilii* to be intermediate between that of *P. prolifera* and *P. velutina*.

Chromosome pairing behaviour in the diploid hybrids *P. glumacea* × *P. prolifera* indicates that no major structural rearrangements of the chromosomes have taken place during the evolutionary divergence of these two species. It is of interest to note that the large and small-flowered forms of *P. prolifera* differ in their phylogenetic relationship with *P. glumacea*. Hybrids with small-flowered *P. prolifera* as a parent show slight disturbances during meiosis and are highly sterile. Large-flowered *P. prolifera* on the other hand, forms highly fertile hybrids with *P. glumacea*. It seems unlikely that selection has been important in the evolution of the breeding barrier between small-flowered *P. prolifera* and *P. glumacea* as their distributions do not appear to overlap. Gene flow may occur, however, between large flowered *P. prolifera* and *P. glumacea* since they grow in close proximity in Greece (THOMAS & MURRAY 1981). The behaviour of the intraspecific hybrids between large and small-flowered *P. prolifera* provides further evidence for genetical differentiation between these two floral forms. Although chromosome pairing appeared to be normal, pollen fertility was very low, presumably as a result of cryptic structural differences or genic sterility. As selfing and backcrossing of the F_1 hybrids produced a proportion of offspring with much improved fertility, it is possible, but nevertheless unlikely, that limited gene flow is occurring in the wild. These findings contrast with those of MOORE & LEWIS (1965) in *Clarkia*

xantiana; hybrids between selfing and outbreeding races were highly fertile. However, as racial differentiation within species is a dynamic and frequently intrinsic part of speciation (WHITE 1978), it is not surprising that intraspecific hybrids vary in their fertility.

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