Cytology and hybridization in the *Juncus bufonius* L. aggregate in western Europe

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ABSTRACT

Chromosome counts in the *Juncus bufonius* L. aggregate (Juncaceae) in Europe are presented. *J. foliosus* Desf. (2n=26), *J. ambiguus* Guss. (2n=34), *J. hybridus* Brot. (2n=34) and *J. sorrentinii* Parl. (2n=28) are considered to be near-diploids, while our concept of *J. bufonius* sensu stricto (incl. *J. minutulus* Krecz. & Gonch.) includes plants at near-tetraploid and hexaploid (and perhaps octoploid) levels. The process of meiosis is described, and the possibility of the existence of diffuse centromeres and inverted meiosis is briefly discussed; the evidence is still equivocal. The results of hybridization experiments between the five segregates of the aggregate are presented. Two hybrid plants were raised from 490 pollinations, both *J. ambiguus* × *J. foliosus*. The plants were more or less intermediate between their parents but totally sterile, with an increased period of flowering and an increased number of flowers per inflorescence unit. Apart from their sterility and floriferousness the hybrids fell within the range of variation of *J. bufonius* sensu stricto.

It is concluded that *J. bufonius* agg. constitutes a polyploid 'pillar' complex, with four diploids in western Europe and a very variable polyploid (*J. bufonius* sensu stricto) at two or three ploidy levels. While the nature of the diploids which originally gave rise to the polyploid complex is not known, it seems likely (judging from the hybridization experiments) that the process is still continuing.

INTRODUCTION

Juncus bufonius agg. belongs to subgenus *Poiophylli* Buch., which comprises annual species with grass-like leaves and a rather diffuse, leafy terminal inflorescence. Also included in the subgenus are *J. tenageia* Ehrh. and *J. sphaerocarpus* Nees, both of which differ from *J. bufonius* agg. in having a spherical rather than oblong capsule. *J. bufonius* agg. is a highly polymorphic group whose morphology has been intensively studied with the result that a number of taxa within it have been recognized at species level. The five species recognized by us in western Europe are (Cope & Stace 1978, 1983): *J. bufonius* L. sensu stricto (incl. *J. minutulus* Krecz. & Gonch.), *J. foliosus* Desf., *J. ambiguus* Guss. (*J. ranarius* Song. & Perr.), *J. hybridus* Brot. and *J. sorrentinii* Parl. These differ in rather critical, but nevertheless constant, ways, mainly on the basis of floral morphology. However, they do share the same weedy habitat and much the same growth form. The inflorescence is of the type technically described as an anthela, but often loosely (and incorrectly) referred to as a panicle. It begins as a dichasial cyme, but after one or two nodes many of the branches become monochasial. Sporadic dichasia may reappear at some of the upper nodes. The internodes between flowers vary considerably in length, and a monochasium may bear from one to six (exceptionally twelve) flowers. Individual plants, especially those that develop into tufts, can remain in flower for a considerable period and seed output is enormous. In the more northerly parts of its range, the flowers of *J. bufonius* agg. are mostly cleistogamous, but there are reports of chasmogamy from warmer latitudes. The evidence, however, is largely anecdotal and to some extent contradictory, so careful observations are still needed (see Laurent (1904) and Shah (1963) for conflicting views).

TABLE 1. PUBLISHED CHROMOSOME COUNTS FOR THE *JUNCUS BUFONIUS* AGGREGATE

J. nastanthus and *J. minutulus* are included by us in *J. bufonius* sensu stricto, and *J. rechingeri* and *J. turkestanicus* occur outside our area of study (Europe). We consider *J. ranarius* and *J. ambiguus* to be synonymous.

Species name used by investigator	Origin of material	Chromosome number ($2n$)	Reference
J. bufonius L. agg.	Sweden	30	Hedberg & Hedberg (1964)
	Rumania	30	Tarnavschi (1948)
	Canada	34	Taylor & Mulligan (1968)
	Finland	c. 54	Hämet-Ahti & Virrankoski (1970)
	—	c. 60	Delay (1947a, 1947b)
	—	c. 60	Rohweder (1937)
	Iceland	c.120	Löve & Löve (1948, 1956)
J. bufonius L. sensu stricto	Finland	c. 54	Ahti & Hämet-Ahti (1971)
	Germany	c. 60	Wulff (1937)
	Denmark	80	Jörgensen *et al.* (1958)
	—	80	Segal (1962)
	Czechoslovakia	80	Uhrikova (1974)
	Netherlands	100–110	Loenhoud & Sterk (1976)
	—	100–110	Snogerup (1971)
	—	104–106	Snogerup (1959)
	Sweden	106	Weimarck (1963)
	Canada	106	Löve & Löve (1981)
	Canada	106	Löve *et al.* (1980)
	U.S.A.	c. 108	Harriman & Redmond (1976)
J. minutulus Alb. & Jah.	Netherlands	c. 70	Loenhoud & Sterk (1976)
	?Iran	72	Snogerup (1971)
J. minutulus Krecz. & Gonch.	—	30	Podlech & Dieterle (1969)
J. nastanthus Krecz. & Gonch.	—	60	Podlech & Dieterle (1969)
J. ranarius Song. & Perr.	Denmark	30	Jörgensen *et al.* (1958)
	Greenland	30	Jörgensen *et al.* (1958)
	—	34	Snogerup (pers. comm. 1967,
	Sweden	34	Weimarck (1963)
	Canada	34	Löve & Löve (1981)
	Netherlands	34	Loenhoud & Sterk (1976)
	Germany	c. 108–120	Wulff (1937)
J. ambiguus Guss.	—	30	Segal (1962)
	—	32	Segal (1962)
	Colorado, U.S.A.	34	Löve *et al.* (1971)
	Iceland	34	Löve (1970)
J. hybridus Brot.	?Iran	34	Snogerup (1971)
J. rechingeri Snog.	Afghanistan	30	Snogerup (1971)
J. turkistanicus Krecz. & Gonch.	Afghanistan	30	Podlech & Dieterle (1969)

The object of this paper is to outline the results of chromosome studies – both meiotic and somatic – and breeding experiments, and briefly to discuss the bearing these have on the evolution of the group.

Numerous chromosome counts have been provided by earlier workers (Table 1) but, since many of them are imprecise and derived from material whose correct identity has not been verified, they were discounted when chromosome numbers were given in the first of this series of papers (Cope & Stace 1978).

TABLE 2. CHROMOSOME NUMBERS OF THE *JUNCUS BUFONIUS* AGGREGATE DETERMINED DURING THIS STUDY

Somatic counts given as 2n, meiotic counts as n. Vouchers are in **MANCH** in addition to those indicated in the Table.

Juncus bufonius L. sensu stricto	
J371 – Knockbrandon, Co. Wexford, v.c. H12, Eire	n=54
SL1 – Merebere, Holbeck, Belgium	n=54
SL4 – Chilly-sur-Salins, Jura, France	n=54
SL10 – Pohjois – Pohjanmaa, Österbotten, Finland	n=54
SL13 – Ficuzza, Palermo, Sicily	n=54
Juncus foliosus Desf.	
J199 – Port, N. of Glencolmalle, W. Donegal, v.c. H35, Eire	2n=26
J313 – Rathlough, E. Donegal, v.c. H34, Eire	2n=26
J355 – Ardmore to Kilkearan,.W. Galway, v.c. H16, Eire	2n=26
J370 – Bargy Commons, Co. Wexford, v.c. H12, Eire	2n=26
J372 – Ballyknockan, Co. Wicklow, v.c. H20, Eire	2n=26
J373 – Near Laragh, Co. Wicklow, v.c. H20, Eire	2n=26
J393 – Barmouth, Merioneth, v.c. 48, Wales	2n=26
J584 – Llyn Peris, Caernarvonshire, v.c. 49, Wales	2n=26
Juncus ambiguus Guss.	
J334 – Lady's Island Lake, Co. Wexford, v.c. H12, Eire	2n=34
J359 – Smerwick Harbour, S. Kerry, v.c. H1, Eire	2n=34
J360 – Ballymacoda, E. Cork, v.c. H5, Eire	2n=34
Juncus hybridus Brot.	
J212 – San Nicola, Messina to Villafranca, Messina, Sicily (**LTR**)	n=17
J214 – San Nicola, Messina to Villafranca, Messina, Sicily (**LTR**)	n=17
J391 – Near C'an Picafort, Mallorca, Spain (**LTR**)	2n=34
J392 – La Puebla del Rio to Isla Mayor, Sevilla, Spain (**LTR**)	2n=34
J599 – Terceira, Azores	2n=34
SL11 – Alfonte, Palermo, Sicily	n=17
Juncus sorrentinii Parl.	
J581 – Near Caniçal, Madeira	2n=8

MATERIALS AND METHODS

MATERIALS

We used a total of 85 accessions representing all five segregates of *J. bufonius* agg. that we recognize. These were collected as seed or sometimes living plants by us and correspondents, or acquired via international seed exchange schemes. Chromosome counts were achieved from 23 accessions, and full details of these are given in Table 2.

SOMATIC CHROMOSOMES

Somatic chromosomes proved to be the more problematical and some considerable experimentation was needed before satisfactory results were obtained. The initial problem encountered was that of preferential absorption of stain by cytoplasmic granules and oil droplets over the chromosomes themselves. The material did not respond well to conventional fixing, mordanting and staining procedures, so a new technique, derived from suggestions made by Thomas (1940) for use with difficult material in Rosaceae, was developed.

The three stages of fixing, mordanting and staining were achieved with a single solution modified from Carnoy's Solution (3 parts absolute alcohol: 1 part glacial acetic acid). The acetic acid fraction of Carnoy's Solution was replaced by a mixture comprising 9 parts saturated aceto-carmine in glacial acetic acid, and 1 part saturated ferric acetate in glacial acetic acid. Once root-tips had been treated they could be stored in this solution in a deep-freeze for up to 2 months without noticeable deterioration.

After fixation, a corresponding lack of response to conventional hydrolyzing agents was also experienced. Root-tips were therefore not hydrolyzed, reliance being placed instead on efficient tapping-out and smearing.

The full schedule is as follows:

1 Sow seeds on filter-paper in petri-dishes.
2 Water with tap-water and place in an incubator set at 20°C with a 24 hour photoperiod.
3 When roots are about 1 cm long place the petri-dishes in a 5°C refrigerator overnight to reduce activity in the meristem.
4 Restore seedlings to room-temperature for 2 hours to allow synchronized cell division to begin.
5 Collect root-tips at intervals of 20–30 mins during the 2 hours to determine the optimum recovery period after cold shock.
6 Fix root-tips in alcoholic iron-aceto-carmine for 48 hours at room-temperature. If need be, after fixation, root-tips can be transferred to fresh solution and stored in a deep-freeze until required.
7 Tap-out root-tips, smear and squash in fresh iron-aceto-carmine (omit the alcoholic fraction from the fixative).
8 Seal cover-glass with rubber solution.

Once preparation of the slide was complete it was scanned for chromosomes and suitable cells were immediately drawn and, if good enough, photographed. No satisfactory technique for preparing permanent slides was found.

MEIOTIC CHROMOSOMES

The only serious problem encountered with meiotic chromosomes, apart from those already described for somatic chromosomes, was discovering the time of day at which meiosis takes place. It was eventually established at about midday, but depended to a great extent on prevailing weather conditions. For meiotic counts, stages 6–8 of the schedule devised for somatic chromosomes were followed.

BREEDING EXPERIMENTS

In order to test relationships between the segregates, a considerable number of artificial cross-pollinations was performed, but the rate of success was very low. Full details of the experimental procedures are given here but discussion of the background relating to floral biology is reserved for later.

Our own observations indicated that *J. bufonius* agg. is almost exclusively cleistogamous, although on occasion the flowers open for 2–3 hours after anthesis. Meiosis commences 40–45 hours before anther dehiscence. Pollen maturation occurs during the day following meiosis. The larger anthers available during the period of pollen maturation were found to be too fragile for safe manipulation, so emasculation was performed on the day of meiosis. The flowers at this time are 3–4 mm long and tightly closed, with the overlapping tepal margins somewhat adherent. Care is needed when handling the flower because it readily disarticulates at the base of the pedicel.

Once the flower had been opened, the anthers were knocked off their filaments and brushed away. Pollen was collected from a mature flower which was inverted over a watchglass and tapped or shaken. The recipient flower was then inverted and gently dipped into the pollen. Sweeping up of pollen had to be avoided because the connection between ovary and stigma is extremely fragile. After pollination the flower was re-closed as far as possible and sealed in clear adhesive tape. Sealing is vital as it prevents both contamination by air-blown pollen and desiccation of the stigmas. Pollination was performed nearly two days before it would normally have taken place in the flower concerned, so pollen had to remain on the stigma during the latter's maturation. The success of cross-pollination therefore depended to some extent on the longevity of the pollen, a factor which remains unknown.

Nearly all interspecific combinations of male and female parent were attempted and the same strain of each species was used throughout. Because of practical difficulties, no cross-pollinations employing *J. sorrentinii* as female parent were possible.

TABLE 3. STOMATAL DIMENSIONS AND PLOIDY LEVEL IN THE *J. BUFONIUS* AGGREGATE

	Range of means of stomatal length (μm)	Overall mean stomatal length (μm)	Number of specimens	Ploidy level
J. bufonius sensu stricto	24–48	38±1	264	6x
J. foliosus	30–47	38±1	64	2x
J. ambiguus	23–39	30±<1	135	2x
J. hybridus	23–40	32±1	80	2x
J. sorrentinii	29–42	35	12	2x

RESULTS

CHROMOSOME COUNTS

The difficulties encountered in obtaining accurate chromosome counts are to some extent a reflection of chromosome morphology. No chromosome structure was visible through an ordinary light microscope and the chromosomes appear as poorly resolved circular bodies averaging 0·5–0·8 μm in diameter. They are thus visible only near the limit of resolution of the microscope and there is no reason to suppose that they are in fact spherical.

Chromosome counts (Table 2) were obtained from all five species, although they were obtained from both root-tips and pollen mother cells only in *J. hybridus*.

Counts reported in the literature were often quite different from those achieved during the present work, and the variation in number indicated hitherto did not become apparent. Published chromosome numbers for *J. ambiguus* are 2n=30, 32, 34 and c.108–120, but all counts made during the present study were 2n=34. Similarly, our counts for *J. bufonius* sensu stricto were all 2n=108 compared with 2n=30, 34, c.54, c.60, 80, 100–110 and c.120 reported in the literature. Only one count (2n=34) has been reported for *J. hybridus* and this we confirmed. Neither *J. foliosus* (2n=26) nor *J. sorrentinii* (2n=28) has previously been studied cytologically to our knowledge.

Karpechenko (1928) and Sax & Sax (1937) first reported the correlations between cell size and level of polyploidy, and suggested that stomatal dimension would prove to be a useful index of polyploidy. This approach was taken up in *Juncus* by Snogerup (1971), who investigated this relationship in the *J. bufonius* aggregate. He attempted to use the information to predict the chromosome number of *J. turkestanicus* and his prediction was, in fact, confirmed by a later chromosome count. Our own investigation, however, indicates a different correlation from that apparently demonstrated by Snogerup. Table 3 shows that mean values for stomatal length fall into three groups: *J. bufonius* sensu stricto and *J. foliosus* with relatively long stomata (overall means both 38 μm); *J. ambiguus* and *J. hybridus* with relatively short stomata (overall means 30 and 32 μm respectively); and finally *J. sorrentinii*, whose values (29–42 μm, overall mean 35 μm) are intermediate. The interesting point is that *J. foliosus*, with a low chromosome number, is more similar to *J. bufonius* sensu stricto, a high polyploid, than to either of the other taxa with low chromosome numbers. It may be worth pointing out that the two species with the longest stomata are both mesophytes, whereas those with short stomata are xerophytes (one, *J. hybridus*, a plant of dry places, the other *J. ambiguus*, a saltmarsh plant subject to physiological drought). Cope & Stace (1983) have given observations on the plasticity of stomatal length.

FLOWERING BEHAVIOUR

The inflorescence of *J. bufonius* agg. is basically cymose with upwards sequential development of flowers. The number of flowers produced by each monochasium is theoretically unlimited, although in *J. bufonius* agg. it rarely exceeds five or six. Exceptionally there may be as many as twelve; quite often there is only one.

The flower terminating the main axis emerges from the sheath of its subtending bract three days before anthesis and, on emergence, the next flowers, the lowest on each monochasium, are already visible. Meiosis occurs in the first flower on the day following emergence, usually at or about midday.

Meiosis seems to be more or less synchronous within a single anther with all pollen mother cells at the same stage (although Shah (1963) disagrees on this point). Meiosis between anthers of a single flower is not synchronous, however, and, in preparations of all six anthers made simultaneously at just the right time, all stages of meiosis can be seen.

After meiosis there is no appreciable pause before pollen mitosis begins, although between first and second meiotic divisions and between meiosis and mitosis the nuclei do enter a short interphase of about half an hour. There is no delay of up to 24 hours before pollen mitosis begins, as seen in some species of *Juncus* subgenus *Genuini* (J. W. Grimes 1975, pers. comm.). By mid-afternoon all six anthers contain 8-nucleate pollen mother cells. The nuclei are arranged four towards the centre of the cell and four at the periphery; the former are the generative nuclei (Malheiros, Castro & Camara 1947), and the latter the vegetative (tube) nuclei. Once this stage has been reached cytokinesis begins and maturation of the pollen grain continues throughout the following day; the four products of pollen mother cell division remain united in a tetrad and are dispersed as such. Anthesis occurs at about 40–45 hours after the beginning of meiosis, sometime between sunrise and 8 or 9 a.m. Dehiscence of the anther is by means of a terminal pore in each theca.

The lowest flower on the lower monachasium undergoes anthesis 24 hours after the first flower, and on the day following that the lowest flower on the upper monochasium is pollinated. Thereafter there is an interval of 2 days between pollination events in each monochasium, and events alternate between flowers on the two monochasia arising from each biparous node.

HYBRIDIZATION EXPERIMENTS

A total of 490 cross-pollinations were undertaken (Table 4) and these yielded 26 probable hybrid seeds. In each successful case *J. ambiguus* had served as the female parent, the male parents being *J. bufonius* sensu stricto, *J. foliosus* and *J. hybridus*; one capsule was obtained from each of these crosses and contained eleven, eight and seven seeds respectively. Only two of these 26 seeds were successfully germinated and both were from the *J. ambiguus* × *J. foliosus* cross. In some respects the plants which ultimately developed from these seeds were intermediate between the parents, but in others they were quite different from either. Most significantly, if the origin of the hybrid plants had not been known, they could quite easily have been mistaken for *J. bufonius* sensu stricto, although they were highly sterile.

Table 5 summarises the characteristics of the hybrids (the two plants were identical) compared with those of their parents. The most important differences were in the inflorescence and comprised enlarged monochasia of five or six flowers instead of three or fewer, a much more profusely branched inflorescence, and a much longer flowering period (June 20–October 11, about two months more than either parent). Neither mature seeds nor mature capsules were produced by the hybrids, although pollen showed a 100% staining with aceto-carmine, a situation found in many other sterile *Juncus* hybrids. In size, the pollen was closer to that of *J. ambiguus* than to that of *J. foliosus*.

The capsules themselves stopped developing when about two-thirds the length of the inner tepals. The larger ones were dissected and found to contain numerous aborted ovules and often a few apparently well-developed seeds. Twenty-five such capsules contained an average of 5·9 of these seeds, but on removal from the capsules they collapsed within a few hours.

TABLE 4. NUMBER OF FLOWERS CROSSED IN HYBRIDIZATION ATTEMPTS BETWEEN THE SEGREGATES OF THE *JUNCUS BUFONIUS* AGGREGATE

One capsule of each combination marked* was obtained; all other crosses were unsuccessful.

	Male parent				
Female parent	*J. bufonius*	*J. foliosus*	*J. ambiguus*	*J. hybridus*	*J. sorrentinii*
J. bufonius	—	45	43	53	1
J. foliosus	42	—	31	50	3
J. ambiguus	28*	35*	—	37*	2
J. hybridus	39	47	28	—	6
J. sorrentinii	0	0	0	0	—

TABLE 5. COMPARATIVE MORPHOLOGICAL CHARACTERS OF THE SEGREGATES *J. AMBIGUUS* AND *J. FOLIOSUS* AND THE HYBRID BETWEEN THEM

Measurements of the two species refer solely to plants of the parental strains grown alongside the hybrid plant.

	J. ambiguus	Hybrid	*J. foliosus*
Inflorescence	Sub-fasciculate	Remote-flowered	Remote-flowered
Mean number of flowers per monochasium	2·5	6·0	2·8
Apex of outer tepal	Acute	Acute	Acute
Apex of inner tepal	Rounded-mucronate	Subacute-mucronate	Subacute
Apex of capsule	Truncate	—	Truncate
Mean inner tepal: capsule ratio	0·93	—	1·02
Stripes on tepals	Absent	Weak or absent	Strong
Mean filament: anther ratio	1·17	1·00	0·35
Mean pollen diameter (μm)	40·98	41·23	48·05
Pollen stainability	100%	100%	100%
Seed morphology	Smooth, barrel-shaped	—	Ridged, obovoid
Mean seed size (μm)	390×310	—	500×330
Mean seed length:breadth ratio	1·26	—	1·53
Leaf width (mm)	0·5–1·0	1–1·5	2·0–3·0
Height of plant (cm)	18	22	15

Another interesting characteristic of the hybrid was the filament: anther ratio. Its mean was 1·00, but in fact it varied from 0·63 to 3·00, embracing not only much of the ranges of both *J. ambiguus* and *J. foliosus*, but also a large part of that of *J. bufonius* sensu stricto.

Cytologically, the hybrids were difficult in that they did not respond as well to the alcoholic iron-aceto-carmine stain-fixative developed for use with their parents. A prefixation of 24 hr in acetic-alcohol before stain-fixation went some way to ameliorating the problem.

The earliest meiotic phase seen was anaphase I, in which the expected total of 30 chromosomes was visible (from 2n=34 and 2n=26 in the parents). On many occasions three lagging chromosomes were seen, but sometimes there were fewer or none at all. At metaphase II, what appeared to be a multivalent structure comprising four chromosomes was seen in each nucleus. The apparent contradiction implied by these last two features is considered in the Discussion.

DISCUSSION

With chromosome numbers ranging from 2n=26 to 2n=108 or 120 in the *J. bufonius* aggregate, it is difficult to decide what the basic chromosome number for the aggregate, or even the genus, may be. An assessment of all known chromosome numbers in the genus *Juncus* reveals peaks of occurrence at n=15, 20, 30, 40 and 60 (31% of all numbers are 2n=40), i.e. multiples of 5, 10 or 15. As *J. decipiens* (Buch.) Nakai has 2n=20 and *J. capitatus* Weig. has 2n=18, it is probable that the basic number for *Juncus* is 5, a conclusion tentatively reached by Löve & Löve (1961). 63% of all numbers fall within the range n=13 to n=25, so clearly there is considerable aneuploidy in *Juncus*.

If the base number of the genus really is to be regarded as x=5, then no strictly diploid species (2n=10) are known to exist. Alternatively, one may consider *J. decipiens* (2n=20) and *J. capitatus* (2n=18) to be diploid or near-diploid. However, in view of the wide and frequent occurrence of aneuploidy, it seems best to consider each taxonomic group within *Juncus* separately, especially as the main features of chromosomal evolution differ between many of the subgenera. On this basis we have treated the species of the *J. bufonius* aggregate with chromosome numbers of 2n=26 and 2n=34 as near-diploids, *J. minutulus* and others in the range 2n=54–80 as tetraploids, and *J. bufonius* sensu stricto (2n=100–120) as hexaploids.

The *J. bufonius* aggregate therefore comprises taxa with at least three ploidy levels, with considerable aneuploidy exhibited at each. Since there is no single base number evident in the aggregate, interpretation of the higher numbers must remain conjectural, e.g. 2n=108 could be a hexaploid based on x=18, but perhaps equally 2n=104 could be an octoploid based on x=13. The precise degree of aneuploidy is similarly impossible to ascertain, because the extremely small size of the chromosomes prevents accurate counting at the higher levels other than in exceptionally favourable preparations.

Consideration of the possibly unusual course of meiosis in *Juncus* is likely to be relevant to an understanding of the evolution of the *J. bufonius* aggregate. Observations of meiotic behaviour in *Juncaceae* and *Cyperaceae* have revealed a level of similarity that has led certain authors to draw conclusions on the nature of the meiosis and structure of the chromosome in *Juncus* from behaviour seen in *Carex*, *Scirpus* and *Luzula*.

The earliest references relating to this aspect come from Heilborn (1928) and Wahl (1940), who worked on *Carex*, and Nordenskiöld (1951), who studied *Luzula*. Having noted the absence of lagging chromosomes at anaphase I in certain hybrids, these authors concluded that meiosis in the taxa concerned was 'inverted', i.e. the equational division preceded the reductional. Battaglia & Boyes (1955) doubted that this was the case in *Carex* but considered it to be so in *Juncus*. Wahl (1940) suggested that inverted meiosis occurred in all Cyperaceae, even though Tanaka (1937, 1938, 1939a,b,c, 1940a,b, 1949) stated that bivalents segregated at anaphase I in *Scirpus*. Malheiros *et al.* (1947) thought inverted meiosis was common to all members of both Cyperaceae and Juncaceae. Davies (1956) and Faulkner (1972) have confirmed that it does indeed occur in *Carex*.

Battaglia & Boyes (1955) have fully described inverted meiosis in *Luzula* (although they referred to it as "post-reductional meiosis"). The important point is that at metaphase I each chromosome is auto-orientated with its two chromatids directed to opposite poles, so that first division is, as a result, equational. If the first division is indeed equational and chiasmata are formed from chromatids originating from different chromosomes (Swanson 1958), then it follows that multivalent configurations should be visible at both metaphase I and metaphase II (Nordenskiöld 1962).

The possession of aneuploid series of chromosome numbers in Cyperaceae and Juncaceae is normally attributed to the presence of a non-localized centromere. Apart from the dicentric chromosomes reported by Piza (1939, 1941) and Malheiros & Castro (1947) in *Luzula purpurea* (Marson ex Buch.) Link (=*L. elegans* Lowe), there are two further possible conditions summarized by Rhoades & Kerr (1949). The 'diffuse centromere' (Löve *et al.* 1957) was described as having sites for spindle-fibre attachment spread throughout the length of the body of the chromosome, while the 'polycentric chromosome' (Godward 1951; LaCour 1953) was envisaged as end-to-end union of extremely small metacentric chromosomes. Malheiros *et al.* (1947) considered the evidence in favour of either type in most examples to be inadequate and preferred to call them both 'non-localized centromeres'.

Heilborn (1924) showed that the chromosomes of *Carex* were without centromeric constrictions, a feature later confirmed by Davies (1956). The absence of a centromere in *Luzula* was demonstrated by Malheiros & Castro (1947), Malheiros *et al.* (1947), Castro (1950) and Castro & Noronho-Wagner (1952), and experimentally confirmed by Castro *et al.* (1948, 1949a, 1949b) and Nordenskiöld (1962). After exposing chromosomes to X-rays, these authors observed that the fragments so produced retained mobility at anaphase. A similar experiment, with the same result, was conducted on *Eleocharis palustris* (L.) Roem. & Schult. by Håkansson (1954, 1958). Further reports of a non-localized centromere in *Luzula* come from Östergren (1949), Berger (1949), Brown (1950) and Thomas (1950).

That there is a non-localized centromere in *Juncus* is now generally accepted (Löve & Löve 1944; Malheiros-Gardé & Gardé 1951; but see also Grant (1971, p. 269) for a conflicting view), but it has never been confirmed experimentally. One reason for supposing it might be present is the existence of aneuploids which all seem to behave as conventional diploids. Löve *et al.* (1957) noted that in *Carex* the frequency of large chromosomes decreased with an increase in chromosome number. A similar correlation was found in *Luzula* by Malheiros & Gardé (1947), Nordenskiöld (1949, 1951), Wagner (1949) and Halkka (1964). This correlation has been attributed to fragmentation of single chromosomes or whole chromosome sets, and the survival of the

fragments, which is only possible in chromosomes with a non-localized centromere. The phenomenon was called 'agmatoploidy' by Malheiros-Gardé & Gardé (1950) and Nordenskiöld (1956), 'endonuclear polyploidy' by Nordenskiöld (1951), and 'pseudopolyploidy' by Battaglia (1956). Agmatoploidy thus signifies an increase in chromosome number without any attendant increase in total chromatin mass or chromatid length (Nordenskiöld 1951; Löve *et al.* 1957; Halkka 1964). We consider that our measurements are not sufficiently accurate to ascertain whether this inverse correlation between chromosome size and number holds true for *J. bufonius* agg. DNA estimation would be desirable.

Certain aneuploids in *Luzula* (Nordenskiöld 1951; Löve *et al.* 1957), *Carex* (Heilborn 1924) and *Scirpus* (Tanaka 1938) are thought to be derived by chromosome fragmentation and are therefore incomplete endonuclear polyploids (agmatoploids); they are quite different from the aneuploids originally circumscribed by Winge (1917, 1940), in which increases or decreases in DNA content are involved.

A number of authors, among them Malheiros *et al.* (1947), Castro *et al.* (1949a, 1949b), Malheiros-Gardé & Gardé (1951), Nordenskiöld (1951) and Davies (1956), have indicated an apparent correlation between the occurrence of a non-localized centromere and inverted meiosis. Castro (1950) went so far as to claim that such a centromere was essential for inverted meiosis. In the Odonata, however, where inverted meiosis is known to occur, the chromosomes each have a single localized centromere (Oksala 1943, 1944).

The evidence for either a non-localized centromere or inverted meiosis in the *J. bufonius* aggregate is still very inconclusive. That no centromere was seen in any chromosome is no evidence that one does not exist, since observation was subject to two severely limiting factors: the method of preparation and the level of microscopic resolution. Our evidence on this point was not only equivocal but sometimes contradictory. The presence of an apparent multivalent at metaphase II in the synthesized hybrid suggests an inverted meiosis, but lagging chromosomes at anaphase I are exactly what one would expect from normal meiosis in a hybrid. Our work originally did not seek to investigate the existence in *J. bufonius* agg. of diffuse centromeres or inverted meiosis, but progress along these lines is now being pursued by one of us (C.A.S.) in *Juncus* subgenus *Genuini*.

On the basis of morphological and geographical data discussed in our earlier papers, and cytological data presented in this, we have established three lines of evidence for the nature of the *J. bufonius* aggregate. Firstly, *J. bufonius* sensu stricto is morphologically the most variable segregate and shows elements of all other European species in its structure. Secondly, *J. bufonius* sensu stricto is a polyploid with the behaviour of an allopolyploid, and presumably of hybrid origin; the other four segregates are considered to be diploid. Thirdly, *J. bufonius* sensu stricto is geographically and ecologically more diverse than any other single taxon, being wholly sympatric with all of them and occurring in places where they do not. These three features together indicate that the group as a whole represents a polyploid 'pillar' complex (Babcock & Stebbins 1938). Although we were not able to confirm an intermediate level of polyploidy between diploids and hexaploids, reports of numbers corresponding to a tetraploid level do appear in the literature, rather more than might be attributed to error alone. Thus it is reasonable to accept that taxa such as *J. minutulus* (which we do not separate taxonomically from *J. bufonius* sensu stricto) do represent the intermediate, tetraploid level. *J. bufonius* sensu stricto is envisaged as having arisen from a sequence of hybridization events, in association with amphidiploidization, from diploid taxa which interbred to form tetraploids. These in turn back-crossed to the diploids to form hexaploids and possibly interbred amongst themselves to form octoploids.

Therefore we believe that *J. bufonius* sensu stricto (including *J. minutulus*) arose from a pool of diploids, including the four included in our studies. It is likely that this origin was both polytopic and a long-continuing process. The ranges of the European diploids overlap at present across a wide area of the western Mediterranean, and there is no reason to believe that *J. bufonius* sensu stricto originated from only a part of this area. However, the Middle Eastern diploid, *J. rechingeri*, which is part of the aggregate, has certain distinctive features, such as a characteristic tepal morphology and testa sculpturing, and a lack of cauline leaves, not found in *J. bufonius* sensu stricto, which suggests that *J. rechingeri* (and perhaps other taxa in that area) has not contributed to the gene-pool of *J. bufonius* sensu stricto. On the other hand, it is probable that the original diploid gene-pool was quite different (possibly more diverse) from that found in the western Mediterranean today.

Some direct evidence for the origin of *J. bufonius* sensu stricto has come from our hybridization experiments. The single synthetic hybrid we produced was completely sterile. Nevertheless, it was morphologically so like *J. bufonius* sensu stricto that it is easy to speculate that, had it been a fertile amphidiploid, it would have been quite indistinguishable from it. Probably many plants ascribed to *J. bufonius* sensu stricto are fertile allotetraploids of this sort; one such plant is possibly *J. minutulus*. If this is so, then new variants of *J. bufonius* sensu stricto are probably still arising today.

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