A novel approach to the determination and identification of Juncus × diffusus Hoppe and J. × kern-reichgeltii Jansen & Wacht. ex Reichg.

M. WILCOX*

32 Shawbridge St. Clitheroe, BB7 1LZ

ABSTRACT

The identity of J. inflexus L., J. effusus L. and J. conglomeratus L. have had a rather chequered past. The differences are now relatively well documented and there is less confusion, though there are still misidentifications, particularly in the latter two species. The hybrids Juncus effusus \times J. inflexus (J. × diffusus Hoppe) and J. effusus × J. conglomeratus (J. × kern-reichgeltii Jansen & Wacht. ex Reichg.) present further difficulties in their identification. The former hybrid has been accepted for some time, but the latter has always been a difficult plant to identify with any certainty. Also, J. × kern-reichgeltii is said to be a fertile hybrid and therefore backcrossing (introgression) is likely with no certain recognition or distinctions that include the potentially introgressed individuals. This article aims to present information that may be useful in the determination of these 5 taxa using morphological and anatomical characteristics. In J. × kern-reichgeltii using both morphological and anatomical characters, there is evidence for hybrids and introgression; ultimately, these characters provide a more critical determination of not only the parent taxa, but also of their hybrids.

KEYWORDS: Sclerenchyma, epidermal ridge cells, introgression.

INTRODUCTION

Within this group of rushes, subgenus Genuini, J. inflexus appears to be the most distinct with stiff glaucous stems that have interrupted pith and lax, suberect branches, (Stace 1997). Two other species, J. effusus and J. conglomeratus have at times been difficult to separate with a suggestion that characters overlap (Stace 1970b, 1972). This is mainly due to these two having forms in species which the inflorescence can be effuse or compact in either species; J. effusus var. subglomeratus DC a compact from and J. conglomeratus var. subuliflorus (Drejer) Asch. & Graebn. having several stalked heads (Stace 1997). There are two sheets of in NMW which are labelled J. conglomeratus var. laxus A & G; Four

specimens on one sheet, No. 2442 - N. Woodhead and two on another sheet No. 4972 - 2428 - G. Claridge Druce. These specimens have been reviewed in MANCH, with the G. C. Druce specimens being attributable to J. effusus and the N. Woodhead specimens are J. conglomeratus (only one of which may vaguely be called, 'var. subuliflorus') The name 'var. laxus' appears to be misapplied. However, very lax forms of J. conglomeratus have been found, in which the pedicels are long, up to 8 cm with flower clusters at the distal ends; these are particularly found in woodland situations. This form needs further investigation. Some of these inflorescence types also occur in the hybrid $J. \times kern-reichgeltii$, though more commonly it has a compact form of inflorescence as in J. conglomeratus.

The hybrid J. × kern-reichgeltii was considered on balance not to exist (Stace 1972) and Tweed & Woodhead (1949) reported they could not find evidence for hybrids in their studies. Ascherson & Graebner (1904) had reported J. \times kern-reichgeltii (and J. inflexus \times J. conglomeratus, for which there is no evidence at present) from various locations in Britain and Europe. Kriša (1962) considered that J. effusus and J. conglomeratus were just end points of the same species. However, Agnew (1968) looked at populations and graphed various characters, such as spathe length against ridge number, which appeared to suggest fertile hybrids existing in populations and that 'introgression' was occurring with J. effusus; given that fertility was high, this could have equally shown two end points of one species or two variable taxa. While Agnew's (1968) work did suggest a range of intermediates sometimes based on subjective qualities such as inflorescence colour, though there was no real way of differentiating any distinct evidence for hybridity, it could have been that one parent was very variable. Also to see and count the ridges in J. effusus the stems would have to be dry as it is more or less smooth stemmed in life.

Fertility in *Juncus* can be interrupted even in the parental taxa as well as in hybrids often for different reasons. Juncus hybrids are often considered sterile or with very low fertility, (Stace 1970a, b, 1972). However, fertility is known in hybrid rushes ranging from partial fertility in some plants such as Juncus articulatus L. × J. acutiflorus Ehrh. ex Hoffm. $(J. \times surrejanus$ Druce ex. Stace & Lambinon), (Blackstock & Roberts 1986) to almost no fertility, e.g. J. balticus Willd. × inflexus, and J. balticus \times effusus (J. \times obotritorum Rothm.), (both these latter hybrids having approx <1% fertility and seeds produced in these two taxa do germinate into full sized phenotypes, which are ± sterile also unpublished data M. Wilcox.) Due to this confusing situation, Stace (1975) comments, that misidentifications are still not that uncommon, though identifications are more defined today at least for the species (Stace 1997). In relation to the hybrids, J. inflexus \times J. effusus is relatively widespread being the most frequently recorded hybrid of the two (Stace 1997, Preston et al. 2002) and is the only well known hybrid of these two with a few likely to be errors for odd or sterile J. inflexus (Stace 1975, 1997). The hybrid J. × kern-reichgeltii on the other hand has always been an uncertain hybrid (Stace 1972) and today it is said to be difficult to identify, though Stace (1997) now supports the idea that it occurs sporadically with the parents and also states that due to its high fertility it is difficult to determine other than in the field with its parents and that many records are likely to be erroneous. More recently, O'Mahony (2002) provided a more up-to-date key based on field observations of external morphological characteristics. This key is likely to be of some use in detecting potential hybrids, but errors are still likely and given there is little conclusive evidence for hybridity due to its fertility, this key again may be describing large scale variation in one of the parent taxa, namely J. cong*lomeratus* due to the presence of ridged stems.

Initially, this project started in the winter of 2006 with the hybrid J. × *diffusus*, which was investigated to see if there were any characteristics that would help to confirm its identity from any odd forms of the parent taxa, especially from sterile J. *inflexus*. Stace (1970b), states that the use of micro-anatomical/morphological features in Juncaceae is clearly of immense taxonomic value not only at the level of subgenus but also at the specific

rank and for interspecific hybrids. The hybrid J. \times diffusus is depicted in transverse section on the cover of Stace (1975) and is an excellent example of an intermediate between the two parents. Thus, while studying the parents of J. diffusus and this hybrid, two main x anatomical-morphological characteristics were noted and these were studied and compared between the parents and the hybrid. These characteristics relate to the form, arrangement and type of subepidermal sclerenchymatous girders, (SeSgs) and the shape (and other characteristics) of the epidermal ridge cells (Ercs) above these longitudinal strands; the latter appearing to be useful and novel in the identification of all these taxa. Therefore, the study was extended to J. \times kern-reichgeltii as the characteristics noted may have had a similar type of inheritance thus possibly separating it from the parents and even putative introgressed individuals. The previous difficulties encountered in this latter hybrid may account for there being no mention of it and therefore a lack of any map in the new Atlas of the British & Irish Flora (Preston et al. 2002) with most records unsubstantiated and based on field comparisons. This study sheds light on the identification of the parent taxa and both hybrids but in particular J. × kernreichgeltii. It shows a relationship between the patterns of the subepidermal sclerenchymatous girders and the epidermal ridge cell patterns and that these in turn will highlight the hybrids. In J. \times kern-reichgeltii, it will show that this hybrid appears to be fertile and that backcrossing can be frequent in suitable places creating a hybrid range, though it appears there is a more distinct form of the hybrid, (variable in fertility) which is recognisable from the introgressed individuals.

METHODS

The methods employed in this study are relatively simple so that they can be repeated by the amateur botanist with access to a compound microscope with approximately $\times 20-100$ magnification; this can be less once familiar with the parts. The photographs illustrated here may have used magnifications higher than this but this was for illustrative purposes.

Material from different populations of approx 60–100 stems was used and other individual specimens from personal collections.

Many stems of the parents were also checked. A length of stem approximately 3 cm was cut from about 1–2 cm below the inflorescence. This material, and if dried, was rehydrated in warm distilled water until soft. These lengths were then sectioned for transverse and longitudinal sections (TS/s LS/s) each being numbered and labelled and kept separate in glass tubes. A normal shaving razor was used and sections were cut as thin as possible and stored initially in the distilled water then later in 70% Ethanol. For J. × *diffusus* ten plants each were viewed from 7 populations with 100 stems each of *J. effusus* and *J. inflexus*.

For population studies, (mainly in J. \times kernreichgeltii) several populations of c. 60 plants, most from Wales was received from Arthur Chater (AOC) BSBI recorder for v.c. 46. These populations for J. × kern-reichgeltii were considered by AOC as having potential hybrids and introgressants, an apparent 'hybrid swarm'. These plants (mostly) had already been measured for spathe length against the number of vascular bundles and graphed accordingly by AOC. This method is based on and modified from Agnew's (1968) work which mainly used ridge number against spathe (bract) length, and is said to suggest introgression within populations of the mixed species J. effusus and J. conglomeratus. This method was partly changed in this study and the spathe length was graphed against the number of subepidermal sclerenchyma girders (SeSgs), partly because they are generally easier to count and are not too different in number to vascular bundles and generally would form the basis of a ridge especially when dry. However, the main reason for the change is that in this study it is believed that the subepidermal sclerenchyma girders are linked to hybridity having a relationship with the shape of the epidermal ridge cells above them. For all taxa the longitudinal length of stem after sectioning was retained to view the epidermal ridge cell characteristics and the TSs for the form, arrangement and number of SeSgs.

RESULTS

In this novel approach, aspects of the species are highlighted here as they are important to the identification of the hybrids. These characteristics are the form, arrangement and number of subepidermal sclerenchyma girders (SeSgs) and the epidermal ridge cell patterns. These lines of epidermal ridge cells, (Ercs) were found to be different from other epidermal cells and have no stomata. The shape of the Ercs above the SeSgs was found to be different for each species.

JUNCUS INFLEXUS:

This species generally stands out as being a pale somewhat grey-green glaucous rush. The stem is clearly ridged and rather stiff and has distinctly interrupted pith (Fig. 1d). The inflorescence is suberect with flowers well spaced out towards the ends of the long branches. The ridges in TS show up well and generally appear to be 'flat-topped' and few in number, c. 10-18 (commonly 15) and the subepidermal sclerenchyma girders are broadly triangular and large, (Fig. 1a-b). The SeSgs generally do not quite reach the main vascular bundles but a few rare smaller more band-like SeSgs can reach the sclerenchyma caps of the vascular bundles and fuse with them. Note that in the main larger ridges there are approximately 8-12 similar sized epidermal cells running over the base of the SeSgs, which are discernable from the rest of the epidermal cells; they are relatively uniform in shape and size, diminishing laterally (Fig. 1b). In a longitudinal plane the Ercs above the main SeSgs are thick walled 'mesh-like' cells, (Fig. 1c). In life, with a hand lens the ridges appear a very dull yellowish colour.

JUNCUS EFFUSUS:

It is generally not that difficult to recognise J. effusus which has a rather smooth, unridged outline, often glossy, waxy stems (Fig. 2d) and it often has a effuse relatively rounded (loose pom-pom-like) inflorescence, though in J. *effusus* var. *subglomeratus* the inflorescence is round-compact, which can cause confusion. With a hand lens, its numerous 'pinstripe' narrow, pale-whitish lines of the sclerenchyma can be seen (Fig. 2d); these show up when dry very low ridges almost touching. In as transverse section (TS) the arrangement of the sclerenchyma strands, (ridge formers), can be seen more clearly (Fig. 2a-b). These strands, which are very numerous, (36-62) show up in TS as more or less oblong-triangular shapes. In this species many are fairly narrow, flat-based and more band-like and they can frequently reach the top of the vascular bundles (VBs) and often fuse with the sclerenchyma caps of the VBs, giving them a superficial look of 'exclamation marks' as shown in Fig. 2a-b. In



FIGURE 1. J. inflexus; a) TS, b) Ercs and 1 SeSg, c) 'mesh-like' cells longitudinally on ridges, d) ridged stem showing interrupted pith.



FIGURE 2. J. effusus; a) TS, b) Ercs and 1 SeSg, c) 'oblong cells' longitudinally on ridges, d) smooth, waxy stem showing narrow 'pin-stripe' pattern.

J. effusus, it was noted that from over 500 stems viewed the epidermal ridge cells above the SeSgs are thin walled cells and rectangular in shape and more or less flat having no discernable profile from the rest of the circumference, (see Fig. 2a and 2c). This pattern is relatively uniform for all the Ercs occurring above all the SeSgs and for this study they are termed 'oblong cells' here. The epidermal ridge cells over the base of the

SeSgs in TS are small rounded and usually only about 2–4 across (Fig. 2c) being small and only slightly larger than the rest of the epidermal cells. The number, form and arrangement of the 'exclamation mark' SeSgs and the 'oblong cells' that occur above all these SeSgs are the most important combined characteristics of all in relation to both hybrids. Bearing this in mind the other two species are quite different in all these characters.



FIGURE 3. J. conglomeratus, a) TS, b) Ercs in TS show large cells, c) Ercs longitudinally showing uneven rugulose-wrinkled cells, d) distinctly ridged stem section – pith is not thick and slightly airy.

JUNCUS CONGLOMERATUS:

This species is difficult to define as there is evidence to suggest hybridisation and introgression, which may obscure the limits of the species. However, in what is assumed to be the species, the ridges in TS are highly elevated, peaked to rounded and very distinct for this species (Fig. 3a and 3b) and commonly with about 14-20(-25) distinct ridges. In considering specimens of J. conglomeratus, it was noted that in specimens thought to be to the species, the form and closest arrangement of the SeSgs became more distinct. It is suggested here that the SeSgs for this species are relatively round-based, often blunt-tipped triangles that are mainly isolated from the vascular bundles in all the distinctly elevated ridges, with (usually) no SeSgs in between (Fig. 3a-b), where only one to a few of these occur in between it may suggest a past hybridization history. The Ercs in this species are very distinct from the other two species in that they are of a 'rugulose-wrinkled' appearance (Fig. 3c). These cells are twisted and have rough projections on them. They appear translucent and often shiny in life and become brownish with age. In TS the cells are large in the middle of the ridge apex

diminishing in size laterally often with a rounded-triangle appearance (like *cheese* triangles' Fig. 3b) much larger than those of the rest of the epidermis and typically 4-8 in number. Late season plants, (i.e. October-December) show that the roughness is worn down (probably decay and abrasion etc) and these cells can appear less 'wrinkled' and appear square to rectangular in shape but clearly in general they are quite distinct from either of the other two species. Some of the variation in shape may be related to introgression, see the hybrid J. \times kernreichgeltii below. Thus, for each species there are three distinct TSs showing distinct form, arrangement and number of SeSgs and the patterns of the Ercs appear to be distinct.

POPULATION STUDIES – HYBRIDITY AND INTROGRESSION:

In relation to $J. \times kern-reichgeltii$, hybridity and introgression can be artificially highlighted by looking at populations. Initially, Arthur Chater (AOC) sent material from Wales v.c. 46 as a 'hybrid swarm' indicating that hybrids occurred in a middle range between the two species by graphing the spathe (bract) length



Welsh Populations 1, 2, & 3 - *J. x kern-reichgeltii* Study

FIGURE 4a. SeSgs graphed against bract length to show variation in population; *J. effusus* is smooth-stemmed and stands alone; shows a relatively intermediate range for hybrids.

against the number of vascular bundles. Whilst this appears to be the case there was no distinction and any of the points on the graph could be a hybrid or even just variation in the two species or as noted by Tweed & Woodhead (1949) two end points in a continuum. In this study the specimens were graphed using the bract length against the SeSgs for the reasons mentioned above in the methods section. More populations from Wales were studied along with plants from other areas, e.g. from Gisburn Forest Complex (GFC) v.c. 64 in the same way and further populations from elsewhere, e.g. Orkney Isles. Note that bract length is not significant but is a useful baseline character to graph against the number of SeSgs as it is very variable in both species.

Having analysed the specimens in the ways described above it is possible to show albeit arbitrary, how introgression appears to progress from one parent towards the other. Two graphs are given, Figs 4a and 4b to show two aspects



FIGURE 4b. SeSgs graphed against bract length to show variation in population; *J. effusus* is smooth-stemmed and stands alone but this shows a high incidence of introgression towards it in the range for hybrids, only one *J. conglomeratus* in this population.

of variation in SeSgs and bract length. The data points on the graphs for all plants are arbitrarily assigned a shape to show introgression but are in fact based on the form, arrangement and number of SeSgs and their relationship with the Ercs and can only be assigned a shape for graphical purposes when the differences were known, otherwise they would appear in some populations to be a relatively even spread of points; *J. effusus* is also assigned not only on the number of SeSgs but also by the fact that it has a smooth stem, all others have a discernable ridged stem. Though there is overlap in the number of SeSgs in the main introgressed population, the distinct hybrids are those that have the 'oblong cells' of *J. effusus* above all the SeSgs longitudinally and ridged stems and are therefore assigned a different point on the graph; those with mixed features and more like *J. conglomeratus* are shown as introgressants; those with up to about 25 ridges (SeSgs) were retained as *J. conglomeratus*. Therefore, the initial population from Wales suggesting that hybrids occurred in a middle band was essentially the case but the characters used here defined the populations further. Note that the points can not be assigned without prior analysis.

In Fig. 4a, there is some introgression towards *J. conglomeratus* but it also shows that there are a number of distinct hybrids sitting in a relatively intermediate position. In Fig. 4b, there are a lot of ridged stemmed plants that had high numbers of SeSgs.

Given the range of SeSgs found in smoothstemmed *J. effusus*, 36-60 (commonly 48-50), it can be seen that the hybrid plants with 'oblong cells' longitudinally on the ridges have very high numbers of SeSgs, up to about 55 in this population (Fig 4b). This suggests introgression with *J. effusus*. It would appear that the hybrids can backcross both ways. Further information on the forms found in SeSgs and Ercs for hybrids is given in the next section. Therefore the range of SeSgs even when graphed for potential hybrids, in stems with ridges, show these can be lower –showing backcrossing with *J. conglomeratus* (Fig. 4a), and in the higher range showing backcrossing with *J. effusus*, (Fig 4b).



FIGURE 5. $J. \times diffusus$, a) Ercs showing 'oblong-cells' inherited from J. effusus on distinct ridges longitudinally, ridges appear slightly flat-topped, b) TS which shows the more 'exclamation mark' like pattern and increased numbers of SeSgs, c) showing more finely ridged stem than in J. inflexus.



FIGURE 6. J. \times kern-reichgeltii, a) Ercs showing 'oblong-cells' inherited from J. effusus on distinct ridges, which are more rounded at the apex, b) showing TS, again showing the more 'exclamation mark' like SeSgs and again an increase in SeSgs (ridges), c) distinctly ridged stem of the hybrid which can be uneven.

THE HYBRIDS:

$J. \times DIFFUSUS:$

This hybrid in the field essentially looks like a somewhat less glaucous form of J. inflexus. It has a similar ridged stem, but more ridges and a similar inflorescence, though in Britain the inflorescence is usually \pm sterile to very low fertility, (see discussion). In TS this hybrid clearly has a ridged stem with more ridges than J. inflexus, (up to about 42, compared to J. inflexus which has approx (10)12-18 'ridge formers' = SeSgs). The TS (Fig. 5b) is almost identical to that of J. × kern-reichgeltii (Fig. 6b), but can clearly be separated on inflorescence type and the stiff semi-glaucous stems. The form, arrangement and number of the SeSgs looks intermediate between the two species with a somewhat more 'exclamation mark' look about them showing the influence of J. effusus (Fig. 5b). The ridges also appear somewhat more flat-topped like J. inflexus. The SeSgs, from the limited material, range from 30-42 at present and this number may change with more specimens. Whilst distinct, this internal feature should be enough to say that it is not a sterile form of J. inflexus. However, the Ercs above all SeSgs now have the 'oblong cells' of J. effusus (Fig. 5a - arrowed) not 'mesh-like' as in J. inflexus, (Fig 1c). This combination of characters appears to be good evidence to define this hybrid from its parents. Being usually almost sterile, though partially fertile plants are known in Britain. The stems are finely and evenly ridged, (Fig. 5c)

J. × KERN-REICHGELTII:

This hybrid is essentially complicated by evidence of introgression. However, it is believed here that the same characters that are inherited in what is considered an F1 type in the J. \times diffusus hybrid are similar for 'F1-like' plants in this hybrid; not strictly an F1 as it is partially to fully (?) fertile and it would be unknown if some are not backcrosses with J. effusus as well, which seems to be the case i.e. crossing both ways (from the hybrid stand point). Note that in TS, the form, arrangement and number of SeSgs are similar to the 'exclamation mark' type found in J. effusus and $J. \times diffusus$, (Fig. 6b) but the stems are always ridged in some form, (Fig. 6c) so they are not the smooth stemmed J. effusus. Where the hybrid is thought to be a good cross the epidermal ridge cell character also shows that they have 'oblong cells' (Fig. 6a - arrowed)

that are clearly evident in J. effusus and J. \times diffusus and just as importantly, these 'oblong cells' occur above \pm all the SeSgs, therefore giving a distinct form of this hybrid. The cells often show some evidence of *J. conglomeratus* in that the cell walls are a little bit more 'wrinkled' but essentially 'oblong cells' in which the longitudinal profile is also relatively flat to slightly undulate, much less than the 'rugulose-wrinkled' very uneven prominently profiled cells of fresh in season J Generalised evidence conglomeratus. for backcrosses with J. effusus seems apparent as shown in Figures 4a and 4b.

INTROGRESSION

One of the complications in this hybrid has always been related to the suggestion that the hybrid is fertile. Like most rushes the parent taxa and some of the hybrids can show at least some degree of sterility due to other factors, such as smut fungus and other environmental reasons. This hybrid from the study of the 'F1like' plants noted above, show that it is fertile to partially fertile. Given this complication, it was noted that in plants more like J. *conglomeratus*, there was a scale of increasing 'exclamation mark' like forms of SeSgs towards J. effusus; (if one looks at it from the upper limits it might be a decreasing scale as J. effusus appears to remain distinct as a species easily identifiable with its smooth stems) but it may be crossing in various ways; L conglomeratus with J. effusus or one of the resultant hybrids crossing with either species, but it usually has a visibly ridged stem even though it is fine ridging in the more distinct hybrids, (Fig. 6a-c)

It has been established above, that the distinct hybrids, with the 'exclamation mark' form, arrangement and number (30)35–42(–55) of SeSgs will have the 'oblong cells' of J. effusus in the Ercs. However, this form becomes more diluted from backcrossing especially when crossing with J. conglomeratus. An increase or decrease in the numbers of SeSgs is considered here a direct result of hybridization of varying stages in this taxon. Figure 7a shows a TS that looks like the hybrid with the form, arrangement and number of SeSgs, though slightly more elevated ridges. The difference is that this specimen shows that in the main more elevated ridges, the Ercs show a pattern closer J. conglomeratus as in Figure 3c but much less so. Apart from the



Figure 7. J. × kern-reichgeltii introgression patterns, a) showing high numbers of ridges (SeSgs) but highly elevated ridges showing Ercs becoming more like those of J. conglomeratus but the smaller ridges showing Ercs to be 'oblong-cells' as in J. effusus, b) shows backcrossing towards J. conglomeratus still with increased SeSgs but these occur as less distinct ridges and as in 8u, the Ercs are more like J. conglomeratus in the main ridges and some of the intermediate ridges but 'oblong-cells' in the smaller SeSgs.

SeSgs being more numerous, the evidence for backcrossing comes from the smaller ridge forming SeSgs between the main ones and the very small ones that do not appear to form ridges (in life) but show up when dried. The evidence shows that in these plants, the main Ercs have squarish wrinkled cells, but retain square-oblong cells similar to *J. effusus* in the smaller ridges and the very small strands longitudinally. Therefore these plants have a combination of the two types of Ercs in the same plant and suggest a backcross.

In Figure 7b, this pattern is much less with the main SeSgs elevated having the 'rugulosewrinkled' cells of J. conglomeratus described above in the species section and as before the 'oblong cells' of J. effusus in the very small SeSgs that tend to occur between the J. conglomeratus type SeSgs. Also, the SeSgs between the main ridges are more 'exclamation-mark-like'. This pattern seems to continue. Therefore, there appears to be a continuum from the more distinct hybrids described above to J. conglomeratus progression, suggesting a a history of backcrossing. are The crosses almost impossible to say which generation as any could potentially cross with another. Those with higher numbers of SeSgs but still ridged may be backcrosses with J. effusus. The inheritance of the 'oblong cells' in the Ercs, is evident in both hybrids above the SeSgs and is

considered a useful diagnostic character for the main hybrid in J. × kern-reichgeltii with ridged stems and having 'exclamation mark' like SeSgs in TS. The high number of SeSgs on a decreasing scale combined with Ercs characteristics becoming more like L conglomeratus is considered a sign of introgression within J. × kern-reichgeltii. Equally it seems any hybrid can cross either way with either parent.

DISCUSSION

From an initial study of the parents of J. \times diffusus and this hybrid, it was clear that there were morphological and anatomical characters that were useful for their separation. The distinct epidermal ridge cells above all the sclerenchyma girders were subepidermal revealed in this study for each species. This novel character in association with the form, arrangement and number of SeSgs evidently provides useful taxonomic identification features. Extending these characters to the parents of J. \times kern-reichgeltii showed a more complicated situation. This was due to the apparent unstable fertile nature of the hybrid in question. The morphological and anatomical characters were similar to the other hybrid studied here. It showed that there appears to be a relatively distinct hybrid where the Ercs are

all of the 'oblong cell' type longitudinally above the SeSgs which are more numerous than in J. conglomeratus and similar in number and form to those in J. \times diffusus, approximately twice as many ridges in each hybrid. This fact should not be underestimated as it is the same character for both distinct hybrids inherited from the same parent, J. effusus; though J. \times diffusus appears to be essentially often sterile in Britain, there is apparently some fertility in some populations and a segregating F2 hybrid is known in Europe, (pers. comm. Clive Stace). However, the study not only in the population studies but mainly in the morphological and anatomical study shows that there are many hybrids due to introgression, 'hybridization histories' in J. \times kern-reichgeltii. All those with ridged stems on an increasing or decreasing scale, (depending on which way it is crossing) might be considered to be hybrids and though there is no distinct cut off point J. conglomeratus can be relatively assigned as described above where the SeSgs more or less only occur in the elevated ridges, though some minor ones in between might be acceptable to a certain level to cover variation, (though these additional SeSgs may be due to a history of introgression in an essentially variably fertile hybrid). The evidence points to possible introgression with J. conglomeratus and J. effusus, but the hybrid always has some kind of ridged stem. However, the graphical data have arbitrarily assigned J. conglomeratus and the other ridged stem specimens but it was based on form, arrangement and number of subepidermal sclerenchyma girders and epidermal ridge cell type, technically on a decreasing scale from the smooth stemmed J. effusus plants, (given that this species seems to stand apart) and this may be useful in retaining the species boundaries for the time being. J. conglomeratus can be retained with 15-24 ridges (SeSgs) as its main separation feature in conjunction with the form and arrangement of SeSgs and patterns of Ercs from any hybrids. Apart from the distinct hybrids defined above, the subsequent crosses are almost impossible to judge, though those closer to the more distinct hybrids are more distinctive and easily assigned as a hybrid as shown, with others being less distinct towards the presumed form of J. conglomeratus. However, even backcrosses are considered hybrids but it may always be difficult to assign some plants. The illustration Fig. 787 no. 3 (Stace 1997) is a reasonable example of J. \times

diffusus which is better illustrated on the jacket cover of Stace (1975) and is also comparable to the TS of J. \times kern-reichgeltii in many respects, though inflorescence type and general colour and jiz would separate them. The illustration Fig. 787 no. 7 (Stace 1997) for J. conglomeratus appears to show a partially introgressed plant of the hybrid rather than being closer to the species as defined here, see Figs. 3a-d. At a very late stage of writing this, the author managed to get copies of Kirschner et al. (2002a, b, c) and as it includes important information is related here. This is an invaluable work in three volumes on Juncaceae including Rostkovia to Luzula, (Kirschner et al. 2002a) with two others being compilations covering species of Juncus, (Kirschner et al. 2002b, c). A good drawing of the TS of J. conglomeratus is depicted on p.86, of Kirschner et al. (2002c) and concurs with the findings here of what are considered the species limits. These latter two volumes raise Juncus to section level, and those of subgenus Genuini are placed in Section Juncotypus, (Kirschner et al. 2002c). Interestingly, the hybrid J. × kern-reichgeltii (the name is maintained here) and $J_{.} \times diffusus$ are mentioned and illustrated in this third volume. The description for $J. \times kern-reichgeltii$ gives 25–35 ridges like J. conglomeratus in structure but not as prominent and with a similar but less dense inflorescence; it also mentions that the are well developed, 'probably capsules sometimes with ±reduced seed set,' and that, 'the frequency and properties of this hybrid need further investigation,' (Kirschner et al. 2002c). While this is a reasonable description, the range in ridge number basically covers most of the introgressed individuals (backcrosses to J. conglomeratus), though the ridges are likely to have been counted externally. The illustration associated with it, p.143 clearly depicts a transverse section, which is a clear backcross to *J. conglomeratus* probably of at least two generations based on the sclerenchyma pattern and epidermal ridge cells shown, (see Kirschner et al. 2002c).

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One of the complications in N America is that the species *J. pylaei* Laharpe (2n=40) is very similar to *J. conglomeratus* (2n=42) and hybrids may be of the former species with *J. effusus* at least in N America and need further investigation. *J. conglomeratus* is a non-native introduction in N America but does occur in some similar areas to *J. pylaei*, (Kirschner *et al.* 2002c). Only one hybrid is recorded,

J. effusus ssp. *solutus* \times *J. pylaei* from Ontario, Canada, though one of the differences in *J. pylaei* is that it is said to have no sclerenchyma girders above the main vascular bundles, but it is not illustrated, (Kirschner *et al.* 2002c).

The transverse section shown for J. inflexus in Kirschner et al. (2002c) seems to be incorrect. The hybrid, $J. \times diffusus$ is reasonably depicted in Kirschner et al. (2002c), but the illustration of the inflorescence is somewhat effuse! It may relate to partially fertile plants. The segregating hybrid in $J. \times$ diffusus said to occur in Europe needs to be studied as it is likely that it would show similar patterns of introgression as highlighted here but it is likely to retain a J. inflexus type inflorescence. Those in Britain appear to be more or less sterile with few or no segregating individuals known. With the evidence provided these two hybrids have been elucidated further and records can now be made for mapping purposes especially for J. × kern-reichgeltii. I would be interested in receiving specimens for determination in relation to recording both hybrids. Populations with ridged stems in the J. × kern-reichgeltii – J. conglomeratus group would be welcome either as individual specimens or no more than 100 stems per population and the bract intact to be analysed. It is likely that herbarium material can also be identified more readily for the parents but all suspected hybrids in $J \times kern-reichgeltii$ may need confirmation using Transverse Sections of the stem as described in the methods section; some J. \times diffusus may also require stem sections for confirmation but less so than the other hybrid. Other rushes would be welcome. The evidence presented for introgression suggests that J. \times kern-reichgeltii is frequent to sporadic in some localised areas and is likely to occur throughout the range of the parent taxa but less common or not at all in some areas dominated by one parent, which is usually J. effusus in many areas with grazing pressures. It seems from AOC's studies that J. \times kernreichgeltii can be quite frequent in parts of Wales. Also, one population received from the Orkney Isles had 39 hybrids from 62 stems suggesting it can be frequent in other areas also, see records below.

RECORDS

CRITICALLY DETERMINED J. × KERN-REICHGELTII RECORDS SO FAR:

The hybrid is likely to occur rarely-tofrequently throughout the range of both parents in suitable areas, records below are from Gloucestershire to the Orkney Isles.

WELSH MATERIAL: – QUOTED FROM ARTHUR CHATER'S MATERIAL

"6 plants, notes, ? Juncus conglomeratus \times effusus. Plants loose – Note – with Juncus effusus and J. conglomeratus in damp, flushed (....) pasture on SE facing slope, RHOS GARGOED SSSI, 700 m NE of CAEMADOG, STRATA FLORIDA, Cards. 290 m 22/758 668 7/9/1994 – v.c. 46 A. O. Chater." Some of these plants were determined as the hybrid, MW-2008.

23 plants, *J. effusus/conglomeratus*, 'Roadside verge in felled conifer plantation, 300 m NW of NW tip of NANT Y MOCH Reservoir, Cards. 350 m 22/736887, 2/7/2003, v.c. 46 A. O. Chater.' 3 plants were the distinct hybrid, MW-2008.

49 plants (labelled up to 50 but number 19 missing).

NOTES

These plants were for the **1998 BSBI Exhibition**, "Population from Rhos Llawr-cwt NNR, apparently of *J*. conglomeratus, *J*. *effusus* and the hybrid; no obvious hybrid swarm or introgression. The hybrid specimens (**marked H**) have their inflorescences clearly intermediate in colour and density. One stem from each of 50 clumps in fen just S of BWDRAM near E boundary of RHOS LLAWR-CWRT NNR, TALGARREG, Cards. 180 m, 22/415499, 15/7/1998, v.c. 46 A. O. Chater." All those marked 'H' were *Juncus* × *kern-reichgeltii* Jansen & Wacht. ex Reichg. MW-2008.

57 plants, "Population from Mynydd Bach of at least 4 plants of *J. conglomeratus*, and of *J. effusus* introgressed with the hybrid. Note the unusually high proportion of plants of hybrid origin with ridged stems that have 40 or more vascular bundles. – Level mire at NW corner of Mynydd Bach, 300 m ESE of PWLLDRAEN LLWYN, TREFENTER. 300 m., 22/616696, 28/6/1998, A.O. Chater, v.c. 46." Many distinct hybrids and some introgressants, MW-2008. 52 plants, "Upland population from Llyn Gynon, apparently of *J. effusus* introgressed with the hybrid; perhaps one plant of *J. conglomeratus*. Blanket mire N of LLYN GYNON, Cards. 440 m, 22/802650, 4/7/1998, A.O. Chater, v.c. 46." Some distinct hybrids, MW-2008

57 Plants, "Juncus effusus/conglomeratus from; Trackside in partially felled conifer plantation, LODGE PARK, TRE'R-DDOL, Cards; 50 m, 22/665936, 30/7/1997, v.c. 46, A.O. Chater." Only a few introgressants in this population, MW-2008.

70 Plants. Notes: Plants with a scattergram. Notes on paper; "Upland population from Camddwr valley, perhaps a hybrid swarm or perhaps just of the two species; *cf*. Diagram from Esgair-las; *Molinia* dominated, NNE facing slope with *Selaginella* flushes, CAMDDWR valley, 1.7 km NNW of SOAR Y MYNYDD, Cards; 350 m, 22/777548, 25/9/1998, v.c. 46, A.O. Chater." Some distinct hybrids present, MW-2008.

About 55 plants, "A lowland population from New Quay, apparently a hybrid swarm of *J. conglomeratus* and *J. effusus* – One stem from each of 60 clumps in a small fen enclosure 250 m SW of CEFNGWYDDIL, CROSS INN, NEW QUAY, Cards. 130 m 22/38625754, 24/8/1998, v.c. 46 – Arthur Chater." 5 distinct hybrids types with others introgressed, and *J. conglomeratus* det. MW 2007.

OTHER POPULATIONS & SPECIMENS:

Isle of Skye, 2 plants NG50 53, NG6008 and one from the Isle of Raasay, NG5641 v.c. 104 – all 3 plants collected and later determined as the hybrid (M. Wilcox July 2007) specifically collected while on the Bradford Botany Group long weekend away.

Hetchell Wood, Thorner, Leeds v.c. 64 -SE373427, Bradford Botany Group trip, originally identified in the field by Geoffrey Wilmore, 2005, (confirmed MW 2007) using the techniques here (a backcross but in TS clearly referable to the hybrid). Woodside Quarry, Leeds v.c. 64 M. Wilcox, July 2002, SE255 385 collected originally as J. conglomeratus var. subuliflorus. Determined 2007, (MW) clearly attributable to the hybrid. Other records of this variety could be this hybrid also.

Gisburn Forest Complex, v.c. 64 October 2007, SD744553 MW specimens 100 *J. effusus* and 100 with ridged stems. 4 Plants referable to the distinct hybrid were found in those collected with approximately 6 also distinct backcrosses.

Gloucester, woodland behind Michael Wood Service station, ST722950 v.c. 34, collected July 2002 Michael Wilcox as an unknown very odd lax form of *J. conglomeratus* var. *subglomeratus* or *J. × kern-reichgeltii*. Later confirmed as the hybrid (MW 2007) but considered as a backcross due to the variable combined Ercs characters.

A record in Shropshire Botanical Newsletter on a Shropshire Botanical Group (BSBI) trip, J. × *kern-reichgeltii* found by Richard Pryce and det. by Arthur Chater; Wildmoor Pool [incorrectly cited as SJ424965 – should be SO424965] 16 September 2007.

J. × kern-reichgeltii; This record is from dampish grass-heath on Berrow Downs on the E flank of the Malvern Hills at SO768386, 28.7.2002; those present Roger Maskew, Christopher Westall and Bill Thompson. Worcestershire Flora Project; Progress Report. Specimen sent to and confirmed by Arthur Chater, therefore accepted here.

Eric Meek – potential hybrids sent for determination: Orkney Isles, ND450882, v.c. 111; 62 stems arrived; the determinations (01/09/08) are as follows: 23 - Juncus conglomeratus (some of these showing some introgression) 39 - J. × kern-reichgeltii (very good hybrids) det. MW-2008.

Eric Meek, Orkney Isles, v.c. 111, NY323184, August 2008: 117 stems; 98= *J. effusus*, 15= *J. conglomeratus*, 4= *J.* × *kern-reichgeltii*, det. MW 18/08/08

Waddington Fell (Bradford Fell) SD720474, August 2008, MW

Stocks Reservoir, SD735561, August 2008, MW

Gt. Horton Country Park, Horton Bank Top, Bradford, v.c. 63, SE125308; Coll. B. A. Tregale & MW, v.c. 63, Sept 2008 det. MW Wagon Lane, Beckfoot, Bingley, v.c. 63, SE115381 Coll. MW & B.A. Tregale, Oct 2008, det. MW Freshfield Dune Heath, SD293090, v.c. 59 Coll. MW & Phil Smith, 27 Aug 2008, det. MW; *J.* × *surrejanus* also present.

Ireland – County Leitrim – Dromahair, (Droim Dha Thiar) – Five Cross Roads, G813279, H29, meadow, collector B. A. Tregale, 4– 5/08/08; det. MW, Oct 2008

Ireland, County Leitrim – Dromahair – Five Cross Roads – G819275, H29, peat bog, collector B. A. Tregale, 4–5/08/08; det. MW, Oct 2008 Freshfield Dune Heath – SD293090, v.c. 59, Phil Smith & MW, 27/8/08, det. MW, Sept/08

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