

***Polypodium* × *font-queri* nothosp. *encumeadense*
(= *Polypodium cambricum* ssp. *macaronesicum* × *P. vulgare*,
Polypodiaceae, Pteridophyta), a new fern hybrid from Madeira**

by

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With 3 figures

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Abstract: A new *Polypodium* hybrid between *Polypodium cambricum* ssp. *macaronesicum* and *Polypodium vulgare* from Madeira is described as *Polypodium* × *font-queri* nothosp. *encumeadense*. This hybrid is triploid ($2n = 111$) and exhibits mainly univalents but also a number of bivalents during meiosis. By examining isoenzyme profiles and micromorphological characters it was shown that the Madeiran hybrid is different from the triploid hybrid *P. ×font-queri* nothosp. *font-queri* (= *P. cambricum* ssp. *cambricum* × *P. vulgare*) occurring in Europe.

Introduction

The circumboreal *Polypodium vulgare* complex is widely distributed in Europe, Asia and North America. Three species of this group are known to occur in Europe: the diploid *P. cambricum* L. ($2n = 74$), the tetraploid *P. vulgare* L. ($2n = 148$) and the hexaploid *P. interjectum* Shivas ($2n = 222$). On Madeira, the same species are found (Manton et al. 1986). The diploid *P. cambricum* is, however, represented by a different subspecies, ssp. *macaronesicum* (Bobrov) Fraser-Jenkins which is, if we disregard a dubious record from southern Spain, endemic to the Macaronesian Islands. This taxon is often regarded as a separate species (*P. macaronesicum* Bobrov), a taxonomic treatment which is rejected here since both diploids are completely interfertile in crossing experiments (Neuroth 1996).

As in other ferns, formation of interspecific hybrids is well documented in the genus *Polypodium* (Knobloch 1976). During an excursion to Madeira in 1991, one of the authors (W.J.) discovered two sterile *Polypodium* plants at the Encumeada Pass which were not readily identifiable. Within a more comprehensive work on the bio-systematic of European *Polypodium* taxa (Neuroth 1996), the morphology, cytology and biochemistry of these two Madeiran plants were investigated in order to establish their identity. The study revealed them to be the triploid cross between the

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diploid *P. cambricum* ssp. *macaronesicum* and the tetraploid *P. vulgare*. In the British Isles and in continental Europe such a triploid hybrid is already known under the name *P. ×font-queri* Rothm. (Page 1982, Kramer 1984). Its parents involve *P. vulgare* and *P. cambricum* ssp. *cambricum*. As the Madeiran plants differ in the diploid parent belonging to ssp. *macaronesicum*, they represent a new hybrid taxon which is described as *Polypodium ×font-queri* nothosp. *encumeadense* in the following.

Material and methods

Rhizome pieces of the two sterile *Polypodium* plants at the Encumeada Pass were collected and cultivated in the greenhouse of the University of Bochum. For cytological analyses, premature sporangia (colour of sori light green to white) were fixed using a mixture of acetic acid and ethanol (1:3). Squash preparations of spore mother cells were stained in ferruginous acetocarmine (Darlington & La Cour 1963) following the technique of Manton (1950). Epidermis preparations for studying leaf micromorphology were made as described by Steinecke & Bennert (1993).

A comprehensive *Polypodium* collection kept at the Botanical Garden of the University of Bochum was available for isoenzyme analyses (Table 1). These included the following taxa (the number of plants are denoted in parentheses): *P. cambricum* ssp. *cambricum* (99), *P. cambricum* ssp. *macaronesicum* (56), *P. interjectum* (69), *P. vulgare* (92), *P. ×font-queri* nothosp. *font-queri* (11) and *P. ×font-queri* nothosp. *encumeadense* (2). Vouchers of these plants are deposited in BOC. A 0.1 M Tris-HCl grinding buffer (pH 7.5) was used for preparing samples for starch gel electrophoresis (concentration of gels 12.6%), whereas a 1 M ortho-phosphoric acid buffer (pH 6.9; see Zentgraf 1986) was used for polyacrylamid gel electrophoresis. The following enzymes were examined: aspartate aminotransferase (AAT, E.C. 2.6.1.1), glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.9), glutamate dehydrogenase (GDH, E.C. 1.4.1.2), hexokinase (HEX, E.C. 2.7.1.1), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), leucine aminopeptidase (LAP, E.C. 3.4.11.1), phosphoglucomutase (PGM, E.C. 5.4.2.2), shikimate dehydrogenase (SKD, E.C. 1.1.1.25), triose-phosphate isomerase (TPI, E.C. 5.3.1.1). Gel and electrode buffers were modified according to Zentgraf (1986) for AAT assays, for GDH, GPI, HEX, IDH, LAP, PGM and TPI according to Adams & Joly (1980) and as described by Gottlieb (1981) for SKD assays. Staining schedules followed Soltis et al. (1983). Details of methods employed for electrophoretic analyses of isoenzymes are given by Neuroth (1996). According to general agreement, loci and alleles were designated sequentially starting with the most anodally migrating bands; numbers were used for loci, letters for alleles.

Results

Formal description

Polypodium ×font-queri nothosp. *encumeadense* Neuroth, Jäger & Bennert, hybr. nov. - Type: On a wall at Encumeada Pass, Madeira (Portugal); 16 September 1991, leg. R. & W. Jäger & H. Glimpf, at an altitude of about 1,000 m; a piece of rhizome was collected (SP 106b/91) and cultivated in the greenhouse of the Botanical Garden of the University of Bochum; fronds of the type specimen pressed 1 October 1996; holotype: B (Fig. 1); paratypes: specimens grown from a second piece of rhizome (SP 106a/91) collected at the same place (kept in the private herbaria of the authors).

Derivatio: named after the Encumeada Pass, NNE of Ribeira Brava, Madeira.

Diagnosis: Planta hybrida, habitu media inter *Polypodium cambricum* ssp. *macaronesicum* et *Polypo-*

Table 1. Collection data for plant material of *Polypodium* taxa sampled for isoenzyme studies (summarized; for more detailed information see Neuroth 1996).

Taxon and collection number	Locality	Year
<i>P. cambricum</i> ssp. <i>cambricum</i>		
K 30; 3a-i/91; 4a-c/91; 23a-l/92; 78a-h/92	France	1962; 1991; 1992
1/86; 117a-d/91	Greece (incl. Crete)	1986; 1991
K 157; 11/86; 12/86; 27/86; 5a-b/92; 7a-c/92; 9a-b/92; 24a-j/92; 25a-j/92; 26a-c/92; 27a-j/92; 28a-d/92; 29/92; 30a-b/92; 32a-i/92; 33a-c/92	Spain (incl. Mallorca and Gibraltar)	1963; 1986; 1992
5/91	Ukraine	1991
<i>P. cambricum</i> ssp. <i>macaronesicum</i>		
K 214; K 215; 13/83; 24a-t/91; 28a-w/91; 48a-t/91	Azores (Pico, São Miguel)	1976; 1983; 1991
K 175-K 177; K 179; K 181; K 203; K 253; 20/82; 1/84; 53/86; 1a-b/91; 2/91	Canary Islands (Gran Canaria, Tenerife)	1970; 1971; 1974; 1982; 1984; 1986; 1991
K 183-K 190; K 192-K 194; K 199-K 200; K 216-K 222; 21/84; 22a-b/84; 99a-f/91; 100a-g/91; 101a-g/91; 102a-f/91; 110a-g/91	Madeira	1972; 1984; 1991
<i>P. vulgare</i>		
K 195; K 196; 23/84; 101a'/91, 101c'/91	Madeira	1972; 1984; 1991
127a-b/91	Denmark	1977
21a-i/92; 22a-k/92	France	1992
14/91; 58a-o/91; 79a-b/91; 82a-q/91; 83a-y/91; 128/91;	Germany	1991
112a-b/91	Hungary	1991
62/91; 63/91	Italy	1991
<i>P. ×font-queri</i> nothosp. <i>encumeadense</i>		
106a-b/91	Madeira	1991
<i>P. ×font-queri</i> nothosp. <i>font-queri</i>		
20a-i/92; 23h', l'/92	France	1992

dium vulgare; *Polypodio* ×*font-queri* nothosp. *font-queri* similis sed numero parviore cellularum basallium in sporangiis ab eo recedit. Sori ovals paraphysibus absentibus; sporis abortivis. Chromosomatum numerus $2n = 111$, meiosi ca. 91-97 univalentibus et ca. 7-10 bivalentibus.

Hybrid plant, its general morphology being intermediate between the parents, *Polypodium cambricum* ssp. *macaronesicum* and *Polypodium vulgare*; hybrid similar to *Polypodium* ×*font-queri* nothosp. *font-queri*, but differs from it by having a lower number of basal cells in the sporangia. The sori are of oval shape and lack paraphyses; the spores are abortive. The chromosome number is $2n = 111$ with c. 91-97 univalents and c. 7-10 bivalents at meiosis.



Fig. 1. Holotype of *P. xfont-queri* nothosp. *encumeadense* (SP 106b/91; B).

Morphology

The gross morphology of nothosp. *encumeadense* is intermediate between the putative parents (Fig. 2). However, in *Polypodium* and particularly in *P. vulgare* fronds display a wide range of form and size, especially with regards to the length to width ratio, probably depending on differences in environmental conditions.

In *Polypodium*, micromorphological characters are generally necessary to diagnose plants with certainty. In the taxa considered here, neither dimensions of the spores

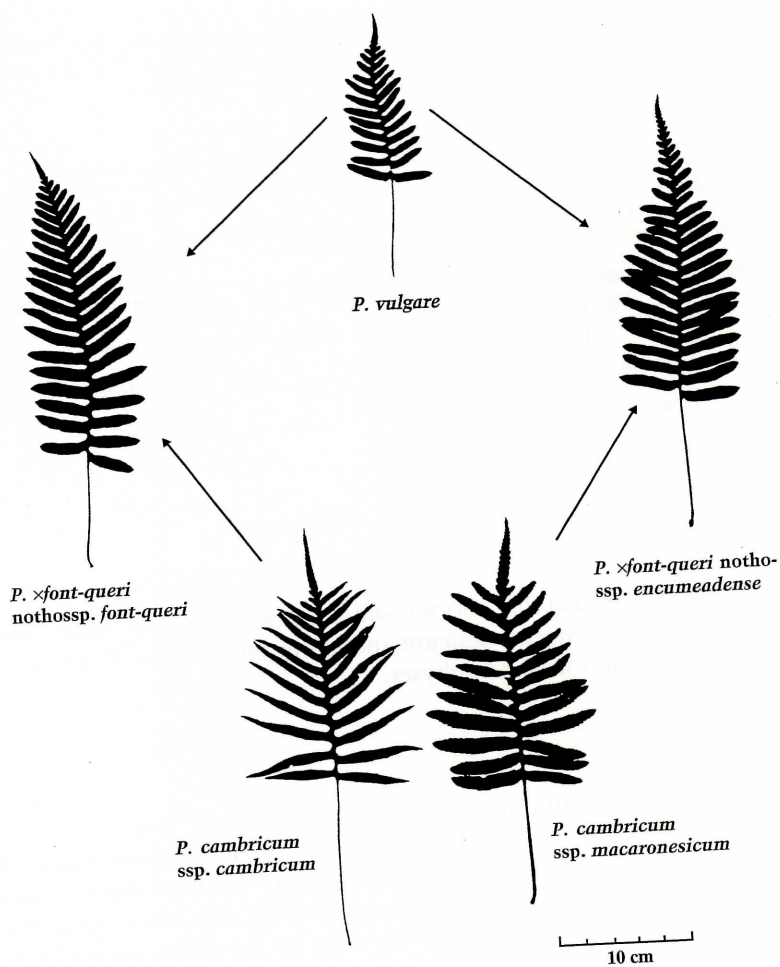


Fig. 2. Silhouettes of fronds of both nothosubspecies of *P. xfont-queri* and of their putative parents. Origin of specimens: *P. xfont-queri* nothosp. *encumeadense*: Madeira (106a/91), *P. xfont-queri* nothosp. *font-queri*: France (20g/92), *P. cambricum* ssp. *cambricum*: France (3g/91), *P. cambricum* ssp. *macaronesicum*: Madeira (101f/91), *P. vulgare*: Madeira (101a'/91).

nor those of the stomata deviate significantly (Table 2). Both forms of *P. xfont-queri* have abortive spores, but number and overall length of the basal cells of the annulus are different. In nothosp. *font-queri*, two basal cells generally are present, whereas in nothosp. *encumeadense* only one occurs. This is due to the differences found in the two *P. cambricum* subspecies with subsp. *macaronesicum* displaying a lower number of basal cells than subsp. *cambricum*. Consequently, in nothosp. *font-queri* the total length of basal cell exceeds that of nothosp. *encumeadense* quite clearly. Paraphyses occur regularly only in *P. cambricum* ssp. *cambricum* where they may attain a length of over 1 mm. In *P. cambricum* ssp. *macaronesicum*

Table 2. Micromorphological characters examined in the two nothosubspecies of *P. ×font-queri* and in their putative parents. For the basal cells, the most frequently observed number is given, more rarely occurring smaller or larger numbers in parentheses; in all other cases the range occupied by 80% of values obtained is given, minima and maxima in parentheses.

	<i>P. cambricum</i> ssp. <i>macaronesticum</i>	<i>P. ×font-queri</i> nothosp. <i>encumeadense</i>	<i>P. vulgare</i>	<i>P. ×font-queri</i> nothosp. <i>font-queri</i>	<i>P. cambricum</i> ssp. <i>cambricum</i>
number of basal cells	(0-) 1 (-3)	(0-) 1	(0-) 1 (-3)	(0-) 2 (-3)	(2-) 3 (-5)
total length of basal cells (µm)	(0-) 40-80 (-120)	(0-) 28-40 (-50)	(0-) 40-80 (-90)	(0-) 52-92 (-105)	(70-) 100-130 (-180)
length of paraphyses (µm)	(360-) 410-650 (-840)*	paraphyses lacking	(320-) 380-405 (-536)*	paraphyses lacking	(500-) 800-1200 (-1400)
length of spore (exospore) (µm)	(45-) 54-65 (-73)	spores aborted	(48-) 56-68 (-73)	spores aborted	(51-) 58-65 (-77)
width of spore (exospore) (µm)	(30-) 36-42 (-46)	spores aborted	(27-) 33-40 (-60)	spores aborted	(27-) 37-43 (-48)
length of stomata (µm)	52-59	55-63	52-60	50-59	55-64
width of stomata (µm)	35-42	39-44	35-45	36-41	38-44

* A number of individuals of *P. cambricum* ssp. *macaronesticum* and *P. vulgare* lacked paraphyses.

and *P. vulgare* they are notably shorter and are present in only some individuals (about two thirds in ssp. *macaronesticum* and about one third in *P. vulgare*). Both forms of *P. ×font-queri* lack paraphyses.

Cytology

Counts of chromosomes during meiotic division showed that *P. ×font-queri* nothosp. *encumeadense* is triploid. This agrees with the assumption that the diploid *P. cambricum* ssp. *macaronesticum* and the tetraploid *P. vulgare* are the parents involved. Examination of four cells revealed that not only univalents but also a number of bivalents are formed (Fig. 3). The number of univalents varied between c. 91-97 and the number of bivalents correspondingly from c. 7-10. Shivas (1961a) reported a number of bivalents ranging from 0 to 6 for nothosp. *font-queri*. Such relatively high numbers of bivalents are unexpected as the parental species do not have a genome in common (Haufler et al. 1995, Neuroth 1996).

Isoenzymes

Nine enzyme systems encoded by fifteen putative loci were visualized electrophoretically. Of the fifteen loci, Tpi-2 and Tpi-3 were omitted because the interpretation of the zymograms was unclear. The unusual banding patterns occurring at these two loci may result from posttranslational modifications or overlapping loci (cf. Bryan & Soltis 1987, Hickey et al. 1989, and Barrington 1990).

The two diploid taxa, *P. cambricum* ssp. *cambricum* and ssp. *macaronesticum*, possess different genotypes for Gpi-1, Gpi-2, Pgm-1 and Pgm-2 (Table 3) with five alleles that were restricted to ssp. *cambricum* (Gpi-1b, Gpi-2a, Pgm-1a, Pgm-1c and

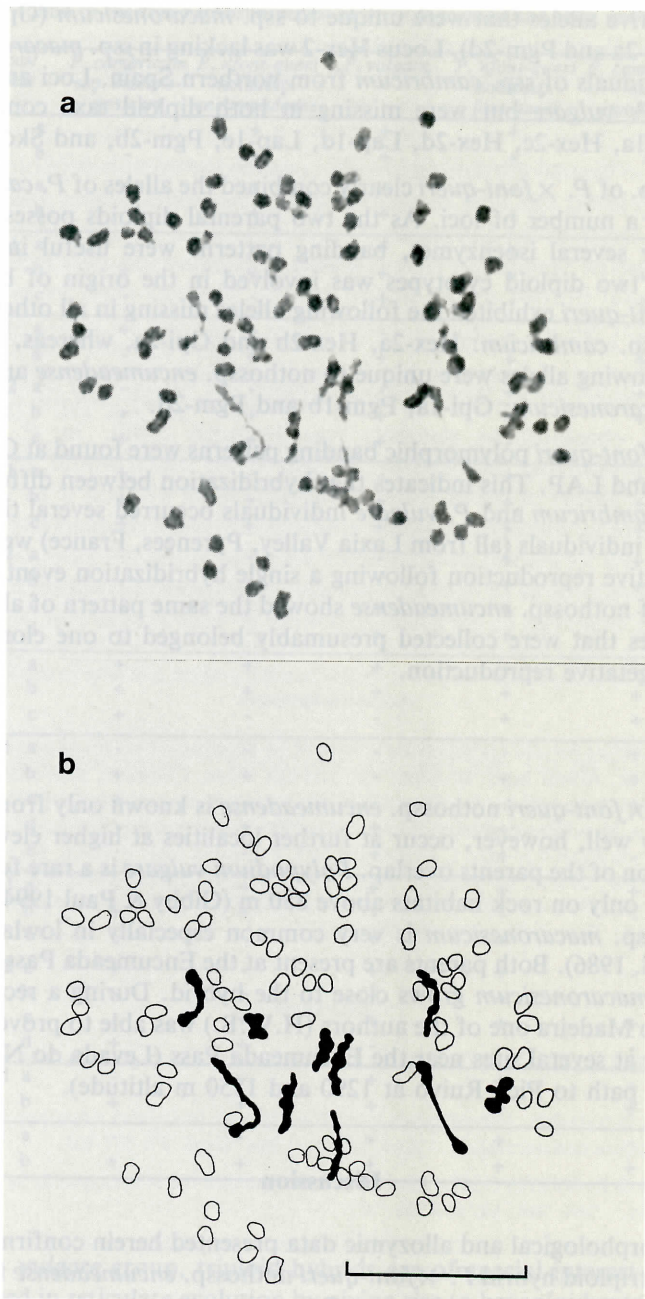


Fig. 3. Cytology of *P. x font-queri* nothosp. *encumeadense* (SP 106a/91). Spore mother cell in first meiotic division with $n = c.91^I$ and $c.10^{II}$; a: photograph, b: explanatory diagram; bivalents black, univalents in outline; length of bar: $10 \mu\text{m}$.

Pgm-2c) and five alleles that were unique to ssp. *macaronesicum* (Gpi-1a, Gpi-2c, Pgm-1b, Pgm-2a and Pgm-2d). Locus Hex-2 was lacking in ssp. *macaronesicum* and in a few individuals of ssp. *cambricum* from northern Spain. Loci and alleles that occurred in *P. vulgare* but were missing in both diploid taxa comprise Aat-1, Aat-2b, Gdh-1a, Hex-2c, Hex-2d, Lap-1d, Lap-1e, Pgm-2b, and Skd-1a.

Both nothosp. of *P. ×font-queri* clearly combined the alleles of *P. cambricum* and *P. vulgare* at a number of loci. As the two parental diploids possessed different genotypes for several isoenzymes, banding patterns were useful in determining which of the two diploid cytotypes was involved in the origin of both hybrids. Nothosp. *font-queri* exhibited the following alleles missing in all other taxa but *P. cambricum* ssp. *cambricum*: Hex-2a, Hex-2b and Gpi-2a, whereas, on the other hand, the following alleles were unique to nothosp. *encumeadense* and *P. cambricum* ssp. *macaronesicum*: Gpi-1a, Pgm-1b and Pgm-2d.

In nothosp. *font-queri* polymorphic banding patterns were found at Gdh-1, Hex-1, Hex-2, IDH and LAP. This indicates that hybridization between different *P. cambricum* ssp. *cambricum* and *P. vulgare* individuals occurred several times and that the 11 hybrid individuals (all from Laxia Valley, Pyrenees, France) were not the result of vegetative reproduction following a single hybridization event. In contrast, both plants of nothosp. *encumeadense* showed the same pattern of alleles; the two rhizome pieces that were collected presumably belonged to one clone which had spread by vegetative reproduction.

Distribution

Polypodium ×font-queri nothosp. *encumeadense* is known only from the type locality. It may well, however, occur at further localities at higher elevations where the distribution of the parents overlap. *Polypodium vulgare* is a rare fern on Madeira and grows only on rock habitats above 850 m (Gibby & Paul 1994), whereas *P. cambricum* ssp. *macaronesicum* is very common especially in lowland situations (Manton et al. 1986). Both parents are present at the Encumeada Pass, and *P. cambricum* ssp. *macaronesicum* grows close to the hybrid. During a recent excursion (July 1996) to Madeira one of the authors (H.W.B.) was able to prove the presence of *P. vulgare* at several sites near the Encumeada Pass (Levada do Norte at 980 m altitude, and path to Pico Ruivo at 1290 and 1550 m altitude).

Discussion

The micromorphological and allozymic data presented herein confirm the assumption that the triploid hybrid *P. ×font-queri* nothosp. *encumeadense* found on Madeira originated from a cross between *P. cambricum* ssp. *macaronesicum* and *P. vulgare*. The hybrid clearly combines the alleles of its parents at Gpi-1, Pgm-1 and Pgm-2, and the banding patterns obtained for these loci rule out the possibility that *P. cambricum* ssp. *cambricum* is the diploid parent. The data obtained for all other loci examined are also consistent with the assumed origin.

Table 3. Alleles found in the two nothosubspecies of *P. ×font-queri* and their parents.

Locus/ Allele	<i>P. cambricum</i> ssp. <i>macarone- nesicum</i>	<i>P. ×font-queri</i> nothosp. <i>encumeadense</i>	<i>P. vulgare</i>	<i>P. ×font-queri</i> nothosp. <i>font-queri</i>	<i>P. cambricum</i> ssp. <i>cambricum</i>
Aat-1	a	-	+	+	-
	b	-	+	+	-
Aat-2	a	+	+	+	+
	b	-	+	+	-
Gdh-1	a	-	-	+	-
	b	+	+	+	+
	c	-	-	+	+
Gpi-1	a	+	+	-	-
	b	-	+	+	+
Gpi-2	a	-	-	-	+
	b	+	+	+	+
	c	+	+	+	-
Hex-1	a	+	-	-	+
	b	+	+	+	+
	c	+	+	+	+
Hex-2	a	-	-	-	+
	b	-	-	-	+
	c	-	-	+	-
	d	-	-	+	-
Idh-1	a	+	+	+	-
	b	+	+	+	+
	c	+	-	-	+
Lap-1	a	-	-	-	+
	b	+	+	-	+
	c	+	-	-	+
	d	-	+	+	-
	e	-	-	+	-
Pgm-1	a	-	+	+	+
	b	+	+	-	-
	c	-	-	+	+
Pgm-2	a	+	-	+	-
	b	-	+	+	-
	c	-	+	+	+
	d	+	+	-	-
Skd-1	a	-	+	+	-
	b	+	+	+	+
Tpi-1	a	-	+	+	-
	b	+	+	+	+

Within the *P. vulgare* group, triploid hybrids are of special interest because they may be involved in reticulate evolution by giving rise to hexaploid cytotypes. In Europe, such a hexaploid species is represented by *P. interjectum* which is commonly believed to have originated from the triploid *P. ×font-queri* nothosp. *font-queri* by chromosome doubling. While the hexaploid derivative is a rather common species in central and western Europe and also in parts of the Mediterranean area (Jalas

& Suominen 1972, Page 1982), the triploid hybrid is scarce and known only from a limited number of widely-scattered sites in Britain and from a few localities in Spain, France, Italy and Switzerland (Shivas 1961b, Page 1982, Kramer 1984, Prelli 1990). On Madeira, a hexaploid, confined to the highest mountains, Pico Ruivo and Pico do Arieiro (Manton et al. 1986, Gibby & Paul 1994, Neuroth 1996), occurs. By applying the isoenzyme technique and considering micromorphological characters, Neuroth (1996) was able to show that this hexaploid (from Pico do Arieiro) is of the same origin as the European *P. interjectum*. As *P. cambricum* ssp. *cambricum* is unknown in Madeira, a long distance dispersal of *P. interjectum* via spores from Continental Europe or the British Isles to Madeira must be assumed.

In *Polypodium* hybrids, the majority of spores is abortive, but a small number of unreduced spherical diplospores are formed. At least under experimental conditions, these diplospores are capable of germinating and may give rise to sporophytes with doubled chromosome numbers (see Neuroth 1996). In *P. ×font-queri* nothosp. *encumeadense* diplospores are also found which would, in principle, allow the establishment of an endemic hexaploid. Such a taxon is as yet unknown; if it exists, it is to be expected at higher elevations where *P. vulgare* occurs. It seems, however, that the formation of hexaploids via diplospores is a rare event. In the Pyrenees, several records of *P. ×font-queri* nothosp. *font-queri* exist, but at no locality it has been observed to be associated with *P. interjectum* (see Neuroth 1996).

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