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<b>Autor:</b>	Brownsey, P.J. / Jermy, A.C.
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# Asplenium *x* khaniense, a new wild hybrid from Crete

P. J. BROWNSEY & A. C. JERMY

## Résumé

Brownsey, P. J. & A. C. Jermy (1975). *Asplenium x khaniense*, un nouvel hybride spontané de la Crète. *Candollea* 30: 21-28. En anglais.

Ce nouvel hybride, *Asplenium creticum* *x* *A. trichomanes* subsp. *quadrivalens*, trouvé dans les Lefka Ori (Crète occidentale, Grèce), est décrit. Il est comparé avec les *A. x aprutianum* et *A. x clermontiae*, morphologiquement semblables. Chez l'*A. x khaniense* on observe, à la méiose, environ 36 bivalents et 72 univalents, ce qui se conforme aux hypothèses de l'origine allopolyplioïde de l'*A. creticum* et autopolyplioïde de l'*A. trichomanes* subsp. *quadrivalens*.

## Abstract

Brownsey, P. J. & A. C. Jermy (1975). *Asplenium x khaniense*, a new wild hybrid from Crete. *Candollea* 30: 21-28.

The new hybrid, *Asplenium creticum* *x* *A. trichomanes* subsp. *quadrivalens*, found in the Lefka Ori (W. Crete, Greece), is described. It is compared with the morphologically similar *A. x aprutianum* and *A. x clermontiae*. At meiosis in *A. x khaniense*, approximately 36 bivalents and 72 univalents were observed, which is consistent with the hypotheses that *A. creticum* is of allopolyplloid, and *A. trichomanes* subsp. *quadrivalens*, of autopolyplloid origin.

## Introduction

During the past few years the mountains of Crete have been subjected to increasingly intensive botanical exploration<sup>1</sup> and this has resulted in a number of interesting additions to the fern flora of the island. In particular, two new species of *Asplenium* were described by Reichstein & al. (1973), and several new records were reported by Brownsey & Jermy (1973). One of these was that of the hitherto unknown wild hybrid, *Asplenium creticum* Lovis & al. *x* *A. trichomanes* L. subsp.

<sup>1</sup> A list of recent collectors, and a comprehensive revision of the documented flora of Crete is given by Greuter (1973).

*quadrivalens* D. E. Meyer emend. Lovis. A single plant of this putative parentage was found by ACJ at 2100 m on Mt Hagios Pnevma in the Lefka Ori, from where it was removed for subsequent successful cultivation at Leeds University Botanic Garden. It is the purpose of the present paper to describe this hybrid and report upon its cytology which was investigated by PJB.

#### *Description of new hybrid*

##### *Asplenium × khaniense* Brownsey & Jermy, hybr. nova

Planta hybrida, inter *Asplenium creticum* et *A. trichomanes* subsp. *quadrivalens* intermedia. Rhizoma erectum, in apice meristematico paleis subulatis vel anguste triangularibus, attenuatis, ad 5 mm longis et 0.75 mm latis, castaneis centro fuscatis vestitum (cf. fig. 12). Stipes c. 1 cm longus. Lamina linearis vel linear-elliptica, 50-80 mm longa, 10-15 mm lata, apice pinnata, basin versus bipinnata (cf. fig. 2-4). Rachis porphyro-castanea, in quartā vel quintā parte superiore virescens. Pinnae paribus sub-oppositis 13-17, approximatae et imbricatae, sessiles; mediae longiores; superiores ovatae profunde laciñatae; inferiores in lobos vel pinnulas tres inaequales (terminali majore) crenatos divisae. Chromosomatum numerus sporophytæ (2n) 144 est.

**Holotypus:** Kriti, province of Khania, Lefka Ori: shaded vertical gully in limestone rock on north face of Mt Hagios Pnevma, 2100 m, 19th August 1971, Brownsey & Jermy J9149, (cultivated at Leeds University Botanic Garden as PJB C55); growing with its parents, *A. creticum* and *A. trichomanes* subsp. *quadrivalens*, and also with *A. aegaeum* Lovis & al. and *A. ruta-muraria* L. subsp. *ruta-muraria*. Fronds deposited in BM.

Fronds of the wild material (fig. 6-7) are somewhat inferior in quality, and are also slightly different from those grown in cultivation (fig. 2-4) upon which this description has been primarily based. In wild material, there are fewer pinnae (only 9-11 pairs) which are more widely spaced, especially at the base where they are also highly reduced in size. The terminal pinnae are considerably larger than those on the cultivated material, and the stipe is much longer, though still less than half the length of the lamina. In all its aspects the original wild plant shows the characteristics of one growing in a crevice in deep shade.

#### *Cytology*

Developing sporangia have been fixed, stained and the resulting chromosome preparations made permanent according to the method described by Manton (1950: 295-296). Fixings were taken from *Asplenium × khaniense* in the wild, and several more have been taken since the plant was brought into cultivation. The hybrid has proved to be tetraploid with  $2n = 144$  chromosomes; approximately 36 bivalents and 72 univalents were observed at meiosis (fig. 13) but an exact analysis has so far proved impossible to be obtained.

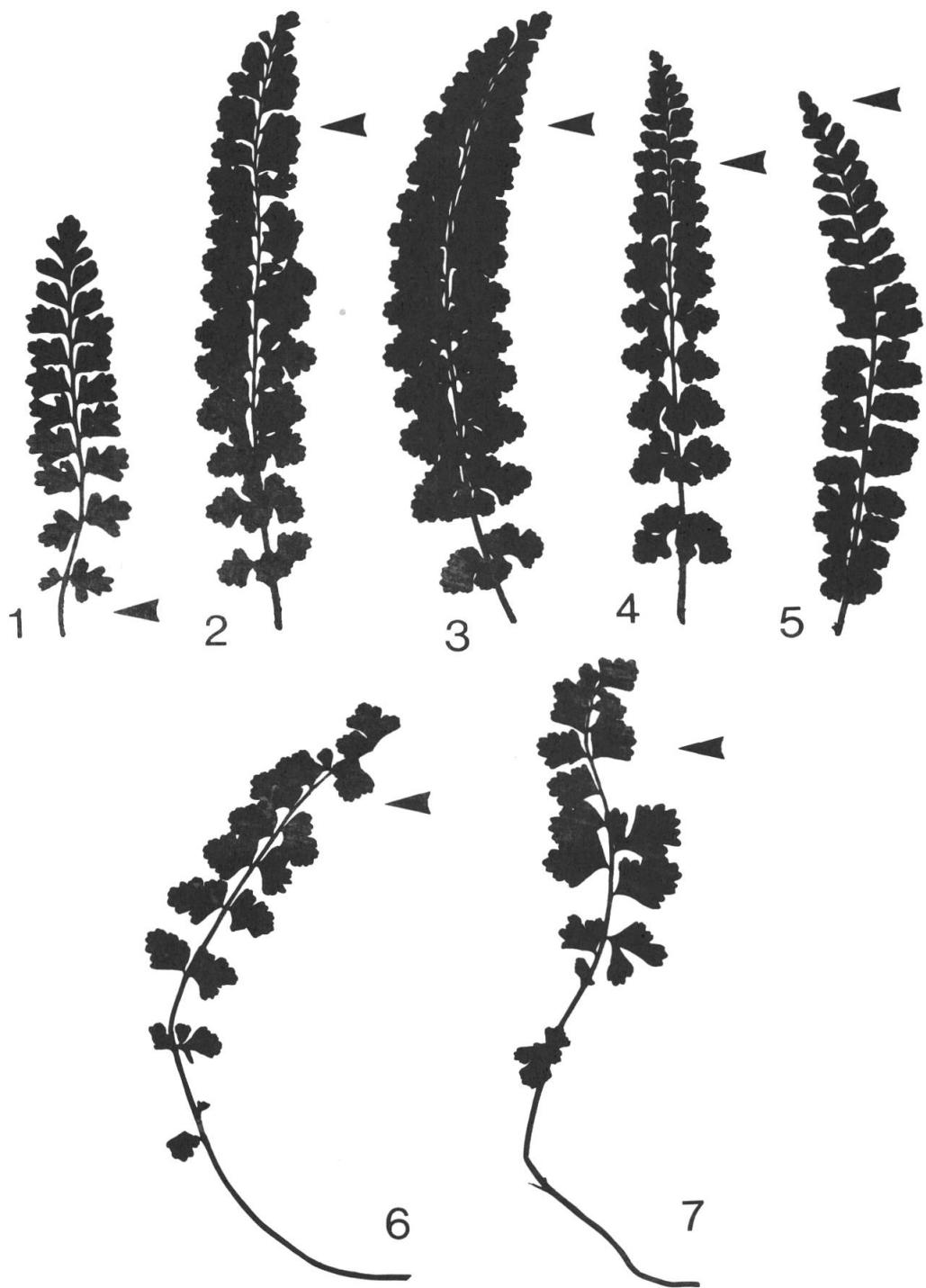


Fig. 1-7. — Silhouettes of fronds in natural size.  
1-5, from cultivation at Leeds (plants originally collected on Mt Hagios Pnevma); 6-7, from plants collected in the wild. 1, *Asplenium creticum* (PJB C53); 2-4, *A. x khaniense* (PJB C55); 5, *A. trichomanes* subsp. *quadrivalens* (PJB C57); 6-7, *A. x khaniense* (J9149). The arrows indicate the position where the colour of the rhachis changes from brown below to green above.

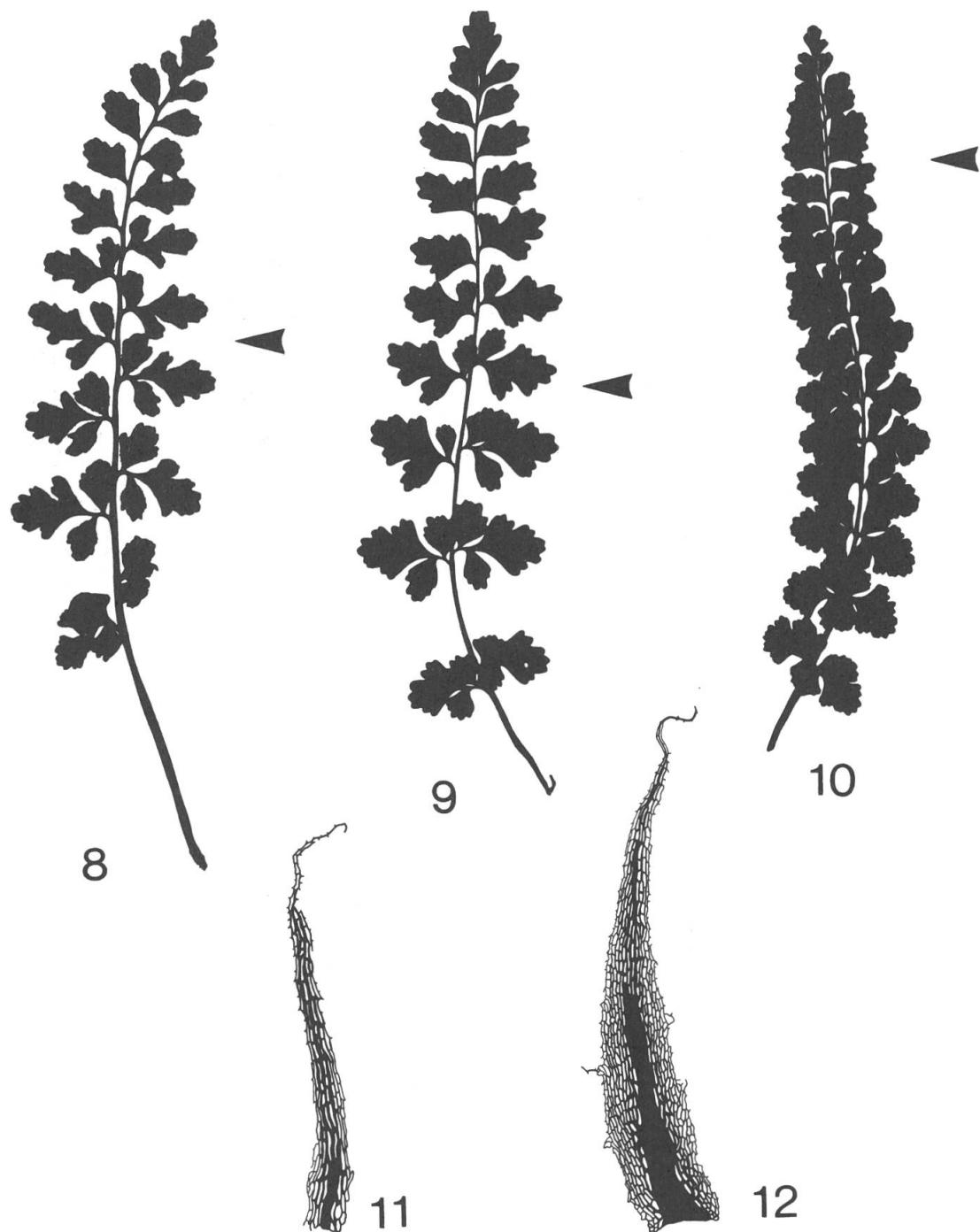


Fig. 8-10. – Silhouettes of fronds in natural size.  
 8-9, *Asplenium x aprutianum*, cultivated in Basel by T. Reichstein (8, from Gole del Sagittario, Abruzzi, Italy, TR 700; 9, from Gahnsleiten near Payerbach, Niederösterreich, Austria, TR 789); 10, *A. x khaniense*, cultivated at Leeds (PJB C55). The arrows indicate the position where the colour of the rhachis changes from brown below to green above.

Fig. 11-12. – Camera lucida drawings from rhizome scales (15 x). 11, *A. x aprutianum* (from Pécs, Hungary, TR 1066); 12, *A. x khaniense* (from Crete, J9149).

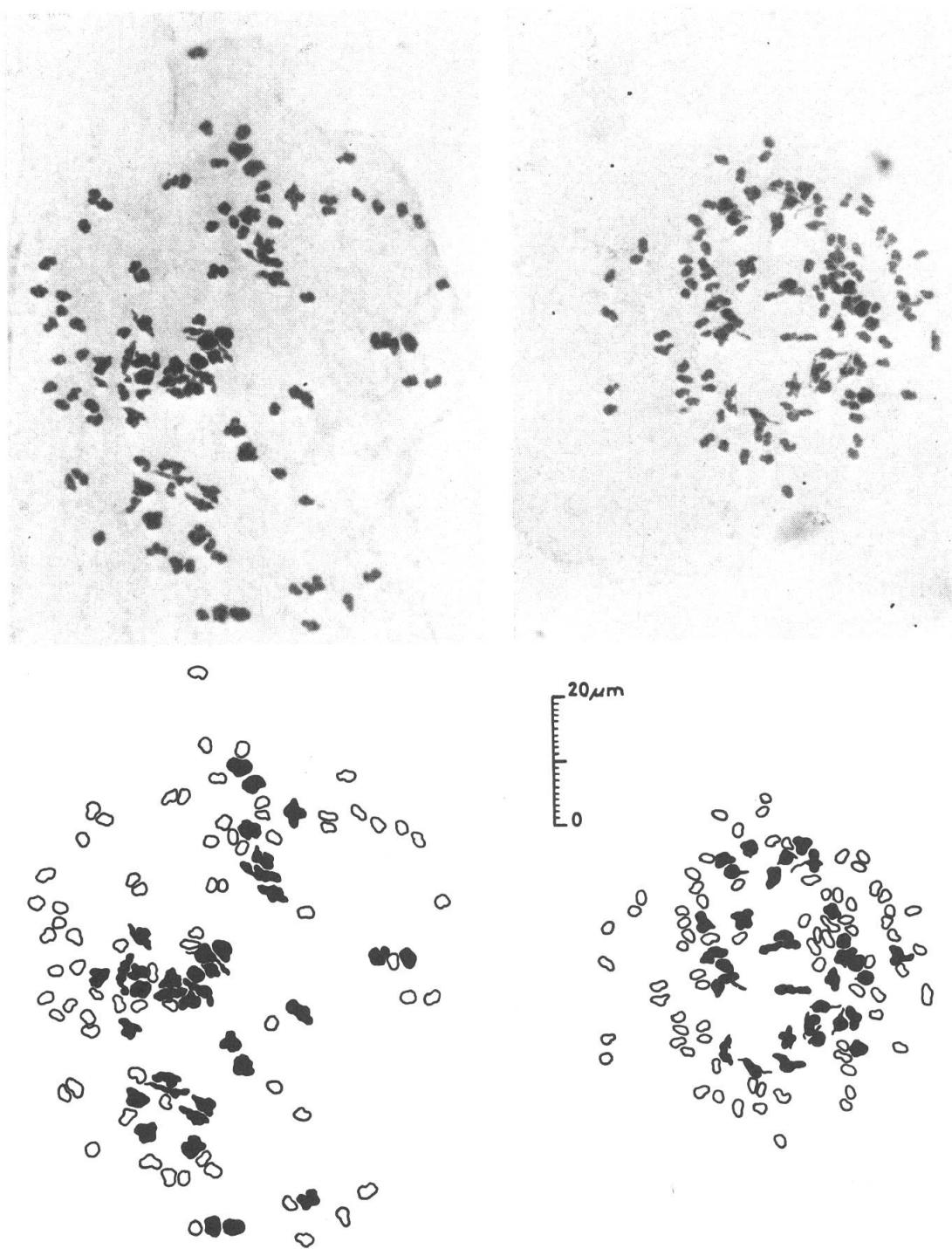


Fig. 13. — Two metaphase plates of meiosis in *Asplenium*  $\times$  *khaniense* (from Crete, J9149), showing approximately 36 bivalents and 72 univalents in each case. Above, photographs from permanent aceto-carmine preparations; below, explanatory diagrams (bivalents in black, univalents in outline).

Cytological investigation of populations of *A. creticum* and *A. trichomanes* subsp. *quadrivalens* from the area in which the hybrid was found has revealed that both species are also tetraploid. Preparations showing 72 pairs of chromosomes at meiosis have been obtained in each case.

### Discussion

In the wild, *Asplenium*  $\times$  *khaniense* appears very similar to another tetraploid hybrid, *A. x aprutianum* Lovis & al. (*A. lepidum* C. Presl  $\times$  *A. trichomanes* subsp. *quadrivalens*) but when these hybrids are grown in cultivation important distinguishing characters become apparent. Thus, fronds of *A. x aprutianum* have longer stipes and fewer pinnae (up to 10 pairs) which are of greater length, more divided and much more widely spaced (cf. fig. 8, 9 and 10). Furthermore, the rhizome scales of *A. x aprutianum* are darker brown in colour and lack the extensive central band of occluded cells characteristic of *A. x khaniense* (fig. 11, 12). Nevertheless, it is not always possible to cultivate wild specimens, and it will always be difficult to identify with certainty hybrids collected from areas where all three putative parents are known to grow together. Indeed, one dried specimen collected by Zaffran from Mt Ornio (Zaffran 82) has been identified as *A. x aprutianum* by Reichstein (Zaffran, 1970; Reichstein & al., 1973). However, this conclusion was drawn without the benefit of having seen specimens of *A. x khaniense*, and examination of Zaffran's material by one of us (PJB) suggests that it may actually be *A. x khaniense* but, since there are no rhizome scales, a conclusive identification is not possible.

A third tetraploid wild hybrid, *A. x clermontiae* Syme (*A. ruta-muraria* subsp. *ruta-muraria*  $\times$  *A. trichomanes* subsp. *quadrivalens*), whose parents grow in the Lefka Ori is also similar to *A. x khaniense*. However, there are adequate criteria for distinguishing this hybrid even in wild material. The tough coriaceous texture and blue-green colour of the frond of *A. ruta-muraria* is also apparent in fronds of the hybrid and the pinnae are more regularly rounded than in *A. x khaniense* with the largest occurring at the base of the frond.

Artificial resynthesis of *A. x khaniense* has been achieved on two separate occasions. Reichstein & al. (1973) reported the synthesis of the hybrid *A. creticum*  $\times$  *A. trichomanes* subsp. *quadrivalens*, but unfortunately this plant grew very poorly in cultivation. Another plant arose spontaneously at Leeds in a pot ostensibly sown with spores of *A. creticum* but in which plants of both parent species and the hybrid developed together. The hybrid has yet to reach full maturity but there can be no doubt of its identity, the fronds appearing indistinguishable from young leaves of *A. x khaniense*, but quite different from those of *A. x aprutianum*.

Cytological investigation of wild material of *A. x khaniense* has revealed the presence of approximately 36 bivalents and 72 univalents at meiosis. A similar result was obtained by Reichstein & al. (1973) from their synthetic hybrid and has also been obtained from the spontaneous plant which arose in Leeds. However, in no case has an exact analysis of a meiotic cell been achieved (the diagrams in fig. 13 merely represent the most likely interpretation of the chromosome pairing). In particular, it has not been possible to rule out absolutely the presence of any trivalents, and therefore the possibility of some degree of homology

existing between the genomes of *A. trichomanes* and one of those from *A. creticum* cannot be excluded on the evidence provided by this hybrid alone.

Recently, however, good evidence has been obtained to show that *A. creticum* is an allotetraploid species which has originated from hybridisation between the diploid species *A. aegaeum* Lovis & al. and *A. viride* Hudson (Brownsey, 1973). Furthermore, complete failure of pairing was observed at meiosis in an artificial hybrid between *A. creticum* and the diploid species, *A. trichomanes* subsp. *trichomanes*, indicating conclusively that *A. creticum* is of allopolyploid origin, and that there is no homology between the genome of *A. trichomanes* and either of those from *A. creticum*.

It may therefore be concluded that only two of the four genomes in *A.  $\times$  khaniense* are capable of pairing at meiosis, and that these have been contributed by *A. trichomanes* subsp. *quadrivalens*, which is known to behave like an autotetraploid in hybrids (Lovis, 1955; Lovis, Melzer & Reichstein, 1966; Lovis & Reichstein, 1969; Reichstein & al., 1973). The unpaired genomes which are incapable of pairing either with themselves or with those of *A. trichomanes* are contributed by the allotetraploid parent, *A. creticum*.

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Addresses of the authors: P.J.B., Department of Plant Sciences, University of Leeds, England (now at Botany Department, Victoria University, Private Bag, Wellington, New Zealand). A.C.J., Botany Department, British Museum (Natural History), London SW7 5BD, England.