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Karyological studies in *Hieracium baeticum* (*Asteraceae*) from the "Parque Natural de la Sierra de Grazalema" (Southern Spain).

Abstract

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Hieracium baeticum is a triploid ($2n = 27$) species restricted to limestone mountains in S and E Spain. Both developing capitula and root tips from seedlings were used for the study. The karyotype suggests an allopolyploid origin of the taxon, and a relative close affinity between its parental is indicated by the possibility of partial pairing of most chromosomes during microsporogenesis. This process is completely disturbed starting from the first phases to degenerative pollen formation: none is formed or shed. In more than 60 % of the flowers, some kind of synapsis occurs in pollen mother cells, while there is total univalence in the rest. The species is considered strictly agamospermous in all the studied populations.

Introduction

Hieracium L. is, together with *Taraxacum* Weber, one of the most complex and widespread genera in the *Asteraceae*. Its complex taxonomy (see e.g. Sell & West 1974, 1976) is due to asexual propagation via agamospermy, known since Raunkiaer (1903) and Ostenfeld & Raunkiaer (1904).

The basic chromosome number in the genus is $x = 9$ (Stebbins 1938, Gadella & Kliphuis 1970), with ploidy levels from 2x to 10x existing in the agamic complex (Gadella 1987). Nevertheless, $2n = 27, 18, 36, 45$, seem in this order to be the most frequent numbers at least in subg. *Hieracium* (Rosenberg 1927, Fernández & Queirós 1971, Delcourt 1972, Queirós 1973, Retz 1973, Mills & Stace 1974, Morton 1974, Auquier & Renard 1979), with a high incidence of agamospermy in the polyploids.

Hieracium baeticum Arvet-Touvet & Reverchon is a member of this subgenus, partially restricted to, limestone mountains in E and S Spain (Betic Mountains) (Talavera 1987, Rivas Martínez & al. 1991, but see Montserrat 1983). It seems to be related to *Hieracium texedense* Pau, *Hieracium elisaeanum* Arvet-Touvet ex Willk., and *Hieracium loscosianum* Scheele, most of which are also triploid (Blanca & Cueto 1984, Blanca & al. 1987, Luque & Díaz 1991) and the diploid ($2n = 18$), presumably sexual, *H. laniferum* Cav. (Merxmüller 1975). Nevertheless, Pajarón (1986) (see Table 1) indicated $2n = 26$ for *Hieracium baeticum*, probably due to a miscount. No previous study concerning microsporogenesis exists in this group of species. With regard to this process, Rosenberg (1927) described three main types of degenerative meiosis in pollen mother cells (PMC) in

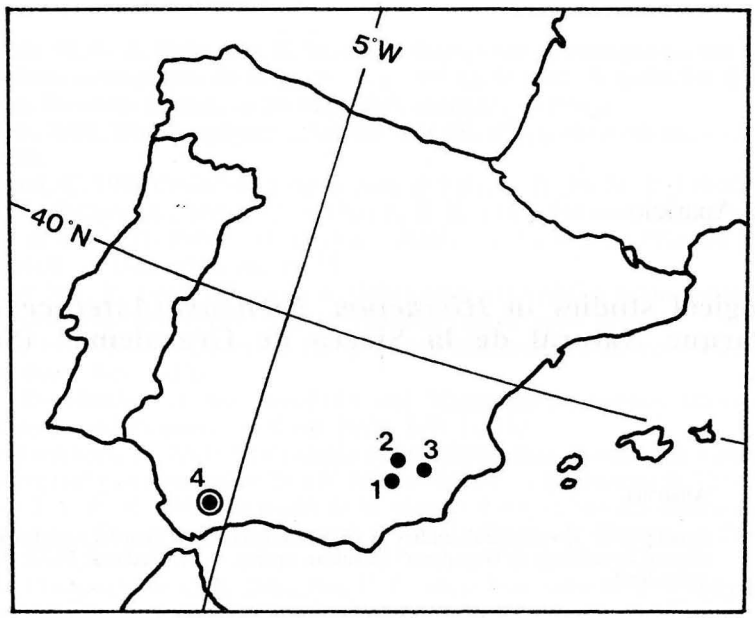


Fig. 1. Provenance of karyologically studied plants of *Hieracium baeticum*. The circled spot indicates the population studied in this paper.

subg. *Hieracium*, to which Gustafsson (1939) added two more. In summary, failure of chromosome pairing and contraction, retardation of meiosis and precocity of meiotic divisions are the main abnormalities in microsporogenesis (Gustafsson 1947, Stebbins 1950), resulting in abnormal sporads sometimes setting some good restitutional or reduced pollen.

All these kinds of alteration are common in other genera of related plants, e. g. *Taraxacum* (Maleka 1964, Koul & Sing 1982), *Chondrilla* L. (Bergman 1950) or *Eupatorium* L. (Rozenblum & al. 1988) and unrelated ones such as *Sorbus* L. (Liljefors 1958).

The aim of the present study was to investigate (1) chromosome number and morphology and (2) chromosome behaviour during microsporogenesis. It is to serve as a starting point in our knowledge of the relationships, biology and taxonomy of Betic *Hieracium* taxa.

Material and methods

Plant material

Plants were collected from the southernmost population of this taxon (Fig. 1), in the Sierra de Grazalema (Cádiz province). Here *Hieracium baeticum* is a perennial herb (Fig. 2) up to 20 cm high. The leaves are all basal, except some which are bract-like on a scarcely-branched flowering stem, entire, densely covered with long white hairs; the involucre is c. 10 mm long, the bracts are shortly glandular, linear-lanceolate with membranous margins; the flowers are up to 14 mm long; the fruits c. 2.5 mm, very dark red to black, shiny and glabrous, with a 4-5 mm long white pappus of short bristle outer rows (see also Talavera 1987).

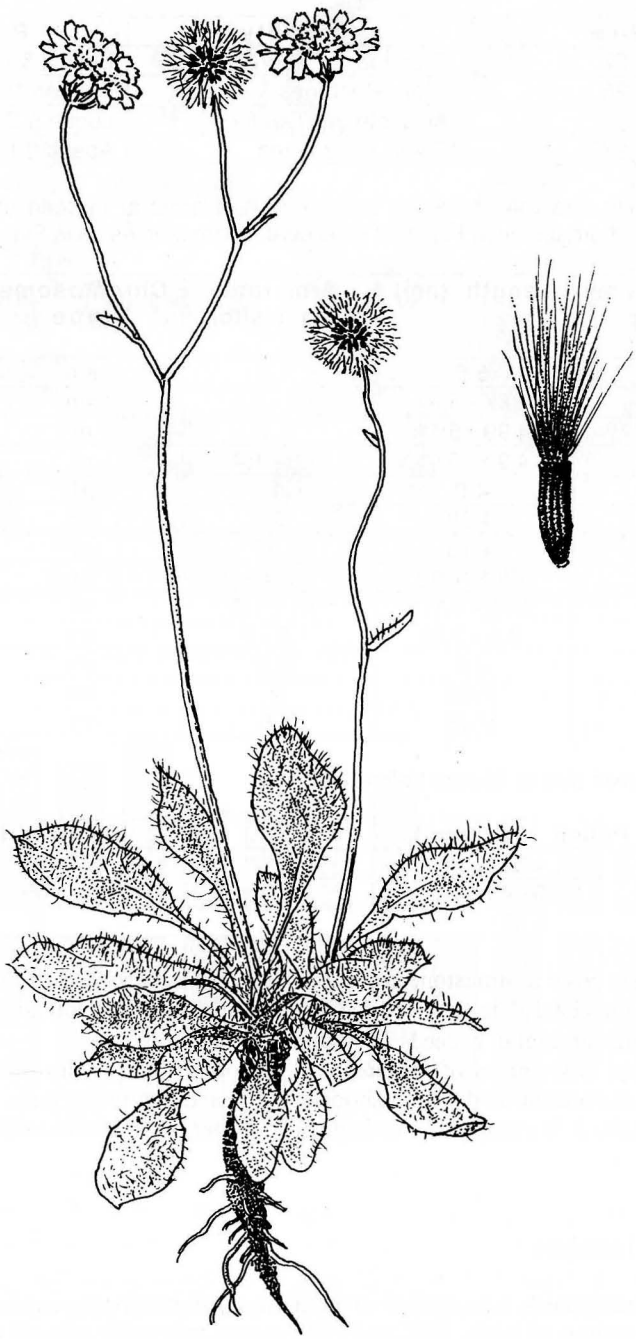


Fig. 2. *Hieracium baeticum*, with a flowering and fruiting capitula and mature cypsela.

Table 1. Chromosome counts in *Hieracium baeticum*

2*n* =	Locality	Reference
27	Granada, La Sagra	Blanca & Cueto 1984
26	Jaén, Pontones	Pajarón 1986
27	Albacete, río Taivilla	Luque & Díaz 1991
27	Cádiz, Grazalema	Aparicio 1993 & this paper

Table 2. Size and ratio between long and short arms of thirteen chromosome groups. Numbering of groups as in Fig. 4, of individual chromosomes as in Fig. 3.

Chromosome Group	Length (nm)	Arm ratio long : short	Chromosome type	Individual chromosome number
1	5.6	1.8	sm	15, 27
2	4.87 - 5.47	2.3 - 2.5	sm	16, 22, 24, 26
3	4.99 - 5.11	1.1	m	2, 21
4	4.99 - 5.11	1.3 - 1.4	m	1, 3
5	4.8	1	M	7, 9
6	4.38	2	sm	13, 19
7	4.13	1.57	m	20, 25
8	3.89 - 4.13	2.4	sm	5, 14
9	3.89 - 4.113	1.1 - 1.3	m	11, 12, 18
10	3.4 - 3.65	1.8 - 2	sm	6, 8, 10
11	3.4	2.5	sm	17
12	2.92	2	sm	4
13	2.43	1.5	m	23

Table 3. Pollen size in flowers before anthesis.

Pollen size (nm)	% (*n* = 150)
< 30	27
30 - 40	65
> 40	8

The plants grow in limestone crevices in very rocky places at 1300 - 1600 m of altitude in the "Parque Natural de la Sierra de Grazalema". The mean annual temperature is 14° C, and mean annual rainfall exceeds 2000 mm.

A detailed description of the area can be found in Aparicio & Silvestre 1987. This population is concentrated within about 1000 ha and comprises three rather scarce groups of individuals. It is completely isolated from other populations, and of other *Hieracium* species.

Cytological analysis

Microsporogenesis was studied in medium-sized developing capitula, fixed in the field during the springs of 1989, 1991 and 1992 in an acetic acid : ethanol mixture 3 : 1, passed to 70 % ethanol after 24 h and stored at 4-6° C.

The developmental and sequential states of this process were established by observing more than sixty flowers from a single capitulum, and the existence of the same events was

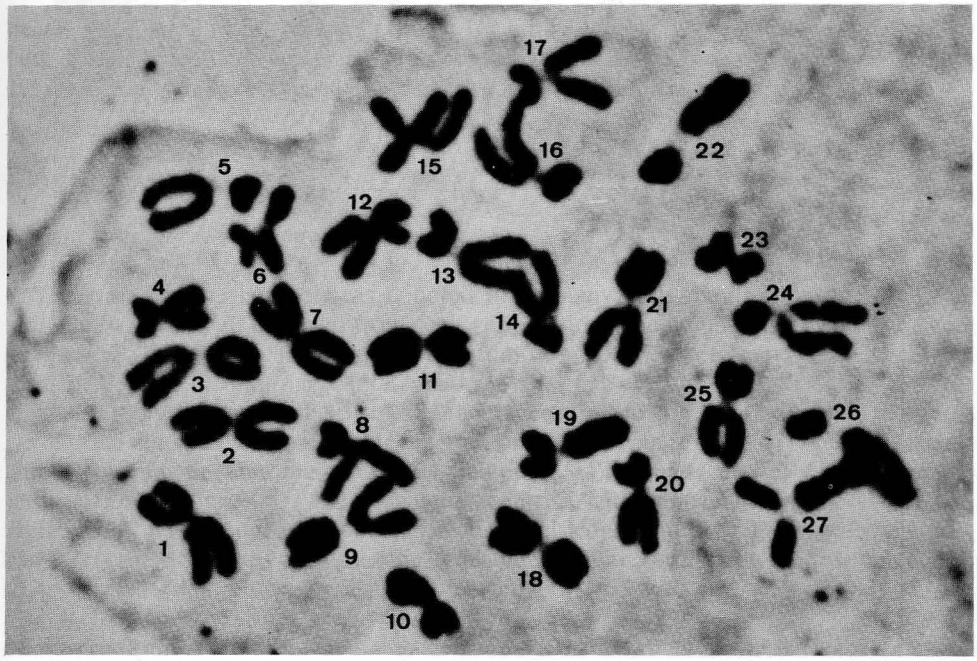


Fig. 3. *Hieracium baeticum*: somatic metaphase, $2n = 27$.

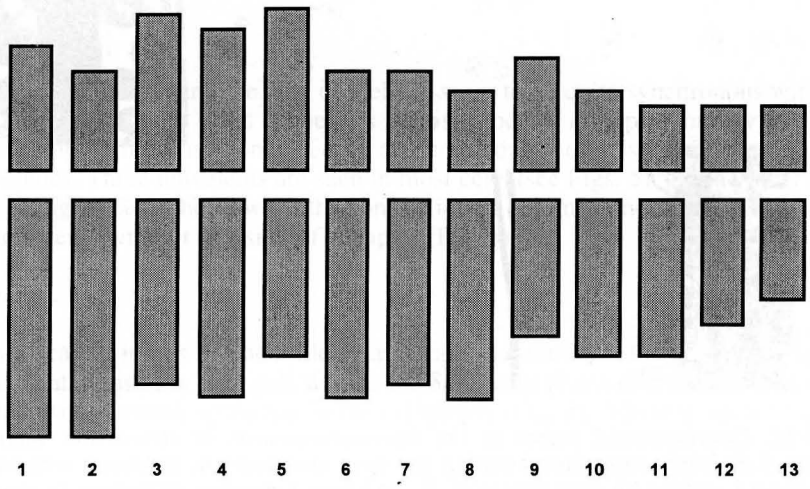
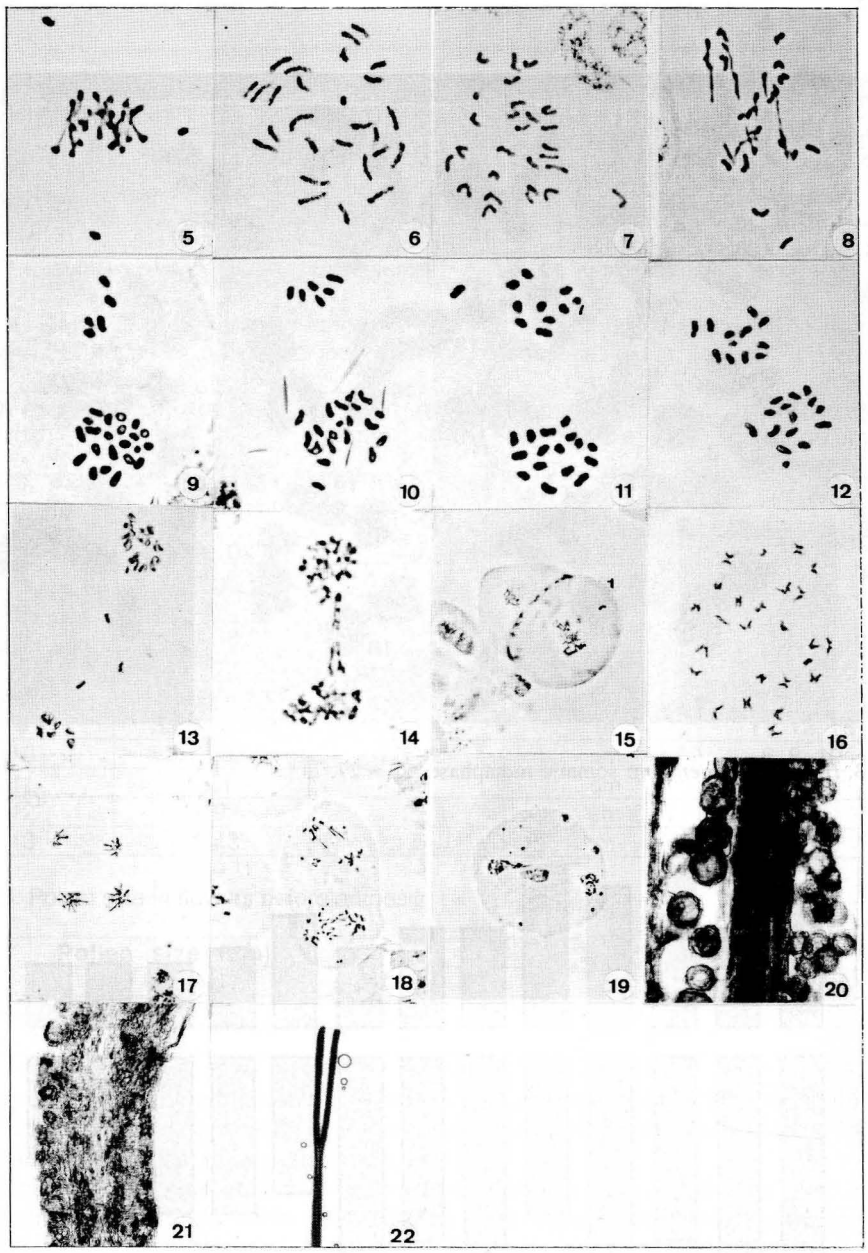


Fig. 4. Idiogrammatic representation of the thirteen chromosome groups detected in the karyotype of *Hieracium baeticum*. The asymmetry is 2B.

checked in 3-14 flowers of another ten individuals in the population (one capitulum from each).

For somatic chromosome analysis, the actively growing root tips of twelve plants, obtained from the germination of randomly gathered cypselas in the population, were



Figs. 5-22. Developmental stages in the microsporogenesis of *Hieracium baeticum*. **5**, metaphase I showing unascertained pairing and three univalents; **6**, diakinesis without pairing. **7**; metaphase I without bivalent formation; **8**, Anaphase I showing difficulties in chromosome separation; **9**, Anaphase I with unbalanced chromosome repartition 5/22; **10**, idem 6/21; **11**, idem 11/16; **12**, idem 12/15; **13**, lagging chromosomes in telophase I; **14**, chromatin bridges in telophase I; **15**, precocious wall formation of sporocytes in telophase I; **16**, metaphase II in a 10/17 sporocyte; **17**, regular repartition of chromatids in telophase II with unbalanced nuclei; **18**, irregular repartition of chromatids in anaphase II; **19**, very irregular sporad with four main nuclei and numerous spreading portions of chromatin; **20**, scarce pollen grains developing in a young anther, with incomplete exine and different sizes; **21**, pollen grains aborted in anther just before anthesis; **22**, stigma just protruding without any attached pollen grains.

treated with 0.002 M 8-hydroxyquinoline for 4 h and then fixed and stored as above. In both cases, the samples were stained in cold alcoholic hydrochloric acid carmine (Snow 1963) studied and photographed under phase contrast light microscopy.

Results

Chromosome number and morphology

The chromosome number was counted in as many cells as possible in the twelve individuals studied all obtained from seed germination. The result was always the same: $2n = 27$ (Fig. 3). The apparent size of the chromosomes varied between 2.43 and 5.60 nm, medium-small to medium-large (Stebbins 1938).

The karyotype presented 2B asymmetry, according to the terminology of Stebbins (1971), and consisted (according to the terminology of Levan & al. 1965) of 2 metacentric chromosomes with centromere in the median point (M), 10 metacentrics with centromere in the median region (m), and 15 submetacentrics with centromere in the submedian region (Sm). Based on the morphology as the total length and the ratio between the long and short arms 13 groups can be arranged (Table 2, and idiogrammatic representation, Fig. 4), to which 1 - 4 chromosomes can be allocated.

Microsporogenesis

As expected, the process was completely disturbed. From the earliest phase to pollen grain development, a series of failures was observed affecting the I and II meiotic divisions, leading to a lack of fertile pollen formation (Fig. 5-22).

Prophase I.

PMC differentiation and the start of meiosis seem to be quite synchronous within each flower but two different ways of meiosis seems to occur: in approximately 66 % of the flowers studied, this phase concluded by an anomalous and unascertainable "pairing" of chromosomes. Three univalents are seen in most cells (see Figs. 5). On the other hand, in the remaining 34 % of the flowers, the contraction of chromosomes ends in the formation of 27 univalents without any sign of synapsis (Fig. 6).

Metaphase I.

In most cases, it was not possible to ascertain the number of multivalents, bivalents and univalents formed in each cell, despite the number of plates observed, except when 27 univalents were formed, spreading in the cytoplasm (Fig. 7). All these seem to attach to the spindle.

Anaphase I.

Stretching of the spindle produced an uneven distribution of chromosomes when "paired", producing portions of chromatin and lagging chromosomes (Fig. 8) due to a considerable difficulty in separation of the "associated" chromosomes. In the cases of total univalence, there was a more or less passive movement of chromosomes to the poles, resulting in an unbalanced distribution (see e. g. Fig. 9-12) from 27/0 to 14/13.

Telophase I.

Fragments and lagging chromosomes (Fig. 13) are common in this phase, resulting in small nuclei set apart from the two main ones. Chromatin bridges and precocious wall formation are also frequent as abnormalities in the development of this phase. Therefore, unbalanced binucleate cells, sometimes with scattered small nuclei or chromatin bridges (Fig. 14), and seldom cells with walls formed (Fig. 15), are the most common cell forms at the end of the first meiotic division.

Prophase and Metaphase II.

These phases seem to be the most regular ones. After a brief interkinesis, the chromosomes undergo condensation and very soon appear to consist of two chromatids (Fig. 16). All of them seem to attach to the spindle.

Anaphase II.

While some cells pass through a more or less normal migration of chromatids to the poles (Fig. 17), many disturbances - mostly in the sporocytes in which synapsis had occurred - were again observed at this stage: chromatids broke into pieces which spread in the cytoplasm (Fig. 18).

Telophase II.

Therefore, the resulting sporads may be either 4 - different-sized nuclei as a result of the "unpaired" metaphase, or multinucleated (Fig. 19) as a consequence of the very irregular previous phases coming from the "synapsis". Very irregular tetrads were also formed when a precocious wall formation occurred in telophase I.

Pollen formation.

As a consequence of all disturbances in the meiotic process, the pollen is extremely variable in size, ranging between 1.25 and 55 µm, although it mostly measures between 30 and 40 µm (Table 3). In the last phase, during exine development, which is mostly poor and incomplete (Fig. 20) as in other apomicts (see e.g. Rousi & al. 1985), a decrease in pollen stainability by safranin was observed (from 81 % to 24 % from young pollen to just before anthesis), followed by comple degeneration within the anther at anthesis (Fig. 21). No viable pollen was, therefore, observed to be formed or shed. Young stigmas just protruding showed a total lack of attached pollen (Fig. 22), while older stigmas were found to bear pollen from other cohabiting species such as *Helianthemum origanifolium* (Lam.) Pers., *Thymus granatensis* Boiss., *Centaurea castellanoides* Talavera, *Brassica repanda* subsp. *confusa* (Emberger & Maire) Heywood, *Scabiosa turolensis* subsp. *grosii* (Pau) Devesa, etc., but none attributable to *Hieracium baeticum*.

Discussion

From the results in this paper, the somatic chromosomes of *Hieracium baeticum* seem morphologically quite similar to each other and could be mostly coupled. If *Hieracium baeticum* is an allotriploid, as is suggested for other *Hieracia* by Gadella & Kliphuis (1970), both parental species likely bear similar chromosome sets judging from the general morphology and partial pairing. If it is eutriploid, relatively high rates of

chromosome rearrangements must have subsequently occurred as indicated by the irregular set of chromosomes, where nine triplets cannot be discerned (see Richards 1972, for a similar situation in Taraxacum).

In the *Hieracium baeticum* plants studied, meiosis in PMC seems to correspond partly to the semiheterotypic division of the ìLevigatumî - type, with total univalence, and partly as the "Boreale" - type with some degree of synapsis, as described by Rosenberg (1927). Nevertheless, restitution nuclei and diploid pollen seem to be very rare (if at all present), as in male organs of other *Asteraceae*, although they seem frequent in other *Hieracium* taxa (Gustafsson 1938). On the contrary, the incomplete pairing or total asynapsis leads to the formation of two to more unbalanced nuclei in telophase I and thus of polysporads with micronuclei at the end of meiosis. The anthers of *Hieracium baeticum* have never been found to shed any pollen, all being completely degenerated within the anther before anthesis. Due to the fact that approximately 62 % of the flowers are able to set fruit with a very high degree of germination (Aparicio 1993), the lack of fertile pollen makes the existence of sexualy produced embryos impossible. Therefore, from the results presented in this paper, *Hieracium baeticum* must be considered in the population studied a strict male-sterile agamospecies.

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