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Hippocrepis balearica aggregate. A statistical analysis

Abstract

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A biometric study of the *Hippocrepis balearica* aggregate from Balearic Island and Alicante (eastern Iberic Peninsula) was carried out. Fiftyeight samples were used in a statistical treatment (principal component analysis and clusterisation) based on 26 variables. The results support the recognition of two species, *H. balearica* and *H. grosii* comb. nova. Two subspecies within *H. balearica* are included, *H. balearica* subsp. *balearica* and *H. balearica* subsp. *valentina*. Factors in evolutionary trends are suggested.

Introduction

The taxonomy of the genus *Hippocrepis* is considered difficult (Ball 1968). Several monographic studies put forward various ideas about the relative affinities among the different species (Bellot 1947, Hrabêtová-Uhrová 1949, 1950a, 1950b, Bolòs & Vigo 1984).

Bellot (1947) notes the similarity between *H. valentina* Boiss. and *H. balearica* Jacq. In a evaluation of the methodology of organising numerical taxonomy, Garrido & Escarré (1973), using data from Bellot (1947) and Ball (1968), confirm the affinities among the various taxa of the genus.

The *H. balearica* aggregate is made up of a variable number of taxonomic units. Bellot (1947) and Ball (1968) accept the existence of two species: *H. balearica*, exclusive to the Balearic islands, and *H. valentina*, which extends through the mountains of the eastern Iberian Peninsula (Fig. 1). Hrabêtová-Uhrová (1950a), Bolòs & Vigo (1984) and Bolòs & al. (1990) consider the two species above mentioned to be subspecies of *H. balearica*.

Pau (1934) describes a new variety, var. *grosii*, basing on material collected by Gros in Ibiza. Bellot (1947) believes that this taxon is only a form of the species. However, Mus & al. (1990) are of the opinion that these populations merit a more important taxonomic position and regard them as a subspecies.

Lainz (1989) studied *H. balearica* in Menorca and proposes that the populations on this island constitute a variety (var. *minoricensis*).

All the taxa show a rupiculous character (Bolòs & Vigo 1984; Bolòs & Molinier 1958, Bolòs & al. 1970). The plants of Mallorca and Menorca, *H. balearica* subsp. *balearica*, behave exclusively as fissure-dwellers.

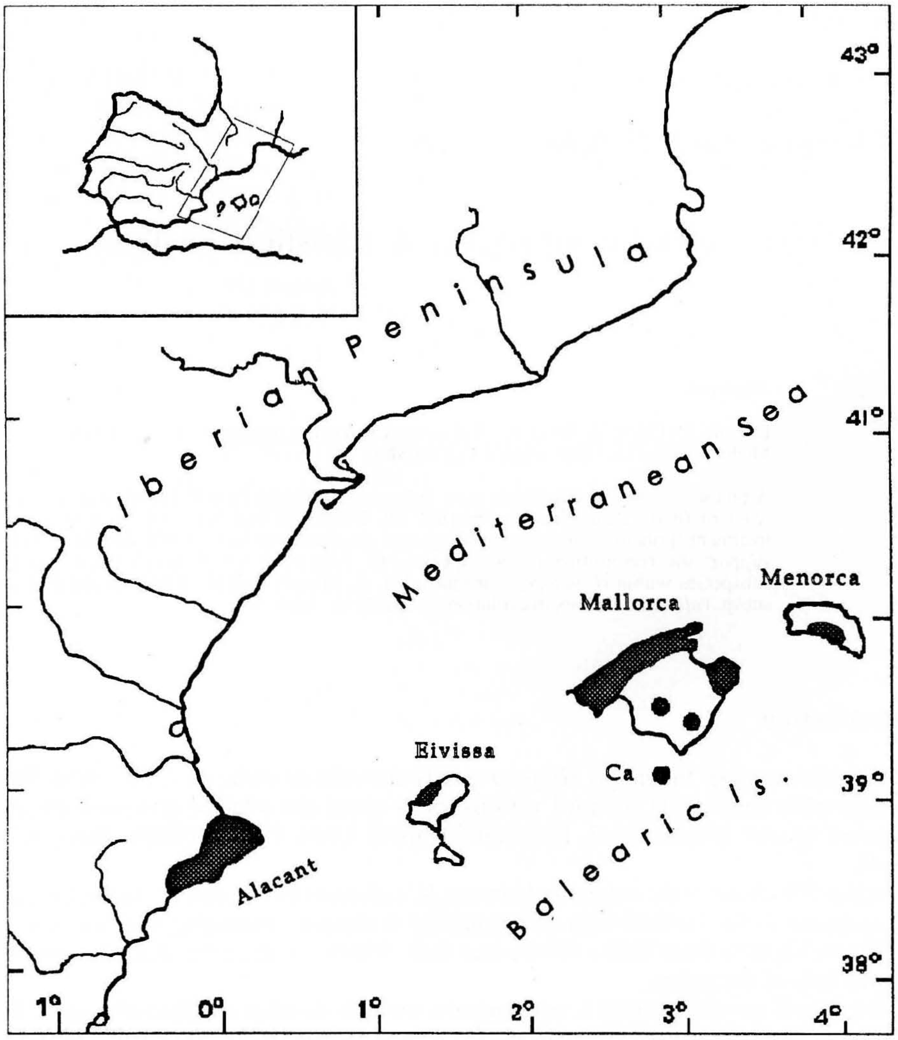

Fig. 1. Map showing the distribution ofH. *balearica* species complex.

Those of Alicante, *H. balearica* subsp. *valentina* (Boiss.) Uhrovà, although preferentially fissure-dwellers too, can be found in scrub close to cliffs (Bolòs 1967).

The Ibiza populations, *H. balearica* subsp. *grosii* Mus, Rosselló & Torres, grow in screes and rocky areas, such as in the littoral scrub.

It is much less common in cracks of the crags (Boira & al. 1989, Rivas-Martinez & al. 1992).

This paper aims to improve the understanding of the taxonomic and ecological/biogeographical relationships among the three taxa which comprise the *H. balearica* aggregate using morphological characteristics and numerical analysis.

Material

For the study, fresh material has been used with the exception of determinations of fruits and seeds.

Fiftyeight samples have been taken from the areas in which the three taxa occur (7 from Alicante, 6 from Menorca, 20 from Mallorca and 25 from Ibiza) including the different habitats in which the plants are found (Table 1) so that the samples would include the maximum diversity of each population.

For each OTU, 26 attributes (characters) relative to the phenotype have been determined (Table 2).

Additionally, length and width of 1650 samples collected evenly throughout the 11 Mallorcan and Menorcan populations have been assessed to establish seed size variability.

Table 1. Codes and geographical location of samples.

B1-B2	Puig de Randa (Mallorca).
B3-B4	Santueri (Felanitx-Mallorca).
B5 to B8	Serra d'Artà (Mallorca).
B9-B10	Serra de Ternelles (Pollença-Mallorca).
B11-B12	Puig Major (Mallorca).
B13-B14	Lluc (Mallorca).
B15-B16	Valldemossa (Mallorca).
B17-B18	Andratx (Mallorca).
B19-B20	Alcúdia (Mallorca).
B21 to B24	Aljandar (Menorca).
B25-B26	Llucalari (Menorca).
G1 to G7	Ses Balandres (Ibiza).
G8 to G16	Ses Fontanelles (Ibiza).
G17 to G20	Cala d'Aubarca (Ibiza).
G21 to G25	Ses Torretes-Cala Sardina (Ibiza).
V1 to V5	Mongó (Dènia-Alicante).
V6-V7	Serra de Bèrnia (Alicante).

Methods

For statistical treatment of the samples, the program NTSYS, version 1.80 (Rohlf 1993) has been used. Principal component analysis (PCA) is applied to the 26 character data matrix (rows = OTU, columns = characters) and previous standardization, separating data intervals from the coefficient of similarity matrix based on the average taxonomic differences (DIST) and obtained using the SIMINT program. Various clusterisation algorithms are used for the cluster analysis which Sneath & Sokal (1973) define as sequential, agglomerative, hierarchical and ringed. This establishes the major grouping of studied objects or groups of objects as a function of their similarity coefficients. From the resulting matrix (TREE), then processed using the COPH program, a matrix of cophenetic similarity values is obtained (ultrametric). Three methods of clusterisation are independently applied with the SAHN program: simple link method, complete link method and UPGMA (unweighted pair-group method, arithmetic mean). Finally to evaluate the clusters obtained using the above methods, a test is run (MXCOMP program) by comparing the cophenetic values matrix (COPH matrix) and the original distances matrix (DIST). In addition, in order to observe the response of the different taxa to grazing, a number of trials were carried out in autumn (October) in which the green parts of 72 plants were removed: 30 in Mallorca, 20 in Ibiza and 22 in Alicante.

Results

The first 3 factors shown in the PCA assume 63.8% of the total variability.

In the spatial arrangement of the OTUs (Table 3, Fig. 2), 3 differentiated populations are established representing the samples from Ibiza, Alicante and Mallorca (which includes samples from Menorca). It is clear that there is a considerable variation within each of them although the space or distance between each of the three populations is greater.

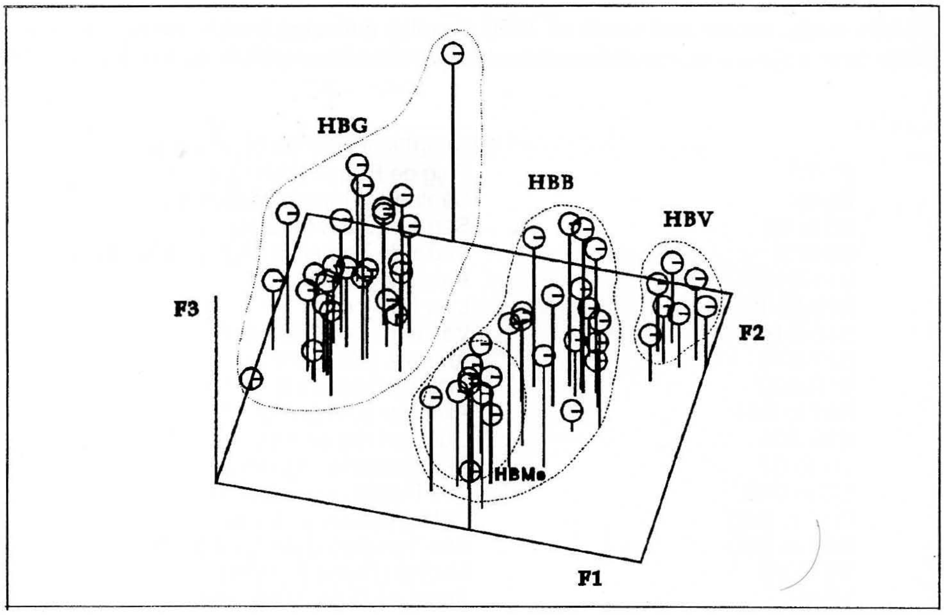

Fig. 2. Distribution in the agreement space with the three primary factors from PCA (Table 3). Origin of populations: Ib, Ibiza. Ma, Mallorca. Me, Menorca. Al, Alicante (Iberian Peninsula).

The OTUs from Menorca form a nucleus with little variation among them; a subgroup within the Mallorcan group. The simple correlation coefficient (Table 4) among the 3 factors, obtained using PCA and the measured variables, gives the greatest weight or taxonomic value to seed curvature ($P<0.01$), pod width ($P<0.01$), leaf length/width relationship ($P<0.01$), length of the claw of the standard ($P<0.05$), width of the standard ($P<0.01$) and flower width ($P<0.05$).

The 3 cluster methods run in the SAHN program show high correlation coefficients (single, r -z Mantel- = 0.846; complete, r = 0.8461 and UPGMA, r = 0.8586). Adopting the latter for its superior fit, the dendrogram (Fig. 3) shows the formation, at a grouping level of K = 7, of 2 groups, G_1 and G_2 with a balanced average taxonomic distance of 1.61. The G_1 group is integrated for the samples (OTUs) from the Mallorcan, Menorcan and Alicante populations. Individuals from the last zone constitute a homogeneous nested differentiated within G_1 at a taxonomic distance of 1.31 from the remainder of the group (level K = 6). The Menorcan samples are somewhat less differentiated (d = 0.676; K = 2).

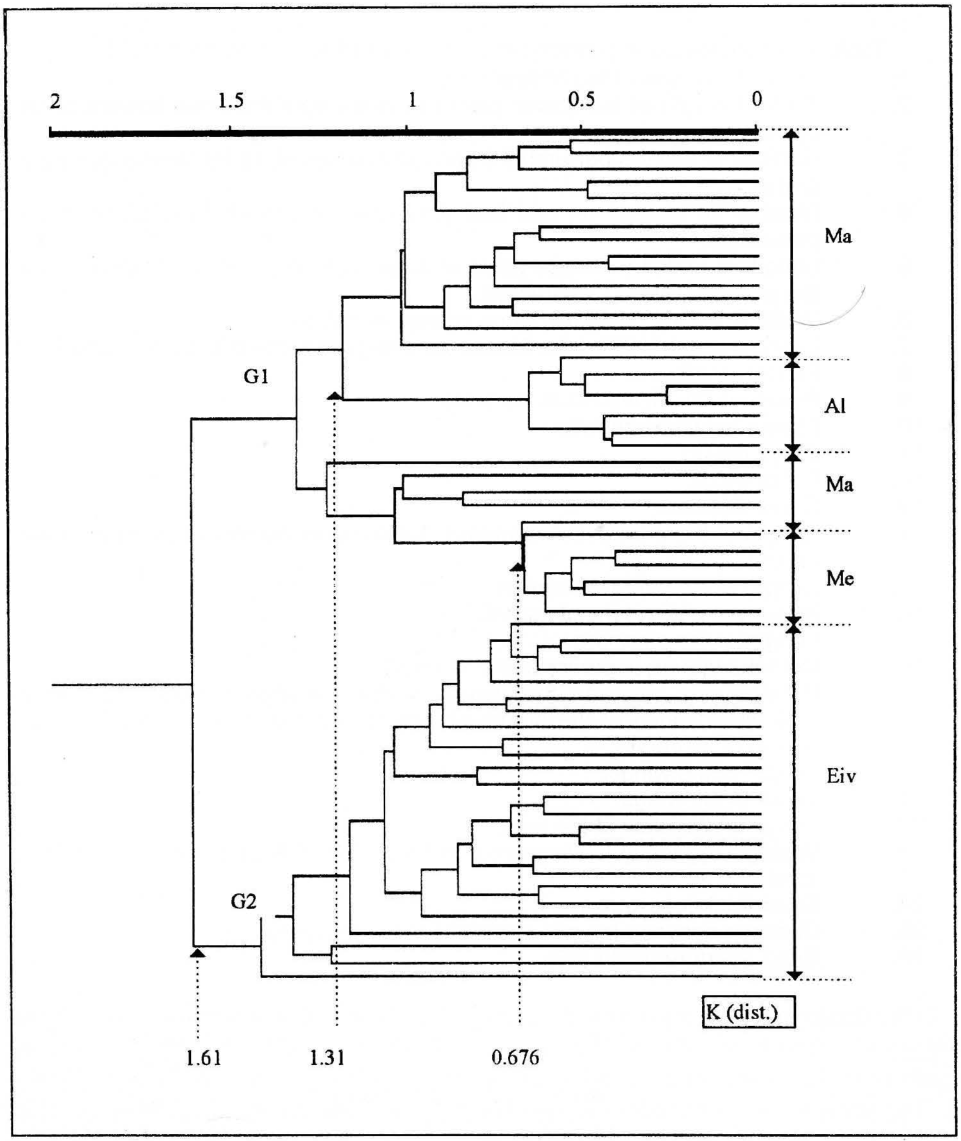

Fig. 3. Dendrogram with UPGMA and average taxonomic distance for coefficient- dissimilarity measures. OTUs from: Ib, Ibiza; Ma, Mallorca; Me, Menorca; Al, Alicante (Iberian Peninsula).

The G_2 group is made up exclusively of Ibizan specimens. The internal differentiation grades in this group are less significant than those of the previous group.

Similar results have been obtained using the OTU correlation coefficients as similarity indices in the same method, within the previously standardized matrix.

Table 2. Relationships of phenotypic characters determined in each OTU.

1.	Peduncle length of the inflorescence.
2.	Pedicel length of the flower (average value from the three flowers of an umbel).
3.	Number of flowers per umbel (rounded average of 10 inflorescences from one plant).
4.	Number of leaflets on one leaf (first ones are taken to have no floral peduncle).
5.	Length of leaflet (average value of those from the same leaf taken as for the previous (No. 4) character).
6.	Width of leaflet, using the same criteria as before.
7.	Leaflets length/width ratio. Measured using the same criteria as 5 and 6.
8.	Flower length.
9.	Flower width (viewed from the front).
10.	Flower length/width ratio.
11.	Calyx length.
12.	Calyx width.
13.	Calyx thickness.
14.	Length of the claw of the standard. Taken up to the fold at the start of the lamina.
15.	Length of the standard lamina.
16.	Maximum width of the standard.
17.	Length of the standard.
18.	Dorsal angle (in degrees) of the standart.
19.	Relationship between the values of the distance between the most projecting edges of the standard lamina and those of the depth of the trough of its curve.
20.	Length of the wings.
21.	Width of the wings.
22.	Length of the claw of the keel.
23.	Width of the legume. Measured on fruits with at least 3 seeds and on the level seeds.
24.	Seed thickness.
25.	Distance between the two extreme arcs of the seed.
26.	Seed aperture/depth ratio.

In the dendrogram obtained from these data (Fig. 4), it is also possible to establish two primary nestings clusters (G_1 and G_2), joined at distance r = -0.382 (K= 6). The G_1 group results from the joining of 2 secondary groups ($G_{1.1}$ and $G_{1.2}$) at distance r = 0,124 (K = 5). The subgroup $G_{1.1}$ includes the specimens from Menorca and some of those from Mallorca. The subgroup $G_{1.2}$ is integrated by the peninsular forms and by some Mallorcan populations. Besides, group G_2 is made up only by Ibizan samples.

The confidence limit of these 2 nestings subclusters is at the level of P < 0'05" since, considering a minimal correlation coefficient to establish a strong dependence relationship of r_0 = 0.8, for n = 58, a threshold correlation coefficient is established at r_L = 0.58 and it is not exceeded by any of the distances considered. The groups at levels less than K=5 can be taken as being due to population variablity.

The measurements of seed length and width from the plants of the Mallorcan and Menorcan populations (Table 5) show that the Mallorcan plants are significantly larger (average confidence interval for the medium values (P<0,05). With lesser confidence levels, they show a tendency to be broader.

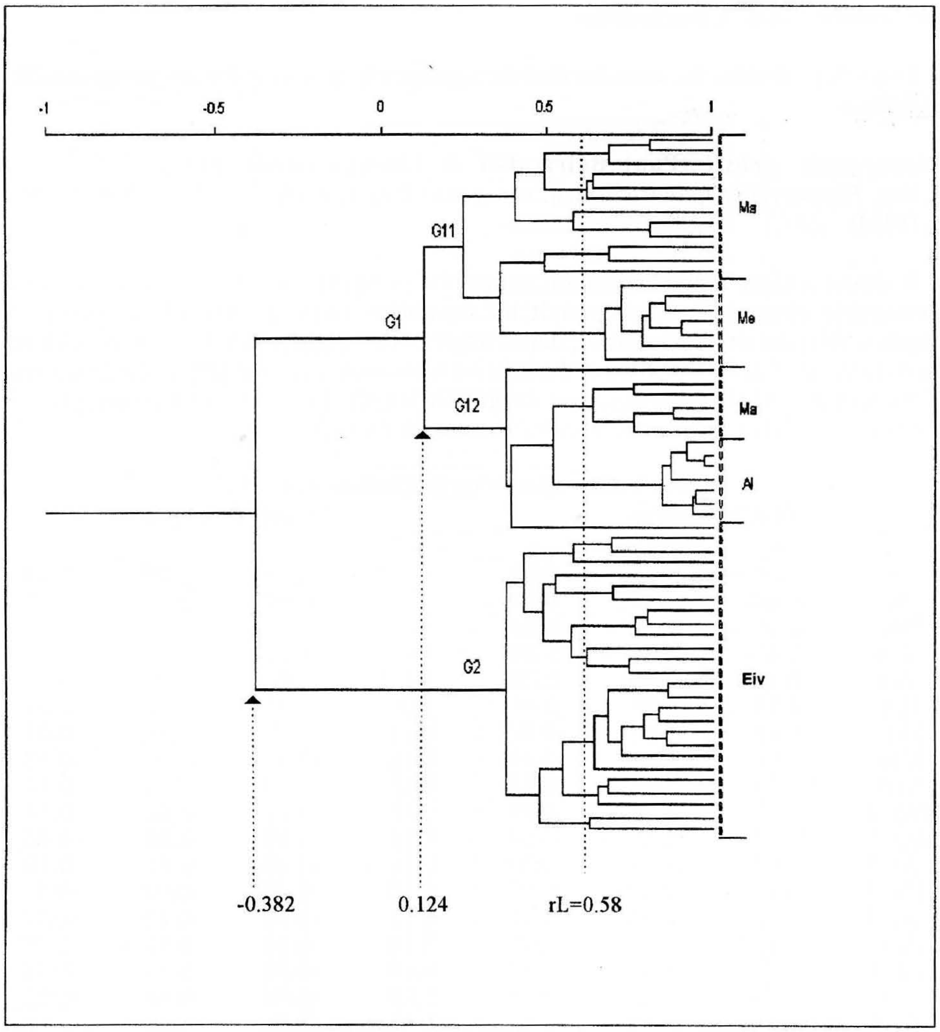

Fig. 4. Dendrogram with UPGMA and correlation coefficient for coefficient- dissimilarity measures. OTUs from: Ib, Ibiza; Ma, Mallorca; Me, Menorca; Al, Alicante (Iberian Peninsula).

The branches of *H. balearica* subsp. *grosii* which were stripped of their greenstuff (they have almost no green stem below the leaves) did not form new buds (0%, n = 20). Those of *H. balearica* subsp. *balearica* and *H. balearica* subsp. *valentina* did form them, in 86.6% (n = 15) and 83.3% (n = 12) respectively of the examples, when they were left with part of the green stem.

The proportion diminished to 20% (n = 15 and n = 10, respectively) when green stems were completely removed.

Discussion and Conclusions

From these results, we consider that the appropriate treatment for the group should be as follows:

Hippocrepis grosii **(Pau) Boira, Gil & Llorens comb. nova.**

Bas. *Hippocrepis balearica* Jacq. var. *grosii* Pau in Broteria Ser. Ci. Nat. 3 (2): 58 (1934)

It shows a clear individualization within the group *H. balearica*. There are certain measurable characters which permit its separation from the rest of the group: the length/width relation of the flower, dorsal angle of the standard, the form of its folding or seed curvature. On the other hand, the relative fruticoseness of the plants, the lesser width of the leaflets and flower wings, and the greater length of the claw of the keel, gives it a much more stylized appearance in comparison with the other taxa.

Table 3. Values of the tree first factors from PCA.

	COMPONENTS				COMPONENTS		
OTUs	1	2	3	OTUs	1	2	3
Ma1	0.56	0.11	0.55	Ei4	-0.72	-0.09	0.04
Ma2	-0.03	-0.72	0.01	Ei5	-0.54	0.37	0.09
Ma3	0.48	0.14	0.54	Ei6	-0.44	0.31	0.32
Ma4	0.35	-0.31	0.31	Ei7	-0.33	0.95	0.73
Ma5	0.32	-0.47	0.39	Ei8	-1.08	-0.22	-0.66
Ma6	0.31	0.09	0.44	Ei9	-0.73	0.57	0.20
Ma7	0.27	-0.02	-0.07	Ei10	-0.56	0.07	-0.01
Ma8	0.62	0.14	-0.45	Ei11	-0.41	0.20	-0.13
Ma9	0.65	0.04	-0.20	Ei12	-0.78	-0.15	0.14
Ma10	0.53	0.08	-0.27	Ei13	-0.38	0.02	0.14
Ma11	0.57	-0.15	-0.54	Ei14	-0.58	0.06	-0.05
Ma12	0.53	0.35	-0.32	Ei15	-0.53	0.31	0.19
Ma13	0.49	-0.42	0.16	Ei16	-0.75	-0.07	-0.17
Ma14	0.25	-0.55	-0.22	Ei17	-0.79	-0.13	-0.47
Ma15	0.43	-0.01	0.15	Ei18	-0.68	0.12	-0.08
Ma16	0.70	-0.10	0.12	Ei19	-0.75	0.13	-0.10
Ma17	0.23	-0.93	-0.24	Ei20	-0.98	0.14	0.22
Ma18	0.62	0.13	0.40	Ei21	-0.68	0.48	0.15
Ma19	0.54	0.13	0.08	Ei22	-0.83	-0.08	-0.07
Ma20	0.25	-0.60	0.12	Ei23	-1.03	0.02	-0.16
Me1	0.16	-0.61	0.20	Ei24	-0.68	-0.22	-0.03
Me2	0.15	-0.66	0.15	Ei25	-0.73	0.22	0.16
Me3	0.09	-0.66	0.04	Al1	1.12	0.43	-0.21
Me4	0.15	-0.62	0.07	Al2	0.87	0.27	-0.33
Me5	0.15	-0.41	0.14	Al3	0.92	0.55	-0.07
Me6	0.25	-0.78	0.19	Al4	1.06	0.46	-0.06
Ei1	-0.48	0.17	-0.32	Al5	0.85	0.48	-0.16
Ei2	-0.39	0.29	0.12	Al6	0.90	0.41	-0.25
Ei3	-0.49	0.40	-0.68	Al7	0.99	0.40	-0.27

The different inclination and curvature of the standard as well as the narrower flowers renders the floral morphology different (Fig. 5) and means that within the same space, there can be a larger number of flowers within an inflorescence. Hence the chances of pollination are increased.

Because of the evident relationships which it shows with other taxa, its geographical and taxonomic isolation gives it adequate space within the species range.

Hippocrepis balearica **Jacq. subsp.** ***valentina*** **(Boiss.) Uhrová in Acta Acad. Sci. Nat. Morav.-Siles. 22: 99 (1950)**

This taxon has been regarded as the best individualized within the *H. balearica* group. However, the analyses carried out have not confirmed these suppositions. Its phenotype is that which it mostly differs from *H. grosii*. The features which make this distinction are: the size of the foliar lamina, the lesser number of leaflets per leaf and of flowers per umbel as well as the opening of the standard and of the curvature of the seeds.

These peculiarities and its geographical isolation favour the proposals of those authors who have treated it as a subspecies.

Hippocrepis balearica **Jacq. in Miscell. Austr. Bot. 2: 305 (1781) subsp.** ***balearica***

Plants from Mallorca and Menorca are included within this subspecies. Minor variations exist between different populations. However, only those of Menorca show significant differences in one attribute: seed size. Thereby, the smallest seeds of the Menorca's plants suggests that the Montserrat's proposal (Laínz 1989), describing the *minoricensis* variety (*H. balearica* Jacq. subsp. *balearica* var. *minoricensis* P. Montserrat ex Laínz, in Fontqueria 24: 2 (1989), it should be considered as correct.

Table. 4. Simple correlation coefficient among the 3 factors, obtained using PCA and the significant measured variables. (*) P< 0.01. (**) P<0.05.

Character	factor 1	factor 2	factor 3
Seed curvature	0.938 *	-0.436	-0.130
Width of legume	0.918 *	-0.520	-0.113
Length/width leaflet	-0.809 *	0.442	0.129
Length of standart thorn	0.032	-0.895 **	-0.241
Width of standart	-0.083	-0.885 *	-0.470
Width of flower	0.302	-0.470	-0.681**

Table 5. Measurements of seed length and width from the *H. balearica* subsp. *balearica* var. *balearica* (Mallorca, HBB MA) and *H. balearica* subsp. *balearica* var. *minoricensis* (Menorca, HBB ME).

	HBB MA		HBB ME	
	Seed length mm	Seed width mm	Seed length mm	Seed width mm
Average	4,22	2,85	3,29	2,27
Stand. dev.	0,25	0,17	0,16	0,18
Err. Stand.	0,04	0,03	0,02	0,02
Maximum	4,61	3,23	3,6	2,78
Minimum	3,78	2,52	2,84	1,83

Taxonomic key

1. Flowers more than 5 times longer than wide. Dorsal angle of the standard greater than 115°. Folding standart strongly concave. Legume (on the level seeds) 5 or more mm wide. Less than 2 mm distance between the extremes of arc curvature of the seeds.
.... *H. grosii* (Pau) Boira, Gil & Llorens

1'. Flowers less than 3 times longer than broad. Dorsal angle of standard less than 100°. Folding standard of slightly concave. Legume (on the level seeds) less than 5 mm wide. More than 2 mm distance between the extremes of arc curvature of the seeds................2

2. Leaflets more than 4 mm wide. Claw of the standard shorter than calyx. ....
.... *H. balearica* Jacq. subsp. *valentina* (Boiss.) Uhrová

2'. Leaflets less than 4 mm wide. Claw of the standard about as long or longer than the calyx. 3

3. Seeds (3.78-4.61) x (2.52-3.23) mm. ....
.... *H. balearica* Jacq. subsp. *balearica* var. *balearica*

3'. Seeds (2.84-3.60) x (1.83-2.78) mm. ....
.... *H. balearica* Jacq. subsp. *balearica* var. *minoricensis* P. Montserrat ex Lainz

Phenetic and biogeographic relationships

The distance and geographical locations of the areas of distribution of the most widely ranging taxa do not correspond to the taximetric distances between them.

Thus it happens that in Ibiza, occupying an intermediate geographical position between the Alicante coast and Mallorca-Menorca, the most differentiated taxon is to be found (*H. grosii*). The phenetically closer populations, however, (*H. balearica* subsp. *balearica* and *H. balearica* subsp. *valentina*) are those which are geographically most separated (Fig. 1).

H. grosii exhibits the greatest set of primitive characteristics. In particular amongst them: greater woodiness and the greatest number of flowers per inflorescence. The remaining taxa of the group are more herbaceous and their organs tend to be less "stylized" since they are larger: broader leaflets, less numerous but larger flowers (estandarts more open and broader wings), less arched seeds etc.

On the other hand, this no-relationship is correlated with a different ecological behaviour. Therefore *H. balearica* subsp. *valentina* and *H. balearica* subsp. *balearica* grow preferentially in fissures and amongst rocks, their lifetime is indeterminate and they can lose part of their greenstuff; on the contrary, *H. grosii* grows forming large round clumps in the shade of crags or integrated within the scrub, behaving as a nanophanerophyte, unable to survive the loss of greenstuff caused by grazing without serious difficulties or death.

This suggests different herbivor pressure. In the east of the Iberian peninsula and in the eastern Balearic islands (Mallorca and Menorca), there are numerous fossil records indicating the ancient and important presence of herbivors such as *Myotragus* or *Capra* (Colom 1964, Cuerda 1989). These, as today, exercised a powerful pressure upon the vegetation (Duvigneaud 1967). In Ibiza, this activity seems to have been more limited. In the past, pressure seems to have been caused basically by small animals such as rabbits (Colom 1964, Cuerda 1989, Alcover 1979), and only later after the arrival of man the effect of introduced domestic animals increased (*Capra hircus*, *Ovis aries*).

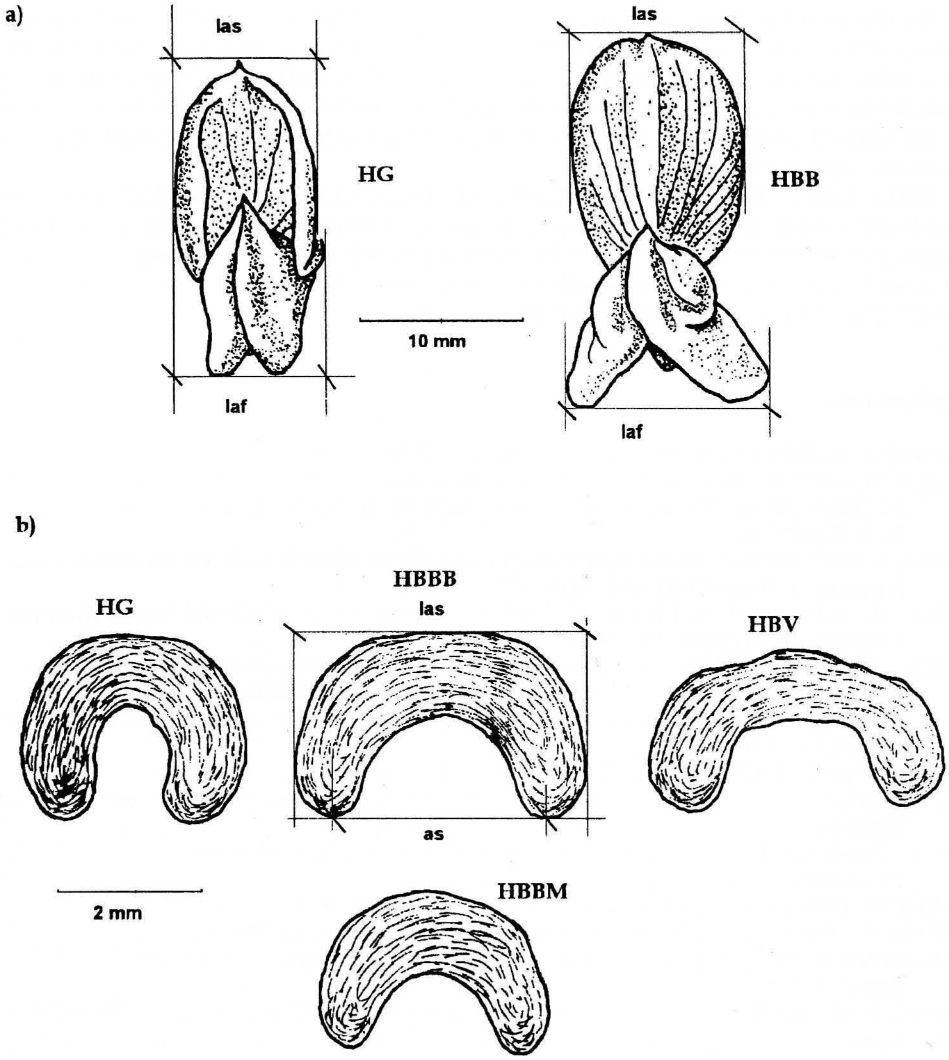

Fig. 5. Some biometric data for *H. balearica* flowers (a) and seeds (b). Flower: laf, width of flower; las, maximum width of standard. Seed: as, distance between the two extreme arcs of the seed; las, seed thickness. Drawn from: *H. grossi* (HG), *H. balearica* subsp. *balearica* (HBB), *H. balearica* subsp. *valentina* (HBV) and *H. balearica* subsp. *balearica* var. *minoricensis* (HBBM)

So it is to be expected that *H.grosii*, whose habitat has been modified but little and recently, maintains a series of attributes such as greater robustness which gives it a more primitive character.

On the other hand, the adaptation to a rupiculous refuge forces floral changes which favour pollination (Faegri & van der Pilj 1979). As a matter of fact, since the plants are of smaller size and are more isolated, their capacity of "advertisement" to possible insect pollinators is reduced. Moreover, since the number of pollinators around cliffs is less than in the scrub, the plants have developed strategies which make their flowers more evident, to ensure cross fertilization.

Hence there is an explanation for the two subspecies of *H. balearica* which answer by diminishing the number of flowers per inflorescence (although not the relative number of them) and increasing the size of corolla parts, especially the wings and keel which thus more effectively reveals the standard (lesser fold angle). In this way, it shows up clearly on the crag surfaces as striking yellow patches of colour.

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