

Variation patterns in the *Phlomis* × *composita* (Lamiaceae) hybrid complex in the Iberian Peninsula

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Hybridization seems to be common in the genus *Phlomis* (Lamiaceae) in the Iberian Peninsula, especially in the *P.* × *composita* complex. In order to detect patterns of morphological variation linked to eco- and geographical variation, ecological (cluster and canonical correspondence) and morphometric (principal component and discriminant function) analyses were performed. Character count procedure was applied to discern between divergence and reticulate events for the origin of the morphologically intermediate plants. Following these analyses clear patterns were detected suggesting the existence of four independent morphological groups also supported by the ecogeographical data. These are *P. crinita* ssp. *crinita* (Levante, eastern Spain), *P. crinita* ssp. *malacitana* (Andalusia, southern Spain) and *P. lychnitis* (widespread) being the extremes of the morphological variation, and *P.* × *composita* including all the morphologically intermediate individual plants. Furthermore, at the population level significant differences in hybrid plant frequencies between areas were also found. © 2004 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2004, 145, 97–108.

ADDITIONAL KEYWORDS: character count procedure – ecological analyses – hybrid zone – morphometric analyses – natural hybridization – Spain.

INTRODUCTION

Morphological intermediacy in nature can be a consequence of either divergence or reticulate events, and several methods have been developed and used to discern between them (e.g. Thorpe, 1984; Wilson, 1992; Rieseberg & Morefield, 1995). One of the most widely employed approaches using morphological data is the ‘character count procedure’ proposed by Wilson (1992) (e.g. Thébaud & Abbott, 1995; Miller & Spooner, 1996; Hawkins *et al.*, 1999; Levin, 1999). However, Rieseberg & Ellstrand (1993) and Rieseberg (1995) have demonstrated that hybridization does not always result in morphological intermediacy, especially when: (1) introgression is extensive (2) the hybridization process happened a long time ago, and (3) the expression of some morphological characters is not under an additive genetic control (e.g. heterosis or dominance) (Levin, 1999). In all these cases, the application of Wilson’s method would produce a type I error (i.e. reject-

ing the hybridization hypothesis even though it was correct). Therefore, and according to Levin (1999: 270): ‘this is a conservative test: when a hypothesis of hybridization is supported, it is very likely to be correct.’

In addition, multivariate ordination techniques such as principal components analysis (PCA), principal coordinates analysis (PCoA) or discriminant function analysis (DFA) have been widely used in hybridization studies to detect putative parental and hybrid taxa (e.g. Thébaud & Abbott, 1995; Gugerli, 1997; Levin, 1999) and backcrossed individuals in hybrid swarms (Wagner, 1983; Wilson, 1992), despite the fact that they cannot distinguish between hybridization and divergence (Wilson, 1992).

The genus *Phlomis* L. (Lamiaceae) comprises more than 100 species, primarily distributed in Eurasia and north-western Africa with two main centres of diversity in southern and eastern Anatolia and north-western Iran (Azizian & Moore, 1982). Although only a few taxa of the genus occur in the Iberian Peninsula, they are surrounded by considerable taxonomic, nomen-

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clatural and ecogeographical confusion, supposedly due to natural hybridization and the existence of frequent intermediate plants. *Phlomis lychnitis* L. is widespread across the Peninsula, whereas *P. crinita* Cav. is restricted to some limestone areas in southern and eastern Spain (Andalusia and Levante, respectively). Here the two taxa are sympatric and intermediate plants have been recognized morphologically since the early 20th century (Pau, 1918a).

Pau (1925) recognized both *P. lychnitis* and *P. crinita*, but within the latter he distinguished and described a distinct variety in the area of eastern Andalusia, *P. crinita* var. *malacitana* Pau, as well as three hybrid taxa: *P. × composita* Pau, *P. × trullenquei* Pau and *P. × almijarensis* Pau (Pau, 1918a, b, Pau, 1922) involving the three former taxa as putative parental taxa throughout the distributional range. Later, in the Iberian revision of the genus by Mateu (1986), *P. lychnitis*, *P. crinita* and four other hybrids were recognized, including *P. × composita* nothom. *malacitana* (Pau) Mateu, *P. × composita* nothom. *composita* (Pau) Mateu, *P. × composita* nothom. *almijarensis* (Pau) Mateu and *P. × composita* nothom. *trullenquei* (Pau) Mateu, without precise information about their geographical distribution. Rivas-Martínez *et al.* (1991) presented a quite different scenario whereby *P. crinita* ssp. *crinita* (Levante and eastern Andalusia) and *P. crinita* ssp. *composita* (Pau) Rivas-Martínez, Asensi, Molero & Valle (western Andalusia) hybridized to produce *P. crinita* nothosp. *malacitana* (Pau) Rivas-Martínez, Asensi, Molero & Valle. They also suggested that *Phlomis lychnitis* hybridized with these two taxa to produce *P. × trullenquei* nothosp. *trullenquei* (eastern Spain) and *P. × trullenquei* nothosp. *almijarensis* Rivas-Martínez, Asensi, Molero & Valle (western and central Andalusia). On the other hand, in Málaga province (central Andalusia) Cabezudo, Nieto & Navarro (1991) considered all *P. crinita*-like plants to be *P. crinita* ssp. *malacitana* (Pau) Cabezudo, Nieto Caldera & Navarro. Those previously named as *P. × almijarensis* were reclassified as *P. lychnitis*, and under *P. composita* (pro. hybr.) they included all hybrids between *P. crinita* and *P. lychnitis*.

The present paper shows the results of a comprehensive morphometric and ecogeographical study of the *P. × composita* complex, formed by *P. lychnitis*, *P. crinita* and their presumed hybrids, in the Iberian Peninsula in order to detect patterns of morphological variation within a geographical and ecological context. First, we characterized various habitats occupied by the studied taxa based on their floristic composition and environmental factors. Second, we employed multivariate ordination analyses (PCA and DFA) for assessing potential putative parental and hybrid groups, checking the existence of ecological and/or geo-

graphical patterns, if any, linked to morphological variation. Third, we applied character count procedure to determine whether intermediacy was due to divergence or hybridization.

MATERIAL AND METHODS

STUDIED PLANTS

All plants of the complex in the studied area are hemi-cryptophytes up to 70 cm high, representing a component of the Mediterranean vegetation in rocky limestone disturbed soils at altitudes of between 200 and 2000 m. They are tomentose with most leaves congested at the stem base, and flowers usually grouped in 6-flowered verticillasters surrounded by two bracts. The shape of the lower leaves was the most widely employed character to distinguish between *P. lychnitis* and *P. crinita* (e.g. DeFilipps, 1972; Mateu, 1986); in the former it is linear or narrowly elliptical, gradually tapering into an indistinct petiole; in the latter it is ovate to lanceolate, cordate or truncate at base, abruptly contracted into a distinct petiole. However, all transitional leaf-shapes could also be found at individual plant level in natural populations. From a karyological point of view, the studied system is a homoploid complex since all plants are diploid ($2n = 20$) (Aparicio, 1997; Aparicio & Albaladejo, 2003).

PLANT COMMUNITIES AND ECOLOGY OF POPULATIONS

A subset of 17 populations out of 28 employed for the morphometric analysis was sampled and characterized (Fig. 1, Table 1). The composition of these communities was studied by recording all woody plant taxa in 0.1 ha plots within each population. Soil data were obtained from three pooled samples (0–15 cm cores) collected in each plot [pH, percentage of sand (S), lime (L), clay (C), CaCO₃ (CARB), and levels of N, P, K, Ca and Mg]. Annual rainfall (AR) and mean annual temperature (T) were obtained from the nearest meteorological stations. The floristic matrix was subjected to a cluster analysis (CA) by means of Sorensen distance coefficient, using UPGMA. A canonical correspondence analysis (CCA) (Ter Braak, 1986) was then conducted on both the floristic and environmental data matrix to explore direct relationships between results of the cluster analysis and environmental variables. CA and CCA analyses were computed by PC-ORD 3.05 (McCune & Mefford, 1997).

MORPHOMETRIC ANALYSES

In total, 154 individual plants collected from 28 populations (Fig. 1, Table 1) were identified according to the model in Mateu (1986), which mainly relies on leaf

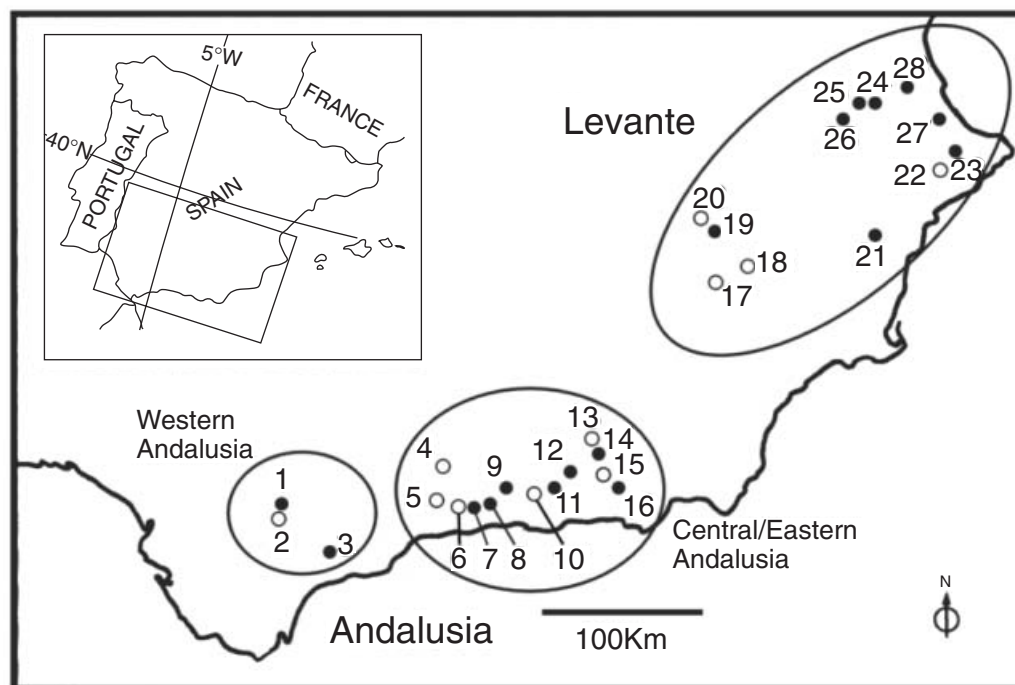


Figure 1. Location of the studied *Phlomis* populations. Populations analysed for (○) morphometric parameters, (●) morphometric, floristic and ecological parameters. Geographic areas supported by CA (western Andalusia, central/eastern Andalusia and Levante) are encircled.

(length, width, base shape), bract (width, colour), calyx (vein number) and trichome morphologies (see Fig. 2) to distinguish between taxa.

In our study, 13 quantitative and 13 qualitative (binary) variables were measured for every specimen (see Table 2). Values for quantitative variables were the averages of three measurements. The qualitative variables were either scored for vegetative and floral characters (INF, KM, UPL) or the presence/absence of ten different types of trichomes (Fig. 2) in seven different plant organs (lower leaf, bract, bracteole, stem base, stem apex, calyx and corolla). The trichome types were: simple (T) (uniseriate, 3-cellular), dendritic (D) (biseriate base and lateral ramifications), stellate (E) or glandular (G). Dendritic hairs either presented equivalent ramifications (D1) or a longer 3-cellular stalk (D2). Stellate hairs were sessile (E1), pedunculate with equivalent ramifications (E2), sessile with a longer 3-cellular stalk (E3) or pedunculate with a pluricellular base (E4). Glandular hairs were simple (G1) or stellate with either a short (G3) or long (G4) glandular stalk.

In order to explore morphological variation, a PCA with a varimax rotation on the correlation matrix was performed. This was carried out with the whole set of variables (quantitative and qualitative), excluding LL, BL, KL and KTL to avoid redundancy, and trichome types E1, E2, D1, G1 and T, which were present in

each specimen. Continuous variables were logarithmic and vector transformed to allow the combination of quantitative and qualitative variables in the multivariate analysis, in view of the fact that the outcome employing this procedure generates scores between 0 and 1 (Brochmann, 1987; Gugerli, 1997). The variables LB, LW and LL/LW were also included in the analysis though normality was not achieved, as multivariate analyses are quite robust for departures from normality (Marcus, 1990).

In order to assess whether an a priori ecogeographical group could be discriminated efficiently and to obtain a classification matrix of the individuals within the groups, a discriminant function analysis (DFA) was performed with the same data matrix. The tested groups were: (I) *P. crinita* and *P. × composita* nothom. *malacitana* from Andalusia, (II) *P. crinita* from Levante, (III) morphologically intermediate plants (*P. × composita* nothom. *trullenquei*, nothom. *almijarensis* and nothom. *composita*) from Andalusia, (IV) intermediate plants from Levante, and (V) *P. lychnitis*.

Character count procedure (Wilson, 1992) was performed to test whether the observed morphological intermediacy in the studied system was due to reticulation or divergence, comparing the assigned putative parental groups I/II and V. This method is based on selection of characters showing significant differences between the supposed parental groups, followed

Table 1. List of *Phlomis* populations and number of collected individuals used for morphometric analysis. Identification of individuals according to Mateu (1986). Asterisks indicate populations also subjected to floristic and ecological analyses. A, *P. crinita*; B, *P. × composita* nothom. *malacitana*; C, *P. × composita* nothom. *composita*; D, *P. × composita* nothom. *almijarensis*; E, *P. × composita* nothom. *trullenquei*; F, *P. lychnitis*

Populations	A	B	C	D	E	F
1* CÁDIZ: Algodonales, Lijar mountain range, 30S TF8676, 1000 m a.s.l.	–	7	2	2	11	5
2 CÁDIZ: Grazalema, Puerto de las Palomas, 30S TF8874, 1260 m a.s.l.	–	1	–	–	–	–
3* MÁLAGA: Torrox, Las Nieves mountain range, 30S UF1761, 1000 m a.s.l.	–	3	1	2	1	1
4 GRANADA: Loja, Loja mountain range, 30S VG0102, 1200 m a.s.l.	–	–	–	4	–	1
5 GRANADA: Alhama de Granada, Almjara mountain range, La Chapa peak, 30S VF1683, 1460 m a.s.l.	–	3	–	–	–	–
6 GRANADA: Alhama de Granada, Almjara mountain range, La Mota peak, 30S VF1981, 1300 m a.s.l.	–	–	–	–	–	1
7* GRANADA: Arenas del Rey, 30S VF2084, 1100 m a.s.l.	2	–	–	2	–	–
8* GRANADA: Between Jayena and Albuñuelas, Las Pilas, 30S VF3488, 1300 m a.s.l.	1	–	–	1	2	2
9* GRANADA: Between Jayena and Albuñuelas, Cañuelo peak, 30S VF3784, 1400 m a.s.l.	1	1	–	1	–	–
10 GRANADA: Lujar mountain range, 30S VF6577, 1600 m a.s.l.	–	5	–	1	–	–
11* GRANADA: Busquístar, Sierra Nevada, Cerros Negros, 30S VF7688, 1400 m a.s.l.	4	2	3	4	1	3
12* GRANADA: Busquístar, Sierra Nevada, Fuentezuelas, 30S VF7792, 1400 m a.s.l.	3	–	–	–	–	–
13 GRANADA: ascending towards Puerto de La Ragua, 30S VG9611, 1600 m a.s.l.	–	–	–	–	1	1
14* GRANADA: Sierra Nevada, Puerto de La Ragua, 30S VG9611, 1800 m a.s.l.	3	–	–	–	–	–
15 GRANADA: Cherín, 30S VF9991, 600 m a.s.l.	–	–	–	–	–	1
16* ALMERÍA: Berja, Gádor mountain range, 30S WF0985, 1600 m a.s.l.	8	4	2	5	1	2
17 MURCIA: Umbria del Obispo, Sierra Seca, 30S WH6515, 1625 m a.s.l.	–	–	–	–	–	1
18 MURCIA: Moratalla, Muela mountain range, 30S WH9234, 1000 m a.s.l.	–	–	–	–	–	1
19* ALBACETE: Between Elche de la Sierra and Gallego, 30S WH8653, 500 m a.s.l.	2	–	1	–	–	–
20 ALBACETE: El Pardo, 30S WH6360, 960 m a.s.l.	–	–	–	–	–	1
21* ALICANTE: Crevillente, Crevillente mountain range, 30S XH8739, 800 m a.s.l.	–	–	–	1	–	1
22 ALICANTE: Between Benimasot and Ballones, 30S YH3391, 650 m a.s.l.	–	–	–	–	–	2
23* ALICANTE: Between Tollos and Beniaya, 30S YH3796, 800 m a.s.l.	2	–	–	–	6	1
24* VALENCIA: Between Quesa and Navarrés, 30S XJ9732, 260 m a.s.l.	4	–	–	–	–	–
25* VALENCIA: Bicorp, Caroché, Portillo house, 30S XJ8330, 800 m a.s.l.	3	–	–	–	–	2
26* VALENCIA: Bicorp, El Barriquet, 30S XJ8530, 600 m a.s.l.	2	–	–	–	–	–
27* VALENCIA: Between Barix and Pinet, 30S YJ3221, 400 m a.s.l.	2	–	1	–	–	–
28* VALENCIA: Tous, 30S YJ0735, 200 m a.s.l.	7	–	–	1	–	1
Total	44	26	10	24	23	27

by a nonparametric one-side sign test (Zar, 1996) of intermediate vs. nonintermediate traits.

Finally, to assess the relative frequency of hybrids in mixed populations (i.e. where one or both parental and hybrid plants grow together) in the studied area, the number of individual plants morphologically regarded as extremes (parentals) and intermediates (presumed

hybrids) was recorded within every correspondent 0.1 ha plot for floristic and ecological analyses. Populations 14 and 24 were not included because only one extreme phenotype *P. crinita*-like was found.

All the univariate and multivariate analyses in morphometric section, except the Dunn test, were computed using STATISTICA 5.1 (StatSoft, 1997).

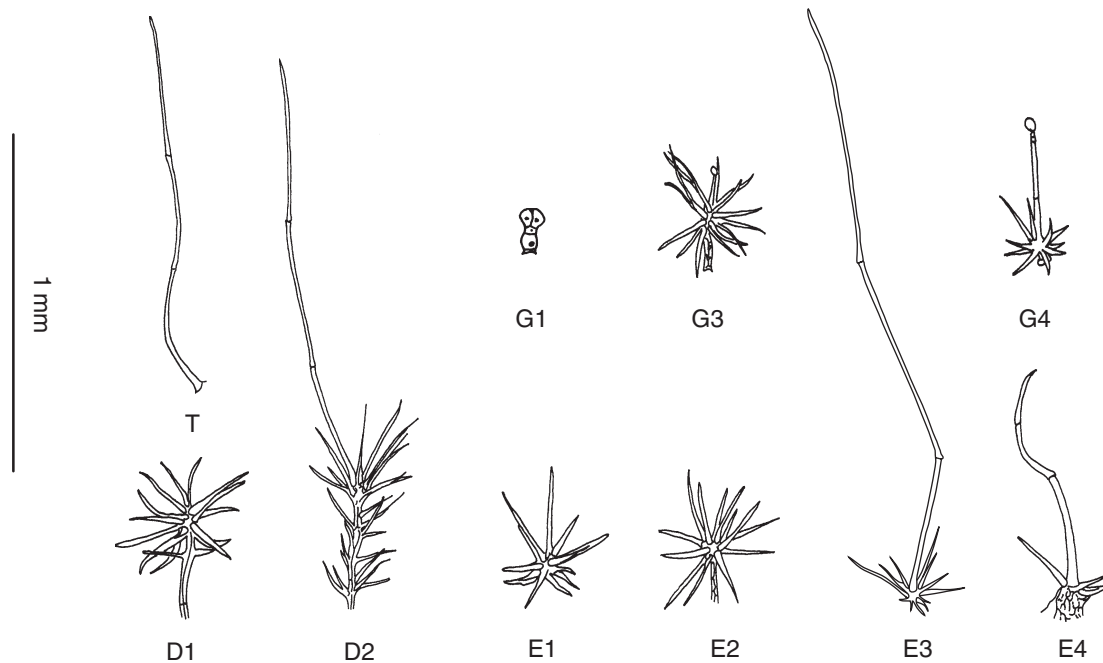


Figure 2. Different types of trichomes present in the studied *Phlomis* taxa. Modified from Mateu (1986).

Table 2. Set of quantitative and qualitative (binary) variables assessed for morphometric analyses

Variable	Description
1. LB	Petiole-leaf blade angle
2. LL	Leaf length (mm)
3. LW	Leaf width (mm)
4. LL/LW	LL/LW ratio
5. BL	Bract length (mm)
6. BW	Bract width (mm)
7. BL/BW	BL/BW ratio
8. KL	Calyx length (mm)
9. KW	Calyx width (mm)
10. KL/KW	KL/KW ratio
11. KTL	Calyx teeth length (mm)
12. KL/KTL	KL/KTL ratio
13. BrL	Bracteole length (mm)
14. INF	Inflorescence; unbranched (0), branched (1)
15. KM	Calyx mouth; open (0), closed (1)
16. UPL	Upper lip; yellow (0), brownish (1)
17. T	Simple trichome; absent (0), present (1)
18. D1	Dendritic trichome D1; absent (0), present (1)
19. D2	Dendritic trichome D2; absent (0), present (1)
20. E1	Stellate trichome E1; absent (0), present (1)
21. E2	Stellate trichome E2; absent (0), present (1)
22. E3	Stellate trichome E3; absent (0), present (1)
23. E4	Stellate trichome E4; absent (0), present (1)
24. G1	Glandular trichome G1; absent (0), present (1)
25. G3	Glandular trichome G3; absent (0), present (1)
26. G4	Glandular trichome G4; absent (0), present (1)

RESULTS

PLANT COMMUNITIES AND ECOLOGY OF POPULATIONS

CA based on floristic composition of communities showed a clear geographical pattern with three main areas: western Andalusia, central/eastern Andalusia, and eastern Spain (here called Levante *sensu lato*, for simplicity) (Figs 1, 3).

The results of the CCA are shown in Table 3 and Figure 4. The three geographical areas showed marked differences in a number of environmental variables (see Table 4). Populations in Levante were

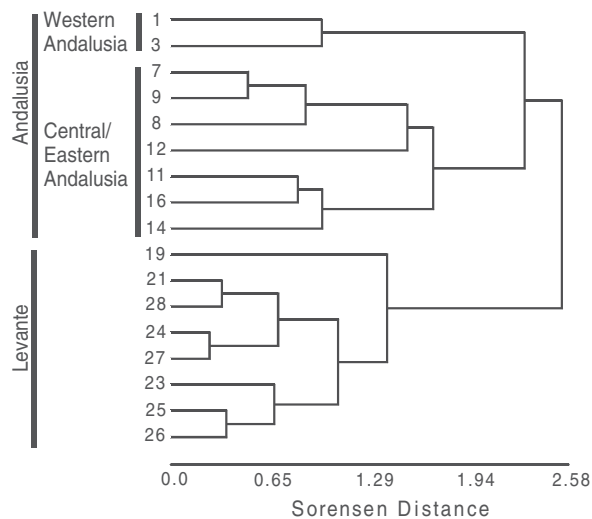


Figure 3. Dendrogram of the UPGMA based on Sorensen distance coefficient for the 17 *Phlomis* populations included in the floristic matrix.

Table 3. Summary of results of the CCA. Only environmental variables significantly correlated with the first two canonical axes are shown ($P < 0.05$). Abbreviations: ALT, altitude; AR, annual rainfall; T, mean annual temperature; Ca, K and Mg: levels of calcium, potassium and magnesium in soil, respectively

	Axis 1	Axis 2
Eigenvalues	0.752	0.565
Pearson's correlation species-environment	0.996	0.996
Cumulative species variance (%)	17.4	30.5
Pearson's correlation with environmental variables:		
ALT	-0.914	-
AR	-	0.582
T	0.624	-
K	0.541	-
Ca	0.568	-
Mg	-	0.501

located at the lowest altitude (<800 m) with the lowest values of annual rainfall, the highest mean annual temperatures, and the highest levels of Ca and K in the soil. Characteristic plant species in this area were *Juniperus oxycedrus* L., *Thymus vulgaris* L., *Globularia alypum* L., *Satureja obovata* Lag. and *Genista valentina* (Willd. ex Spreng.) Steud. The two western Andalusian populations analysed were at 1000 m and harboured the highest annual rainfall and Mg soil content. Representative species were *Linum suffruticosum* L., *Thymus baeticus* Boiss. ex Lacaita, *Halmium atriplicifolium* (Lam.) Spach and *Cytisus fontanesii* Spach. Finally, central/eastern Andalusian populations were at higher altitudes (1100–1800 m) and had the lowest mean annual temperature and K, Ca and Mg values. Characteristic species in this area were *Artemisia campestris* L., *Rosa sicula* Tratt., *Teucrium hyeronimi* Sennen, *Prunus ramburii* Boiss., *Thymus membranaceus* Boiss. and *Astragalus granatensis* Lam.

MORPHOMETRIC ANALYSES

Factor loadings and eigenvalues for the first two components (PCs1 and 2) extracted in the PCA are shown

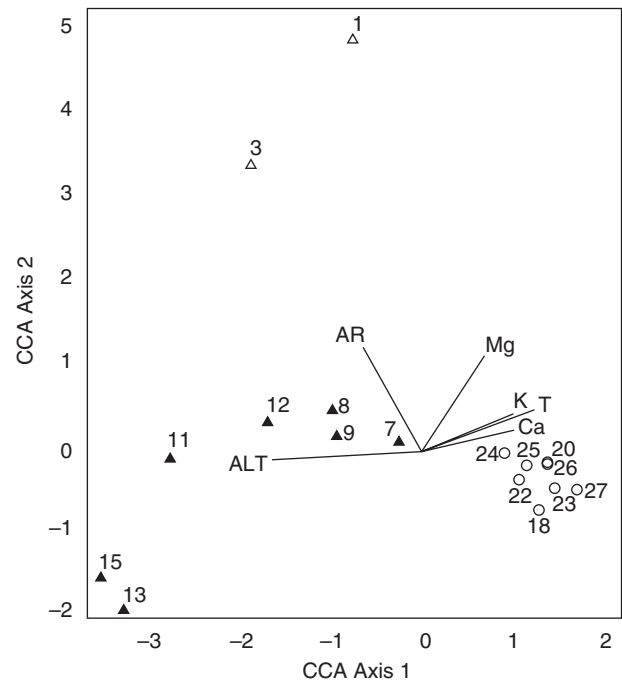


Figure 4. CCA join-plot of studied *Phlomis* populations and environmental variables significantly correlated with the first two canonical axes (environmental scores $\times 4$). (Δ) western Andalusia, (\blacktriangle) central/eastern Andalusia, and (\circ) Levante. ALT, altitude, AR, annual rainfall, T, mean annual temperature, Ca, K and Mg: levels of calcium, potassium and magnesium in soil, respectively.

Table 4. Environmental values for the three geographical areas resulting from CA and CCA. Only environmental variable values significantly correlated with the first two canonical axes are shown ($P < 0.05$). *N*, number of populations analysed in each area; ALT, altitude (m); AR, annual rainfall (mm); T, mean annual temperature ($^{\circ}\text{C}$); Ca, K and Mg: levels of calcium, potassium and magnesium in soil (p.p.m), respectively

Geographical Area	<i>N</i>	ALT	AR	T	Ca	K	Mg
Western Andalusia	2						
mean \pm SD		1000 \pm 0	964 \pm 279.9	15.4 \pm 0.6	7862.5 \pm 364.2	243.5 \pm 143.5	407.5 \pm 236.9
min-max		1000–1000	766–1161.9	15–15.8	7605–8120	142–345	240–575
Central/eastern Andalusia	7						
mean \pm SD		1428.6 \pm 221.5	659.3 \pm 138.5	14.3 \pm 2.4	5833.4 \pm 3312	112.7 \pm 72.8	249.1 \pm 152.5
min-max		1100–1800	410–796.2	10.3–16.4	1246–9310	7–254	100–506
Levante	8						
mean \pm SD		520 \pm 243.3	493.8 \pm 149.5	15.9 \pm 2	9386.9 \pm 1993.3	251.4 \pm 128.5	288.8 \pm 112.2
min-max		200–800	309.1–724	13.4–18.4	7195–13825	104–471	125–475

in Table 5. These accounted for 47.6% of the total variance. Leaf characters (LB, LW, LL/LW) and the calyx mouth (KM) character showed the highest (either positive or negative) correlations with PC1, and those related to bracts (BL/BW, BW, BrL) and the presence/absence of trichomes E3 and G4 showed the highest correlations with PC2. The scatterplot for these two components showed that plants identified as *P. crinita*, *P. lychnitis* and *P. \times composita* nothom. *malacitana* constituted the extremes of the morphological variation, whereas those identified as *P. \times composita* nothom. *trullenquei*, nothom. *almijarensis* and nothom. *composita* were intermingled and occupied an intermediate position between the extremes, especially along PC1 (Fig. 5). Individuals of *P. crinita* and *P. \times composita* nothom. *malacitana* were placed in two distinct groups, corresponding to their geographical origin: group I was formed by both *P. crinita* and *P. \times composita* nothom. *malacitana* from Andalusia and group II by *P. crinita* from Levante. On the other hand, *P. lychnitis* (group V) did not show any ecogeographical pattern (data not shown). The morphologically intermediate taxa (*P. \times composita* nothom. *trullenquei*, nothom. *almijarensis* and nothom. *composita*) overlapped each other closely but were grouped separately according to their geographical provenance either from Andalusia or Levante for further analyses (groups III and IV, respectively).

Factor structure for the 17 variables and eigenvalues for the first two factors (DF1 and 2) extracted in the DFA are shown in Table 5. DF1 was positively correlated with LW, and negatively with LL/LW and LB, whereas DF2 was positively correlated with the presence of trichomes G4 and E3. DFA classification was 100% for individuals from groups I, II and V, whereas for groups III and IV it was 91.5% and 50%, respectively (Table 6). The scatter diagram reveals the same basic pattern previously found in PCA. Along DF1,

Table 5. Factor loadings in PCA and factor structure coefficients in DFA for the 17 morphological variables. CV, cumulative variance

Variable	PC1	PC2	DF1	DF2
LB	-0.866	0.003	-0.583	-0.25
LW	0.926	-0.142	0.704	0.102
LL/LW	-0.928	0.143	-0.753	-0.163
BW	0.154	0.845	-0.027	0.339
BL/BW	-0.236	-0.768	-0.003	-0.261
KW	-0.001	0.391	-0.017	0.151
KL/KW	-0.101	0.008	-0.031	-0.028
KL/KTL	-0.447	0.463	-0.223	0.149
BrL	-0.209	0.582	-0.087	0.183
INF	0.534	-0.093	0.154	-0.003
UPL	0.639	0.192	0.186	0.151
KM	0.722	-0.074	0.257	0.019
E3	-0.215	0.733	-0.179	0.568
E4	0.061	0.027	0.035	0.08
G3	0.239	0.34	0.04	0.16
G4	-0.352	0.724	-0.276	0.595
D2	0.417	-0.182	0.152	0.021
Eigenvalues	4.78	3.31	11.45	5.13
CV (%)	28.14	47.62	62.89	91.07

groups I and II were extremes with regard to group V, whereas DF2 separated groups I and II (Fig. 6). Groups III and IV were intermediate along DF1 between groups I/II and V.

ANOVA results for the quantitative variables among groups are shown in Table 7. Putatively assigned parental groups I and V were statistically different for five out of 13 variables and the array of morphologically intermediate plants between them (group III) was intermediate for all five characters. Likewise, the putatively assigned parental groups II and V were statistically different for ten out of 13 characters and

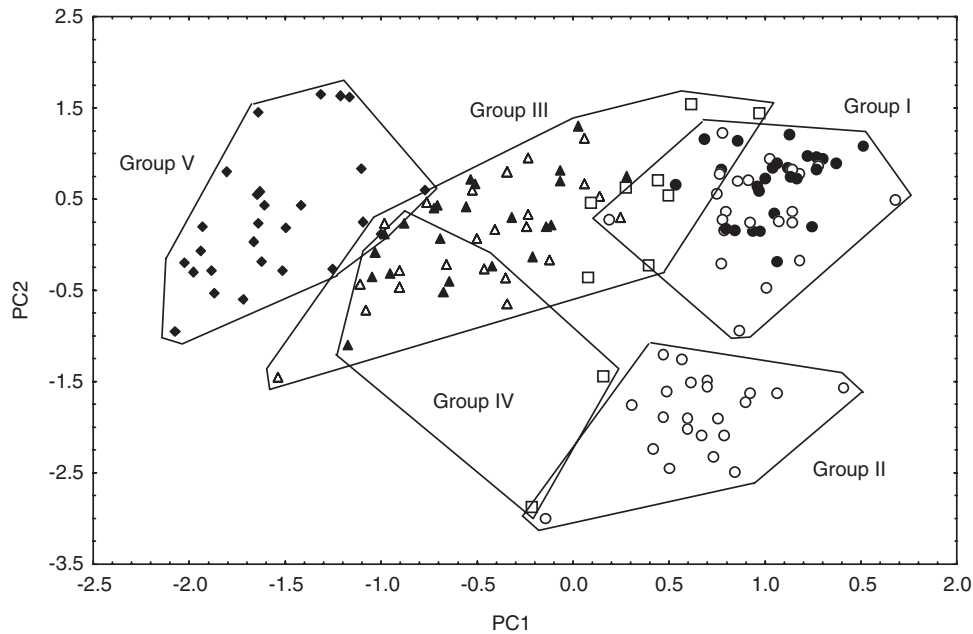


Figure 5. PCA scatterplot for the first two extracted components. Polygons represent the ecogeographical groups I–V. Symbols follow nomenclature of Mateu (1986); (○) *P. crinita*; (●) *P. × composita* nothom. *malacitana*; (□) *P. × composita* nothom. *composita*; (△) *P. × composita* nothom. *almijarensis*; (▲) *P. × composita* nothom. *trullenquei*; (◆) *P. lychnitis*.

Table 6. Classification matrix of *Phlomis* individuals provided by DFA

Group	A priori classification	Group					Percentage correct
		I	II	III	IV	V	
I	48	48	0	0	0	0	100
II	22	0	22	0	0	0	100
III	47	2	0	43	1	1	91.49
IV	10	0	1	4	5	0	50
V	27	0	0	0	0	27	100

group IV was intermediate for all ten characters. In both cases, one-side sign tests of intermediate vs. nonintermediate characters provided $P < 0.05$.

Figure 7 shows the frequency of hybrid plants in 15 mixed populations. Populations from Levante showed a clear lower percentage of hybrid plants ($2.1\% \pm 2.5$, mean \pm SD) than those from Andalusia ($21.6\% \pm 11.0$). On the other hand, western Andalusian populations showed higher percentages of hybrid plants ($34.2\% \pm 1.7$) than those from central/eastern Andalusia ($17.5\% \pm 9.3$).

DISCUSSION

The present study demonstrates the existence of morpho-ecogeographical patterns of variation in the *Phlo-*

mis complex in the Iberian Peninsula. The three regions distinguished by floristic and ecological analyses belong to different Iberian chorological provinces, which are based on historical, anthropogenic, geological and floristic differences (Rivas-Martínez, 1987). We found most of the Levante populations (Catalano-Valenciano-Provenzal and Murciano-Almeriense chorological provinces) growing in degraded stages of thermo-mediterranean holm-oak (*Quercus rotundifolia*) forests. Andalusian populations (Betic chorological province) were mostly found in mid- and high-mountain mediterranean vegetation, primarily in degraded meso- or supra-mediterranean holm-oak forests.

Multivariate analyses (PCA and DFA) revealed the existence of four morphological groups, of which I, II and V constituted the extremes and III and IV the intermediate position between them. Groups I and II fit the ecogeographical pattern obtained by CA and CCA. Following Mateu's taxonomic concept, group I includes individual plants identified as *P. crinita* and *P. × composita* nothom. *malacitana* from Andalusia, while group II includes plants identified as *P. crinita* from Levante. Morphological characters distinguishing these two groups were the presence of trichomes E3 and G4 in bracts, bracteoles, stem apex and calyx in group I, as well as the presence of wider middle bracts. Univariate analyses also provided significant differences for seven out of 13 quantitative traits between groups I and II. In contrast, morphological

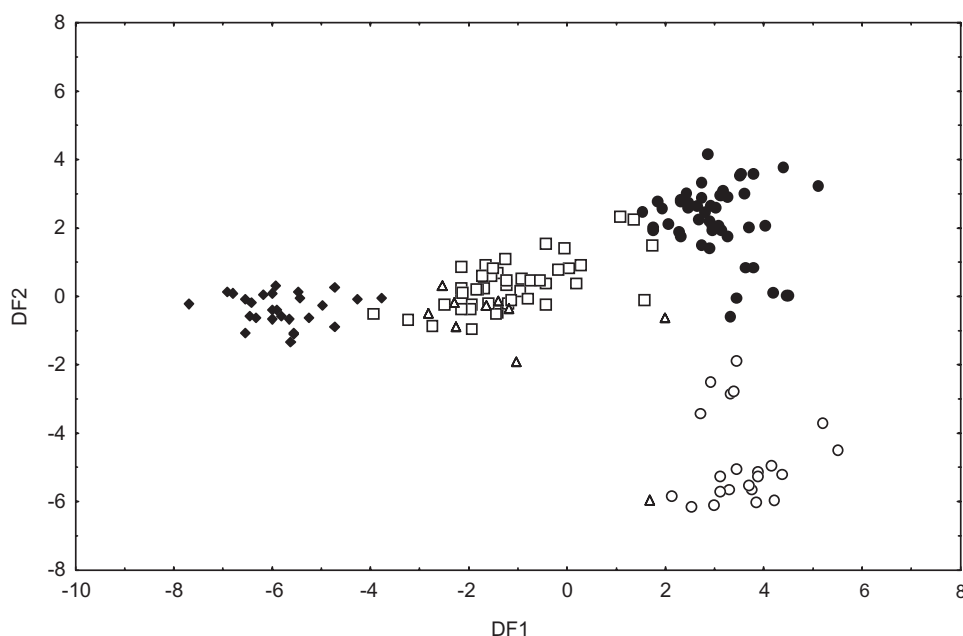


Figure 6. Discriminant function analysis (DFA) scatterplot for the first two extracted factors (●) group I; (○) group II; (□) group III; (△) group IV; (◆) group V.

Table 7. Mean \pm standard deviations and statistical analysis of the 13 quantitative variables for *Phlomis* groups. Sample size in parentheses

Variable	Group I (48)	Group II (22)	Group III (47)	Group IV (10)	Group V (27)
LB \ddagger ***	53.13 ^c \pm 16.7	76.18 ^c \pm 21.35	145.58 ^b \pm 19.39	148 ^{ab} \pm 16.55	170.22 ^a \pm 3.3
LL \ddagger NS	79.24 \pm 16.5	85.89 \pm 14.2	85.5 \pm 19.8	90.23 \pm 23.31	79.46 \pm 22.79
LW \ddagger ***	40.12 ^a \pm 8	37.18 ^a \pm 6.03	21.52 ^b \pm 6.57	21.19 ^{bc} \pm 6.86	8.12 ^c \pm 1.74
LL/LW \ddagger ***	1.98 ^c \pm 0.2	2.35 ^c \pm 0.45	4.22 ^b \pm 1.27	4.48 ^{ab} \pm 1.27	9.81 ^a \pm 2.19
BL \ddagger **	25.16 ^a \pm 4.4	21.86 ^b \pm 2.97	24 ^{ab} \pm 3.38	24.6 ^{ab} \pm 2.47	25.21 ^a \pm 3.9
BW \ddagger ***	20.58 ^a \pm 3.27	13.55 ^c \pm 1.86	18.65 ^b \pm 3.31	15.9 ^{bc} \pm 3.82	18.70 ^{ab} \pm 3.71
BL/BW \ddagger ***	1.23 ^c \pm 0.16	1.63 ^a \pm 0.26	1.31 ^c \pm 0.25	1.63 ^{ab} \pm 0.43	1.38 ^{bc} \pm 0.24
KL \ddagger ***	11.62 ^a \pm 0.95	10.30 ^b \pm 0.68	11.79 ^a \pm 1.18	11.27 ^{ab} \pm 0.64	11.67 ^a \pm 0.95
KW \ddagger ***	5.57 ^a \pm 0.84	4.86 ^b \pm 0.55	5.69 ^a \pm 0.74	5.07 ^{ab} \pm 0.55	5.34 ^{ab} \pm 0.76
KL/KW \ddagger NS	2.12 \pm 0.26	2.14 \pm 0.27	2.09 \pm 0.23	2.24 \pm 0.19	2.22 \pm 0.32
KTL \ddagger ***	3.18 ^{ab} \pm 0.59	3.57 ^a \pm 0.64	2.95 ^b \pm 0.67	3.32 ^{ab} \pm 0.63	2.24 ^c \pm 0.52
KL/KTL \ddagger ***	3.77 ^b \pm 0.75	2.95 ^c \pm 0.44	4.22 ^b \pm 1.14	3.51 ^{bc} \pm 0.69	5.41 ^a \pm 1.07
BrL \ddagger ***	13.06 ^a \pm 1.58	11.21 ^b \pm 1.07	13.59 ^a \pm 1.58	12.99 ^{ab} \pm 1.33	13.32 ^a \pm 1.57

For each variable, parametric ANOVA (\ddagger) or Kruskal–Wallis ANOVA (\ddagger) was performed among groups followed by Tukey and Dunn multiple comparison test, respectively. Variables BL, BL/BW and KL/KTL were log-transformed before parametric ANOVA.

Different superscripts indicate significant differences within rows.

NS not significant; ** $P < 0.01$; *** $P < 0.001$.

differences between plants from western and central/eastern Andalusia within group I were not supported by PCA (data not shown). These results support to some extent the nomenclatural treatment in Cabezudo *et al.* (1991) where *P. crinita*-like plants in central Andalusia were treated as *P. crinita* ssp.

malacitana. Following this taxonomic approach group I should be identified as ssp. *malacitana* (Andalusia), and group II as ssp. *crinita* (Levante).

Group V included all plants identified as *P. lychnitis*, regardless of the geographical origin of the samples. In addition to the usually considered diagnostic charac-

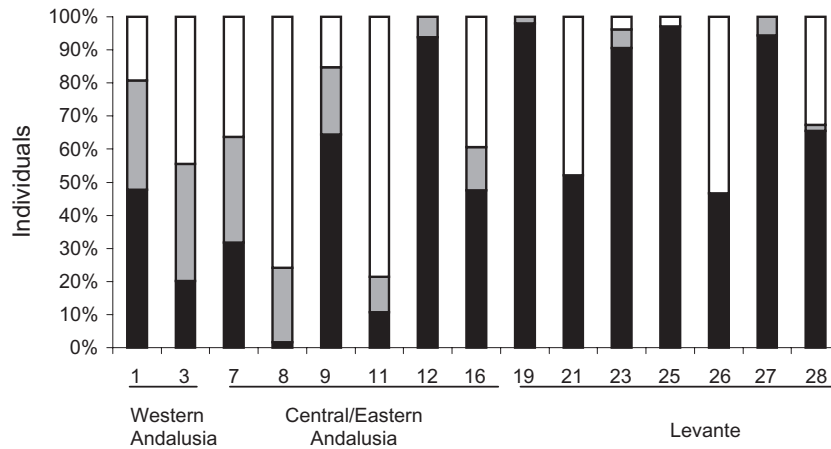


Figure 7. Population structure for 15 mixed *Phlomis* populations. Black and white bars = frequency of morphologically extreme (parental) plants, grey bars = frequency of morphologically intermediate (hybrid) plants.

ters (narrow basal leaves with decurrent blade) (e.g. DeFilippis, 1972; Mateu, 1986; Uibera, 1987; De Bolòs & Vigo, 1995), the open calyx mouth, the unbranched inflorescence and the golden-coloured flowers could be used to distinguish *P. lychnitis* from *P. crinita*, usually with constricted calyx mouth, branched inflorescence and brownish or brick-orange corolla upper lip.

Groups I (*P. crinita* ssp. *malacitana*), II (*P. crinita* ssp. *crinita*) and V (*P. lychnitis*) should be considered the parental taxa of the hybrid complex. In contrast, groups III and IV included plants identified as *P. × composita* nothom. *almijarensis*, nothom. *trullenquei* and nothom. *composita* in Mateu (1986), but PCA did not support this nomenclature. All morphologically intermediate plants between *P. lychnitis* and ssp. *malacitana* from Andalusia included in group III and between *P. lychnitis* and ssp. *crinita* from Levante included in group IV were highly intermingled. DFA wrongly classified four out of ten plants between groups III and IV, which were only significantly different in one out of 13 studied quantitative traits. These results led us to consider all intermediate phenotypes between *P. lychnitis* and *P. crinita* throughout their distribution range in the single nothospecies *P. × composita*.

Some authors have recently pointed out potential problems in hybridization studies with the use of morphological data (Rieseberg, 1995; Brochmann, Borgen & Stabbe, 2000). Nevertheless, morphological intermediacy is generally considered the first evidence of hybridization (Gottlieb, 1972; McDade, 1990, 1995; Dibble *et al.*, 1998; Hawkins *et al.*, 1999). Although the character count procedure is very conservative in its assumptions (Levin, 1999), in the present case it has given support for the hybridization hypothesis between ssp. *malacitana* and *P. lychnitis* in Andalusia,

and between ssp. *crinita* and *P. lychnitis* in Levante. In other studies the results provided by this method have been confirmed with other data sources such as in *Parkinsonia* L. where the hybridization hypothesis supported by the character count procedure was also confirmed by ecological, cytogenetic and pollen viability data (Hawkins *et al.*, 1999).

Furthermore, it appears noteworthy to point out the substantial biased hybrid plant frequencies in mixed populations between Levante and Andalusia. It remains to be investigated whether this bias is due to the existence of stronger reproductive barriers between *P. lychnitis* and ssp. *crinita* than between *P. lychnitis* and ssp. *malacitana* (at either the pre- or postzygotic stage). Mixed populations in Levante apparently corresponded to bimodal hybrid zones (i.e. hybrid zones with a higher frequency of parental than hybrid individuals) where barriers against hybridization should be mainly found at a prezygotic stage (Jiggins & Mallet, 2000). Accordingly, preliminary results of a reproductive biology study involving artificial crosses showed higher seed production in hand-crosses between *P. lychnitis* and ssp. *malacitana* compared to those between *P. lychnitis* and ssp. *crinita* (R. G. Albaladejo & A. Aparicio, unpubl. data). Furthermore, the analyses of chromosome number and meiotic behaviour in this hybrid complex showed that meiotic abnormalities were markedly more frequent in plants from Andalusia than from Levante (Aparicio & Albaladejo, 2003). This was interpreted in terms of higher levels of gene flow in Andalusian populations, which can be indicative of extensive introgression and backcrossing. Systems in which hybridization and introgression are frequent and appear to be associated with great morphological variation represent multi-generational crosses between parental taxa (Grant,

KEY FOR IDENTIFYING THREE PARENTAL TAXA

1. Lower leaves linear or narrowly elliptical up to 12 mm wide, tapering into an indistinct petiole; inflorescence usually unbranched; calyx mouth not constricted; corolla golden-yellow *P. lychnitis* (Iberian Peninsula and southern France, 200–2000 m)
 - Lower leaves ovate or lanceolate, truncate or cordate, abruptly contracted into a distinct petiole; inflorescence branched; calyx mouth usually constricted; corolla brownish to yellow 2
2. Middle bracts >(15) 17 mm wide; glandular (G4) and stellate (E3) trichomes on calyx, bracteole and stem apex present *P. crinita* ssp. *malacitana* (southern Spain, 1000–2000 m)
 - Middle bracts 9–15 mm wide; glandular (G4) and stellate (E3) trichomes on calyx, bracteole and stem apex absent *P. crinita* ssp. *crinita* (eastern Spain, 200–800 m)

1981; Albert *et al.*, 1997). Thus, the continuous morphological variation found between *P. lychnitis* and ssp. *malacitana* might reflect the existence of extensive introgression in Andalusia, while the lack of such morphological pattern between *P. lychnitis* and ssp. *crinita*, with only some intermediate individuals, would reflect lower introgression rates, if any, in Levante.

Further studies dealing with hybrid formation, maintenance and fitness are needed to ascertain the evolutive input of hybridization in *Phlomis*. In this sense, the hybrid zones illustrated by the present study fulfil the theoretical requisites for recombinational homoploid hybrid speciation (Grant, 1981; McCarthy, Asmussen & Anderson, 1995) given that: (1) the hybrid zone interface is large, (2) the organisms involved show relatively high levels of selfing (R. G. Albaladejo & A. Aparicio, unpubl. data; but see Gallez & Gottlieb, 1982; Rieseberg, Carter & Zona, 1990), (3) the hybrids are relatively fertile (Aparicio, 1997; R. G. Albaladejo & A. Aparicio, unpubl. data), and (4) the differences in chromosomal structure between the parental species are low (Aparicio & Albaladejo, 2003).

In conclusion, based on patterns of geographical, ecological and morphological variation, four taxa could be identified within the *Phlomis* hybrid complex in the Iberian Peninsula. A short dichotomous key for the identification of the three parental taxa is presented above.

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