Plant Syst. Evol. 235: 35–51 (2002)
DOI 10.1007/s00606-002-0218-9

Plant Systematics and Evolution
Printed in Austria

Cone morphology variation in *Pinus canariensis* Sm.

L. Gil[1], **J. Climent**[1], **N. Nanos**[1], **S. Mutke**[1], **I. Ortiz**[1], and **G. Schiller**[2]

[1]Unit of Plant Anatomy, Physiology and Genetics, ETSI de Montes, UPM, Madrid, Spain
[2]Agriculture Research Organization, The Volcani Centre, Best-Dagan, Israel

Received June 27, 2001; accepted June 29, 2002

Abstract. Morphological variation of *Pinus canariensis* cones was studied, based on a sample of 891 cones collected at 23 populations covering the entire natural range of distribution of the species. Both categorical and quantitative traits were used for the analyses. The categorical traits of the apophysis and umbo were subjected to Multiple Correspondence Analysis (MCA). The categories related to apophysis type were found to be the most important variables for ordination. Three cone groups were defined through clustering, generally differentiated according to apophysis prominence. Approximately half of the studied cones were classified within one group, denominated form *gibba* and corresponding to the majority of previous descriptions of the species. In addition, we found some other, less frequent cone forms (called *plana*, *gibberosa protuberans* and *reflexa*), which have not been traditionally considered in *Pinus canariensis*. Cones and seeds were larger in stands at higher altitude, possibly adapted to unfavourable (cold and dry) environment. No clear geographical structure was found in the studied traits at the population level. However, variation of cone morphology among islands was found to be related to the extension of pine forests.

Key words: Canary Islands pine, cone scales, apophysis, umbo, cone size, seed and wing size.

Introduction

Canary Islands pine (*Pinus canariensis* Sm.) is today an endemic species of the canary archipelago. Besides its reduced distribution, this species occupied a larger extension along the northern Tethys shore according to the fossil records of the species or close ancestors in Tertiary deposits (Page 1974, Klaus 1982). The relationship of *Pinus canariensis* to Mediterranean pines (*P. halepensis*, *P. brutia*, *P. pinea* and *P. pinaster*) has been highlighted by means of morphological traits (Klaus 1989, Frankis 1999) and DNA analyses (Krupkin et al. 1996, Strauss and Doerksen 1990, Liston et al. 1999). In spite of these phylogenetic links, a number of morphological and physiological traits (long thin needles in fascicles by three, resprouting ability, tall habit, etc.) underline the singularity of Canary island pine among Mediterranean species. It has been pointed out that *Pinus canariensis* is a living survivor of a pine type (subtropical mountain pine) already present in the Tertiary at an ancient Mediterranean evolutionary centre (Page 1974, Klaus 1989). This explains its close relationship to the

Himalayan pine (*Pinus roxburghill*) and its links with other Diplo- and Haploxylon pines in Central America and the so-called "Tethys shore pines" (Frankis 1999).

The Canary Islands are situated in the Atlantic Ocean, 100 km off the northwest cost of Africa, at about 28 °N. The Islands are of volcanic origin, dating from various ages as far back as the Precretaceous era (Dañobeitia and Canales 2000). Natural pine forests occur nowadays on the islands of Tenerife, La Palma, Gran Canaria, El Hierro and La Gomera. Stands of *Pinus canariensis* grow under extremes of climatic variation because of the huge altitudinal range from 200 to 2,000 (2,500) m and the geographical position of each island (from the humid north-western to the dryer south-eastern islands). The presence of a mist layer caused by Trade Winds provokes frequent wet conditions in mid altitudes of north-eastern slopes, this effect being reduced at higher altitudes (above 1,500 m) and totally absent at the xeric southern slopes (Marzol 1988). Intense destruction in the past led to the present fragmentation of some Canary pine forests within the islands (Climent et al. 1996). In other cases, small relict groves in unusual climatic conditions have been interpreted as colonisations favoured by particular edaphic circumstances, e.g. acid or recent volcanic soils (Arco et al. 1992).

Seed cone morphology has been traditionally considered a valuable trait for taxonomic purposes or genetic discrimination in conifers (Shaw 1914, Khalil 1984, Borghetti et al. 1988, Carlson and Theroux 1993). In the genus *Pinus*, the shape of the apophysis and the umbo of the seed scales have been widely used to study inter- and intraspecific differentiation (Beaulieu and Simon 1995, Endlicher 1847, Farjon and Styles 1997, Klaus 1989, Mirov 1967). Today, morphological based taxonomy has been surpassed by methods based on genetic markers; nevertheless, DNA analysis in genus *Pinus* (Liston et al. 1999, Strauss and Doerksen 1990) have tended to confirm a phylogeny based on one morphological traits (Klaus 1989). On the other hand, paleobotanical studies normally lack the means to obtain DNA, thus methodology is still limited to the morphological and anatomical descriptions of materials which are often scarce or incomplete. Accurate knowledge of current variation in living species is crucial for a correct classification of the fossils. Some authors have taken this into account and have pointed out the need to revise the ascription of numerous fossil pine remnants (some of them described in the 19th century) related to *Pinus canariensis* (Page 1974, Morla et al. unpubl. data).

There are some facts suggesting that cone morphology (especially apophysis thickness) may have adaptive significance related to cone opening and the resistance of closed cones to high temperatures (Beaufait 1960, Linhart 1978, Dawson et al. 1997). Thus, apophysis traits could show geographical differences linked to ecological factors.

The objectives of this paper are, firstly, to describe as precisely as possible the variation of cone morphology in *Pinus canariensis* throughout its range of natural distribution and, secondly, to assess the existence of geographical and/or ecological trends in that variation.

Material and methods

Plant material. Sampling took place in twenty-three populations of *P. canariensis*, covering the natural range of species (Fig. 1). Each sampled population consisted of mature natural stands over an ecologically homogeneous area. They included wet stands in north-east slopes, high altitude stands above the fog belt (> 1,500 m), dry southern stands and a few north facing relic stands, below the fog belt (Table 1). Cones were collected from twenty-five to thirty trees per population, spaced at least 100 m apart to minimise consanguinity. The lower number of trees was in some cases due to recent fires, insect pests and the small size of the stands. Two mature cones were collected at random from the lower part of the crown of each tree. The presence of serotinous cones in each population was evaluated as a percentage of the trees bearing serotinous cones (rate of serotiny, RS).

Data description. After seed extraction, cones were re-closed by soaking them in warm water. Cone length (Cl) and width (Cw) were measured

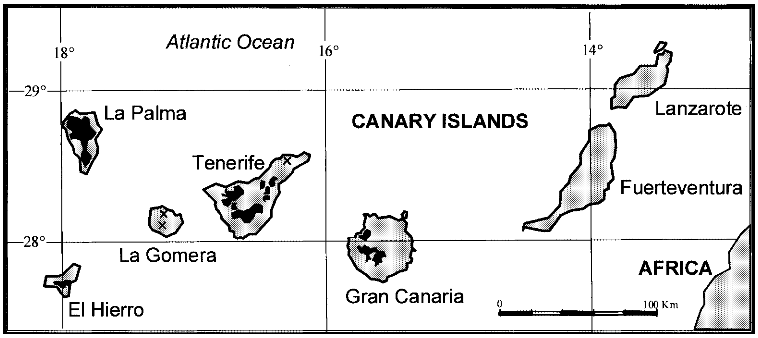

Fig. 1. Range of distribution of *Pinus canariensis*. Main area (black) and relic stands (crosses)

Table 1. Studied populations by island (*Tf*, Tenerife; *LP*, La Palma; *EH*, El Hierro; *GC*, Gran Canaria, *Go*, Gomera), mean altitude (*Alt.*), mean annual rainfall (*R*), temperature (*T*) and number of sampled trees. Asterisks represent small isolated populations

Population	Island (orientation)	Alt. (m)	R (mm)	T (°C)	No. of trees
1. La Orotava	Tf (N)	1,400	700	12	22
2. La Guancha	Tf (N)	700	500	16	29
3. Garachico	Tf (N)	1,350	400	14	24
4. Vilaflor	Tf (S)	1,900	450	10	30
5. Adeje	Tf (S)	1,900	380	12	26
6. Arico	Tf (S)	1,600	380	12	29
7. Candelaria	Tf (E)	1,300	600	14	16
8. La Esperanza	Tf (E)	1,100	600	16	18
9. R. de los Pinos*	Tf (N*)	500	500	18	16
10. Punta Llana	LP (N)	1,900	600	10	21
11. Barlovento	LP (N)	1,900	800	12	13
12. Garafia	LP (N)	1,500	800	12	36
13. Punta Gorda	LP (N)	800	500	16	33
14. El Paso	LP (S)	1,100	700	16	27
15. Fuencaliente	LP (S)	1,050	500	16	33
16. S. Salvador	EH	1,000	450	14	30
17. R. De las Playas	EH	900	600	18	7
18. Tamadaba	GC (N)	1,100	500	14	27
19. Tirma	GC (N)	850	500	16	32
20. Tejeda	GC (S)	950	370	16	31
21. Mogán	GC (S)	900	300	16	30
22. Garabato*	Go (N*)	500	500	20	17
23. Imada*	Go (S*)	1,180	300	14	3

with an image analyser. Two scales from neighbouring helices were removed from the central section of the exposed face of each cone. On these scales, apophysis width (Aw) and apophysis height (Ah) were measured at a precision of 0.01 mm, with an electronic calliper (Fig. 2). Apophysis height was then divided into umbo height (Uh) and apophysis height up to the umbo (Ah'). Further analyses were based on mean values of the two measured scales per cone. Twenty seeds per tree were randomly selected and measured (both seed and wing) with the image analyser. Seed and wing colour patterns were described qualitatively.

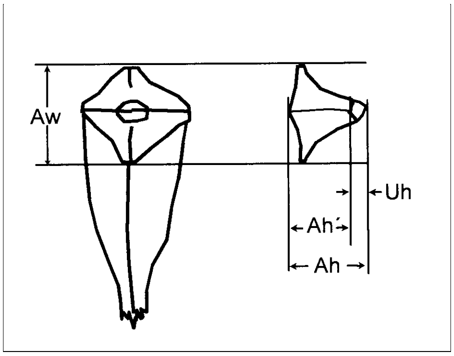

Fig. 2. Apophysis quantitative traits, *Aw*: apophysis width; *Ah*: apophysis height; *Ah*': apophysis height up to the umbo; *Uh*: umbo height

A qualitative description of apophysis and umbo was given from the basal, central and apical scales on the exposed side of the cone. A previously trained person carried out all observations, aided by drawings and photographs. Both apophysis and umbo were described on a radial projection and on a transverse projection, distinguishing flatter or more prominent apophyses and umbos (Fig. 3, Table 2). Prickle was often eroded by weathering (especially in serotinous cones) and it was therefore excluded from the analyses.

Data analysis. Correlations were calculated at the individual level to evaluate relationships between morphological and quantitative traits. Additionally, the correlations between environmental variables

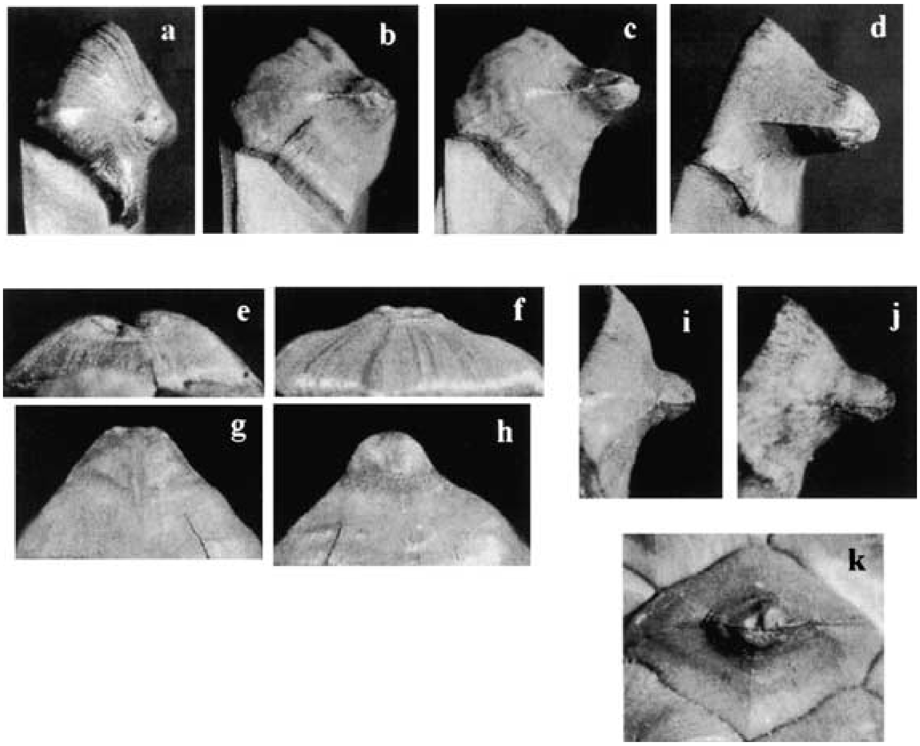

Fig. 3. Levels of the categorical variables of the apophyses.

- Apophysis shape of central scales in a radial projection (APOc): flat, level 0 (**a**); pyramidal, level 1(**b**); prominent, level 2(**c**); reflexed, level 3(**d**).
- Apophysis shape in transversal projection (KEEL): convex, level 0 (**e**) and (**f**); straight, level 1 (**g**); concave, level 2 (**h**).
- Transversal projection of the umbo (UMBOt): depressed, level 0 (**e**); flat, level 1 (**f**) and (**g**); rounded, level 2 (**h**)
- Radial projection of the umbo (UMBOr): even, level 0 (**a**), (**e**) and (**f**); slightly raised, level 1 (**b**), (**d**) and (**k**); prominent, level 2 (**c**) and (**i**); protuberant, level 3 (**g**), (**h**) and (**j**).
- A view of the typical excentromucronate umbo (**k**)

Table 2. Categorical variables and their acronyms, used in ordination. Photographs of the main categories are included in Fig. 3

Description	Cone scale	Variable	Categories (Levels)
Apophysis shape in a radial projection	Apical	APOa	0-Flat 1-Pyramidal 3-Reflexed
	Central	APOc	0-Flat 1-Pyramidal 2-Prominent 3-Reflexed
	Basal	APOb	0-Flat 1-Pyramidal 3-Reflexed
Apophysis shape in a transversal projection (keel)	Central	KEEL	0-Convex 1-Straight 2-Concave
Umbo shape in a radial projection	Central	UMBOr	0-Even 1-Slightly raised 2-Prominent 3-Protuberant
Umbo shape in a transversal projection	Central	UMBOt	0-Depressed 1-Flat 2-Rounded

and the mean values of quantitative cone variables were examined at the population level.

The categorical variables used to describe cone morphology were subjected to Multiple Correspondence Analysis (MCA). This method is an extension of correspondence analysis (CA); but while CA is applied to a contingency table, MCA is applied to a full disjunctive table. The technique of MCA, also known as homogeneity analysis, dual scaling or reciprocal averaging (Hill 1973) is based on a singular value decomposition of the *Burt table* (the equivalent of a covariance matrix for categorical data) (Gifi 1990). The basic premise of the technique is that complicated multivariate data can be made more accessible by displaying their main regularities and patterns in two- dimensional plots. Both the categories and the observations can be plotted on a low-dimensional space (usually two dimensions are retained) and the resulting plots are used to relate the different categories to each other, based on the distances between the row points, i.e., between the individual cases. Contrary to CA, in MCA the total variance explained by the resulting axes is a function of the number of variables and the total number of categories. Consequently, the variance explained by each MCA axis has no significant meaning and it should not be taken into account (Bouroche and Saporta 1983).

The MCA scores for every observation (cone) were used as the input variables for the subsequent cluster analysis. The Euclidean distance was used in order to compute the distance matrix between cones. Classification was carried out using an agglomerate algorithm based on Ward's method (Everitt 1980). The number of groups was judged visually based on the resulting dendrogram. After cone groups were defined, one-way analyses of variance were applied to detect differences of quantitative traits among the previously established groups.

To detect geographic patterns of variation in cone morphology, a clustering analysis following Ward's method was performed. For this purpose, mean score values per population and per island derived from the MCA were used. Since MCA axes are orthogonal, the distance matrix was calculated using the Euclidean, instead of the Mahalanobis distance (Everitt 1980).

Results

Cone size ranged from 5 to 20.5 cm in length and 3 to 8.6 cm in diameter. Apophysis height (Ah) ranged from 4.1 to 11.9 mm, including an umbo (Uh) ranging from 0.3 to 5.2 mm. The prickle (mucro) was normally blunt and always located above the horizontal keel (excentromucronate apophysis). Seeds were rhomboidal, colored differently on both sides, frequently brown, less frequently grey, either spotted or plain. They were 8.2 to 20.0 mm long. Adnate wings were 7.3 to 28.7 mm long, frequently ($>80\%$) with curly stripes, and sometimes plain. Seed and wing color patterns were very constant within trees but extremely variable between trees, with similar levels of variation throughout populations.

Cone size was correlated with both seed and wing sizes but the correlation was generally stronger with the later (Table 3). In fact, the correlation coefficient between seed and wing sizes was only 0.637 ($p < 0.01$). Apophysis dimensions (Aw, Ah and Ah') were positively and significantly related to cone size while the correlation between umbo and cone size was weak. The correlation between cone size (mean cone length and width) was positive and highly significant with the stand altitude and negative with the mean temperature (Table 3). Trees bearing closed cones were present in all the sampled populations with a percentage (RS) ranging from 1 to 60% . Serotinous trees were very scarce in Gran Canaria and more frequent in northern relict stands (populations 9 and 22). RS was negatively correlated ($P < 0.01$) with mean cone size and apophysis height at population level (Table 3). No significant correlations could be found between RS and altitude, rainfall or temperature.

Ordination. The *Burt table* for the 891 cones (Table 4) shows that most of the measured cones had pyramidal or prominent central apophyses (APOc) (37% each), while 17% were reflexed only 8% were flat. The apical apophyses (APOa) of the cones were usually pyramidal (73%) and to lesser extent prominent (10%). Basal apophyses (APOb) were predominantly reflexed (69%). The keels of the studied scales were found to be predominantly convex (54%) or straight (36%) and less frequently concave (10%). The radial view of the umbo was slightly raised or prominent in a large proportion of the cones (44% and 35%, respectively); 17% of the cones had an even umbo, while protuberant umbos were found only in 5% of the cones. In a transversal view, umbos were predominantly rounded (73%) while only a small proportion

Table 3. Correlation coefficients between morphological traits at the individual level and mean serotiny and environmental traits at population level (values in italics). All coefficients significant at 99,9% level except when stated with asterisks and n.s. (non-significant). *Cl*, Cone length. *Cw*, cone width. *Ah*, apophysis height. *Aw*, apophysis width. *Ah'*, apophysis height up to the umbo. *Uh*, umbo height. *Ss*, seed size. *Ws*, wing size. *RS*: rate of serotiny, *Alt.*: altitude, *R*: annual rainfall, *T*: Mean temperature

	Cl	Cw	Ah	Aw	Ah'	Uh	Ss	Ws	RS
Cl		0.783	0.530	0.735	0.568	0.121*	0.377	0.460	−0.667
Cw			0.637	0.622	0.615	0.257	0.428	0.550	−0.672
Ah				0.540	0.885	0.568	0.287	0.329	−0.613
Aw					0.595	0.142**	0.387	0.452	−0.544*
Ah'						0.133**	0.283	0.296	−0.667
Uh							0.122*	0.188	_n.s._
Ss								0.637	_n.s._
Ws									_n.s._
Alt.	_0.666_	_0.724_	_n.s._	_0.786_	_0.535*_	_n.s._	_n.s._	_0.656_	_n.s._
_R_	_n.s._	_n.s._	_n.s._	_n.s._	_n.s._	_n.s._	_n.s._	_n.s._	_n.s._
_T_	_−0.750_	_−0.743_	_−0.445*_	_−0.699_	_−0.581**_	_n.s._	_n.s._	_−0.552*_	_n.s._

Table 4. Burt's table used for the Multiple Correspondence Analysis (MCA) of *Pinus canariensis* cones. This table is a symmetric matrix consisting of a total of Jh × Jh partitions (being Jh the total number of categories) created by each category tabulated against itself and against all other categories. The sum of the diagonal elements in each diagonal partition is constant and equal to the total number of cases. The off-diagonal elements of each diagonal partition are all zero

		APOa			APOc				APOb			KEEL			UMBOt			UMBOr			
		0	1	3	0	1	2	3	0	1	3	0	1	2	0	1	2	0	1	2	3
APOa	0	147	0	0	60	36	35	16	47	37	63	115	27	5	39	27	81	41	61	41	4
	1	0	654	0	9	294	241	110	31	152	471	336	264	54	99	61	494	96	304	228	26
	3	0	0	90	1	2	58	29	3	8	79	28	35	27	10	0	80	7	24	46	13
APOc	0	60	9	1	70	0	0	0	32	15	23	60	10	0	23	17	30	30	19	16	5
	1	36	294	2	0	332	0	0	26	118	188	241	90	1	67	53	212	66	149	102	15
	2	35	241	58	0	0	334	0	15	60	259	122	156	56	37	14	283	37	135	142	20
	3	16	110	29	0	0	0	155	8	4	143	56	70	29	21	4	130	11	86	55	3
APOb	0	47	31	3	32	26	15	8	81	0	0	61	16	4	20	18	43	25	33	20	3
	1	37	152	8	15	118	60	4	0	197	0	139	51	7	48	33	116	48	85	61	3
	3	63	471	79	23	188	259	143	0	0	613	279	259	75	80	37	496	71	271	234	37
KEEL	0	115	336	28	60	241	122	56	61	139	279	479	0	0	107	67	305	106	210	146	17
	1	27	264	35	10	90	156	70	16	51	259	0	326	0	35	21	270	33	146	132	15
	2	5	54	27	0	1	56	29	4	7	75	0	0	86	6	0	80	5	33	37	11
UMBOt	0	39	99	10	23	67	37	21	20	48	80	107	35	6	148	0	0	105	22	21	0
	1	27	61	0	17	53	14	4	18	33	37	67	21	0	0	88	0	38	46	4	0
	2	81	494	80	30	212	283	130	43	116	496	305	270	80	0	0	655	1	321	290	43
UMBOr	0	41	96	7	30	66	37	11	25	48	71	106	33	5	105	38	1	144	0	0	0
	1	61	304	24	19	149	135	86	33	85	271	210	146	33	22	46	321	0	389	0	0
	2	41	228	46	16	102	142	55	20	61	234	146	132	37	21	4	290	0	0	315	0
	3	4	26	13	5	15	20	3	3	3	37	17	15	11	0	0	43	0	0	0	43

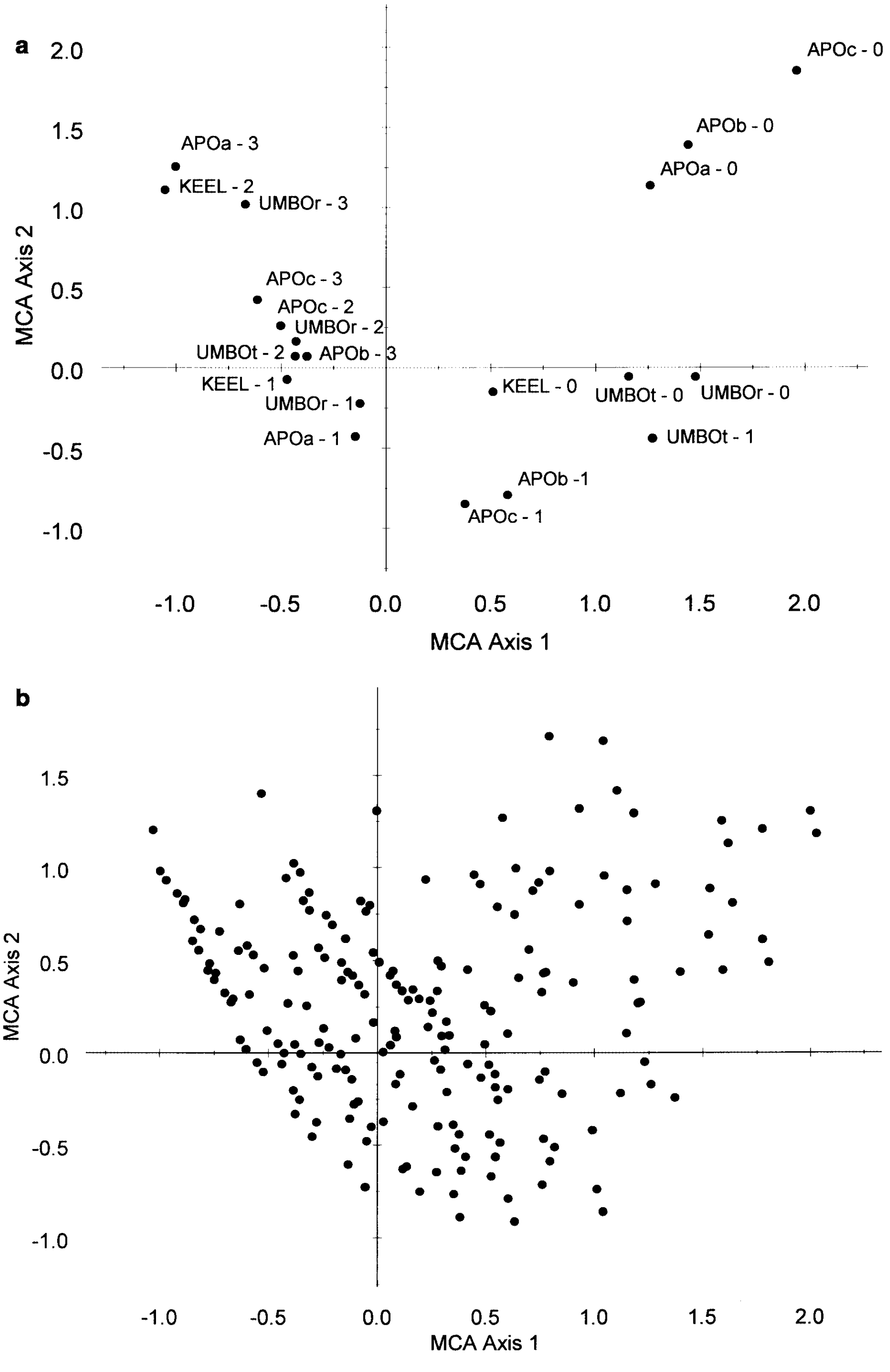

Fig. 4. Multiple Correspondence Analysis: ordination of categories (**a**) and cones (**b**). Only the first two ordination axes were retained

of the cones (10%) had a flat umbo. Depressed umbo (type 0) was found in 17% of the cones.

The categories related to apophysis types were found to be the most important variables for the first MCA axis (Fig. 4a). Flat apophyses (apical, central or basal) were located together on the top-right part of the diagram, while the pyramidal apophyses were located in the centre of the ordination diagram. Depressed or flat, radially even umbos were situated in the right part of the diagram. Another highly correlated group of variables can be found in the top left of the ordination diagram (APOa-3, UMBOr-3 and KEEL-2). All these variables define the cones with the most prominent apophyses and/or umbos.

In Fig. 4b, we present the MCA ordination diagram for cones (only the first two axes were retained). Cones located on the right part of this diagram have flat apophyses while when moving to the left part of the diagram we find firstly cones with pyramidal and then protuberant or reflexed apophyses.

Classification. Cone classification into homogeneous groups was based on the MCA scores. Since the diagram for the first two MCA axes is directly interpretable, we used only these two axes for performing clustering, in spite of the loss of some variability contained in other axes. The cluster analyses showed that three cone groups can be distinguished, following the aforementioned trends of the MCA results (Fig. 5a). The most numerous group is Group 2 with 248 cones followed by Group 3 with 390 cones and Group 1 with 73 cones. The same groups have been depicted on the ordination diagram (Fig. 5b), in order to understand the nature of each group and interpret its main characteristics, which can be resumed as follows (Table 5):

- Group 1. Cones in this group had mainly flat central scales (86%); in fact, this group included 90% of all analysed cones with this trait. Apical apophyses were equally flat (96%), never reflexed. Basal apophyses were also frequently flat (58%). The umbo in this group was predominantly even (44%) or slightly raised (30%), but a significant percentage of cones had prominent (22%) or protuberant (4%) umbos. The keel was mainly convex (88%) and never concave.

- Group 2. Cones characterised by pyramidal apophysis both in the central (73%) and in apical scales (88%). This group included 94% of all sampled cones with pyramidal apophysis in central scales. A lower proportion (21%) showed prominent central apophysis, whereas flat or reflexed apophysis were rare (1% and 5% respectively). Basal apophyses were reflexed (58%) or pyramidal (40%), rarely flat (3%). Cones of this group included 87% of the sampled cones with pyramidal basal apophyses. Umbos were even (25%), slightly raised (44%) or prominent (30%), while protuberant umbos were extremely rare (only one case). In the transversal projection, the umbo was mainly rounded (58%) or depressed (26%); the cones in this group with flat umbos (17%) accounted for most of the occurrences (82%) of this trait in the entire sample. The keel was convex or straight, much rarely concave (1%).

- Group 3. Includes the most prickly cones with prominent (62%) or reflexed (34%) central apophyses, or more rarely pyramidal or flat (4 and 1%, respectively). Basal apophyses were mainly reflexed (94%). Reflexed apical apophyses were scarce (22%), but 97% of all the cones with this character were included in this group. The umbo was mainly rounded (97%) in traversal projection and slightly raised, prominent or protuberant in radial projection (91% of all cases with a protuberant umbo were classified in this group). Group 3 includes nearly all cones with concave keels (95%), but straight or convex keels were frequent too.

Thus, apophysis type discriminated between the three established groups, whereas keel type separated group 1 and 3 and the umbo separated group 3 from 1 and 2. In addition, various quantitative variables were significantly different among the three cone groups (Table 6). The third group included larger (longer and broader) cones compared to the other two groups. As expected, cones classified within group 3 had the thickest apophysis and umbos (Ah and Uh).

Geographical distribution of cone types. A high variability of cone morphology was found within most populations (data not shown). Moreover, the relative frequencies of cone groups varied significantly among neighbouring populations (Table 7). For example, the relative frequency of group 1 cones varied from 0 to 22% among the three populations from northern Tenerife. In northern Gran

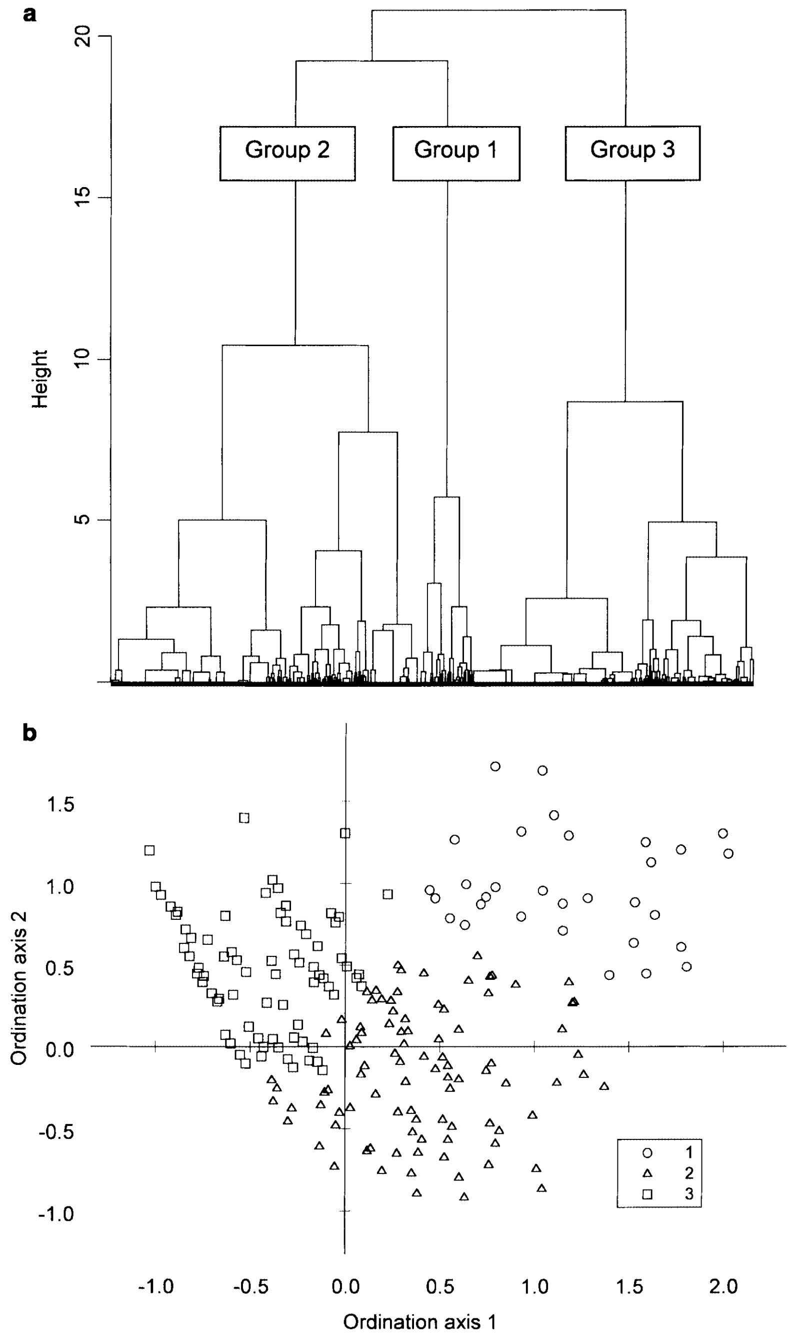

Fig. 5. Clustering of the sampled cones into three groups: (**a**) dendrogram; (**b**) representation of the cone groups over the MCA ordination diagram

Canaria, most cones (95%) of population 18 belonged to the group 2, while all the cones of population 19 belonged to group 3.

The results of cluster analysis conducted at population level (Fig. 6a) confirm this lack of geographical aggregation pattern. Populations 1, 2 and 3, although belonging to the same area of Tenerife, were found to be very distant in respect to their cone morphology. Similarly, the two populations of El Hierro (16, 17) seemed to be very different in respect to their cone morphology. Especially, the most different population (17) had the highest proportion of group 1 cones among all populations.

Table 5. Frequencies (absolute and relative) of categorical cone variables among cone groups derived from cluster analysis (Fig. 5)

Group		APOa			APOc				APOb			KEEL			UMBOt			UMBOr			
		0	1	2	0	1	2	3	0	1	3	0	1	2	0	1	2	0	1	2	3
1	Freq.	70	3	0	63	4	4	2	42	13	18	64	9	0	28	16	29	32	22	16	3
	Rel. Freq.	0,96	0,04	0,00	0,86	0,05	0,05	0,03	0,58	0,18	0,25	0,88	0,12	0,00	0,38	0,22	0,40	0,44	0,30	0,22	0,04
2	Freq.	49	376	3	5	313	88	22	28	171	229	295	129	4	110	72	246	108	89	130	1
	Rel. Freq.	0,11	0,88	0,01	0,01	0,73	0,21	0,05	0,07	0,40	0,54	0,69	0,30	0,01	0,26	0,17	0,58	0,25	0,44	0,30	0,00
3	Freq.	28	275	87	2	15	242	131	11	13	366	120	188	82	10	0	380	4	178	169	39
	Rel. Freq.	0,07	0,71	0,22	0,04	0,04	0,62	0,34	0,03	0,03	0,94	0,31	0,48	0,21	0,03	0,00	0,97	0,01	0,46	0,43	0,10

Table 6. LSD multiple range tests for quantitative variables among cone groups derived from clustering. Means with the same letter are not significantly different ($P < 0.05$). *Cl*, cone length; *Cw**, cone width minus twice the apophysis height; *Ah*, apophysis height; *Uh*, umbo height; *Ah'*, apophysis height up to the umbo

Group	Cl	Cw*	Ah	Uh	Ah'
1	11.16 a	4.25 a	6.03 a	1.55 a	4.48 a
2	10.90 a	4.14 a	7.01 b	1.79 b	5.22 b
3	12.11 b	4.44 b	7.94 c	2.22 c	5.72 c

Table 7. Relative frequencies of cone groups per population and in the whole sample

Population	Location	1	2	3
1	Tf (N)	6,5	84,8	8,7
2		22,2	55,6	22,2
3		0,0	70,6	29,4
4	Tf (S)	11,1	44,4	44,4
5		0,0	43,8	56,3
6		12,2	39,0	48,8
7	Tf (E)	18,2	45,5	36,4
8		20,8	58,3	20,8
9	Tf (N*)	9,1	45,5	45,5
10	LP (N)	25,0	56,3	18,8
11		0,0	69,2	30,8
12		3,2	38,7	58,1
13		4,8	40,5	54,8
14	LP (S)	0,0	23,5	76,5
15		17,5	49,2	33,3
16	EH	5,7	20,8	73,6
17		42,9	42,9	14,3
18	GC (N)	0,0	95,0	5,0
19		0,0	0,0	100,0
20	GC (S)	3,6	32,1	64,3
21		3,7	25,9	70,4
22	Go (N*)	0,0	70,6	29,4
23	Go (S*)	0,0	50,0	50,0
Total		8,1	47,3	44,6

Clustering at island level (Fig. 6b) revealed that Tenerife and La Palma form a small cluster themselves and a larger one together with Gran

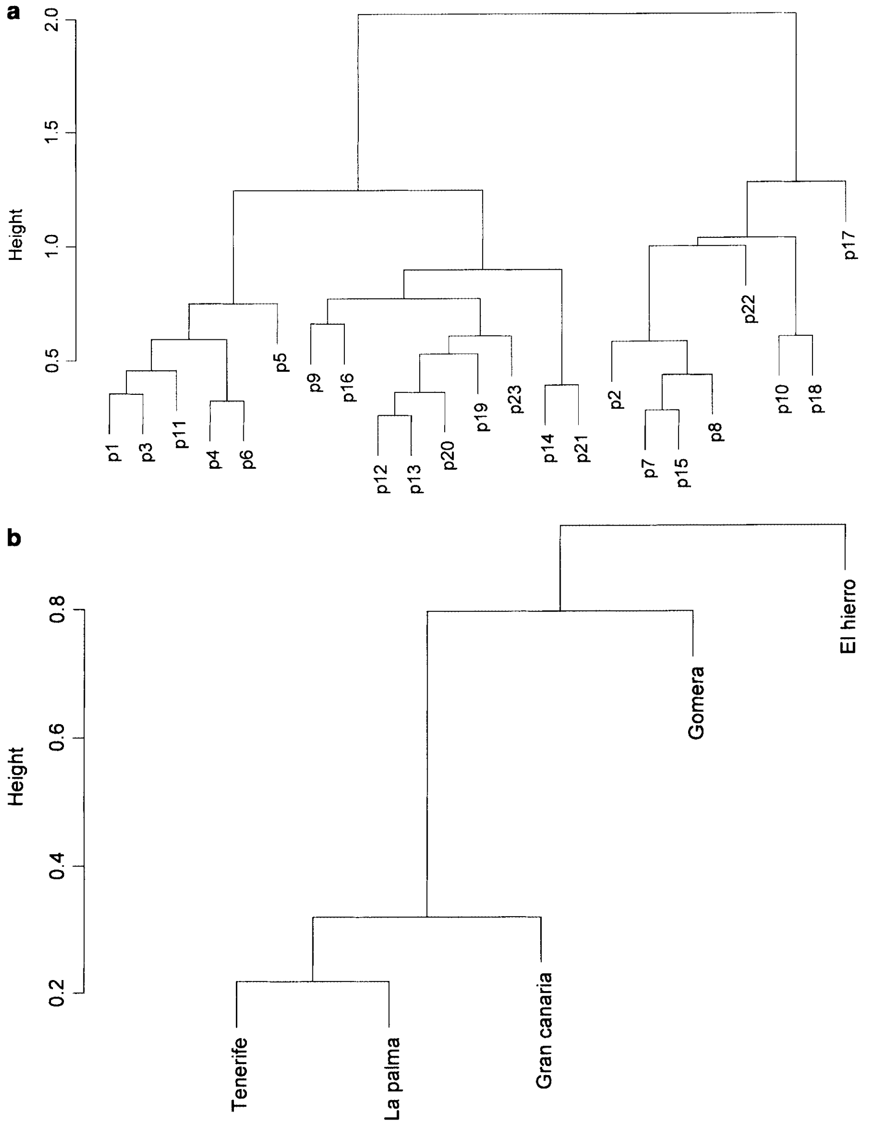

Fig. 6. Results of the cluster analysis for the cone morphology at population (**a**) and island (**b**) level

Canaria. These three islands are clearly separated from La Gomera and El Hierro.

Discussion

The analysis of cone morphology variation in conifers has until now been carried out based on either an univariate description of their main morphological attributes (Forde 1964) or through quantitative traits of the cone and/or the cone scales (Beaulieu and Simon 1995, Khalil 1984, Melzack et al. 1981, Piedra 1984). The use of quantitative traits for the analysis of cone morphology in *Pinus canariensis* previously attempted (Climent et al. 2001) presented serious drawbacks, since some categorical variables were always necessary in order to fully describe existing variation. In addition, categorical variables are more stable than quantitative traits among cones of the same individual. The use of MCA, although being purely descriptive, allowed an objective characterisation of *Pinus canariensis* cones based on multiple characteristics. For the most part, variability is due to the apophysis type in a radial view, since all the related categories were located on the extremes of the first two MCA axes (Fig. 4a). The three cone groups defined by the cluster analysis are separated mostly by the prominence of central apophyses on the exposed side of the cone.

When studying the natural variation of cone morphology, interest is usually centered on the rarest types of cones (apart from the most frequent types). However these cones are the least numerous and consequently the usual data reduction techniques (in this case the MCA) fail to discriminate them even if more than two ordination axes are retained. This is the case of some of the cones within group 1 and 3, which are statistically similar but visually distinct (Fig. 7). Although the first group consists mainly of smooth cones (f. *plana*), some of these showed raised umbos over flat apophyses, a form we have called f. *protuberans*. The third group (cones with thick apophyses and/or umbos) is even more heterogeneous with three distinguishable forms. Most cones showed prominent apophyses and correspond to the form *gibberosa* (Fig. 7) but about 34% showed reflexed (downturned) apophyses (f. *reflexa*). Furthermore, a limited number of cones showed prominent or protuberant umbos over less prominent apophyses, corresponding to f. *protuberans*. In contrast, the second group (the most frequent in *Pinus canariensis*, with a 47% occurrence) is rather homogeneous, corresponding to the form *gibba*, i.e. cones with moderately thick apophyses and inconspicuous umbos.

Given its higher frequency, it is the *gibba* type of cone which has been reported in previous botanical descriptions of the species (Ceballos and Ortuño 1976, Mirov 1967,

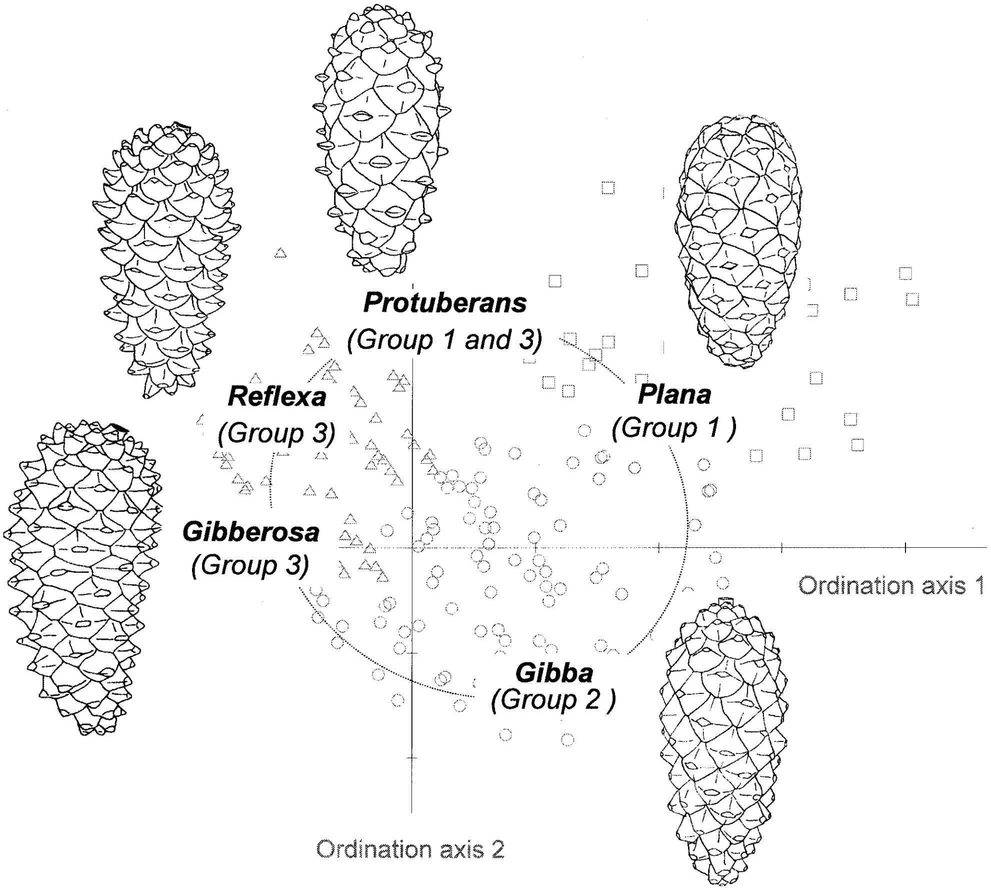

Fig. 7. Graphical representation (over the ordination space) of the main forms of cones found for *Pinus canariensis* and their distribution to the three groups defined by the cluster analysis

Vidakovic 1991). The description and drawing of the Canary islands pine by Farjon and Burgh (1984) seem to correspond to form *gibberosa* (group 3). The forms *plana* and *reflexa* had not been previously mentioned in this species except by Klaus (1989). In fact, the morphological differentiation between *Pinus canariensis* and *P. roxburghii*, from the monsoon belt of the outer Himalayas is practically characterised by the markedly downturning apophyses of the cones present in the Himalayan pine (Farjon and Burgh 1984, Page 1974, Vidakovic 1991). *Protuberans* cones seem to have been also neglected. The acceptance of these cone forms in *Pinus canariensis* is important to the discussion on former or future fossil records since *gibba* and *plana* are the only forms currently attributed to this species (Klaus 1989). Interestingly, various *P. canariensis* cones recently described from a Pliocene deposit in Alicante, south-eastern Spain, seemed to include most of the groups and variants commented in this paper (Morla et al. submitted).

Cone size variation reflected in this paper is greater than has been pointed out in former descriptions (Ceballos and Ortuño 1976, Farjon and Burgh 1984, Page 1974, Pilger 1926). Moreover, the neglected proposal of a variety *microcarpa* for an individual with tiny cones (5 cm long) planted in Portugal (Franco 1943) had pre-empted this variability. The positive relationship between cone size and altitude in natural populations of this species contrasts with comments by Klaus (1989) and with relationships reported in other *Pinus* species (Stockwell 1939, Lanner 1998). The fact that many *Pinus canariensis* populations found at high altitudes grow under drier conditions than those at lower altitudes on northern slopes (Marzol 1988) could explain this divergence of results. Also observed in other pine species (Benkman 1995, Keely and Zedler 1998, Perry 1991, Piedra 1984) is the fact that larger cones of *Pinus canariensis* contain larger seeds, but mainly bigger wings. The adaptive significance of both aspects is clear: bigger seeds produce more vigorous seedlings (Griffin 1972, Reich et al. 1994) and bigger wings allow a higher dispersion capacity (Benkman 1995, Lanner 1998). Even considering environmental effects, e.g. light intensity and duration, a selective pressure towards larger cones may have occurred in unfavourable habitats of *Pinus canariensis*. This assertion has been supported by the strong genetic influence over cone and seed size observed in other conifer species (Khalil 1984, Matziris 1998).

Cone serotiny is a feature often absent in literature on *Pinus canariensis* (Keeley and Zedler 1998, Klaus 1989, Page 1974) but which is however widespread in the sampled stands. The methodology applied in this paper did not permit detection of further relationships between cone morphology and serotiny, as observed in *Pinus radiata* (Linhart 1978) and *P. pinaster* (Tapias et al. 2001). Correlations between apophyses dimensions and seed and wing sizes were significant although weak (Table 4). Relationships between cone morphotypes and seed size may be satisfactorily explained through cone size itself. A high variability in seed and wing patterns (independently of other cone or seed traits) has been observed in *Pinus canariensis*. It is supposed that the variability in seed and wing appearance is naturally selected and serves as a way of escaping predation in *P. radiata* (Forde 1964) and *P. ponderosa* (Ager and Stettler 1982).

In this paper, a high variability in cone morphology both within and among populations was observed. Sharp differences were frequently observed between neighbouring populations. Both facts are coherent with the lack of geographical structure found through clustering analysis at population level. This runs in parallel to findings in other species of the genus such as *Pinus radiata* (Forde 1964), *P. halepensis* (Melzack et al. 1981), *P. tecunumanii* (Piedra 1984), *P. strobus* (Beaulieu and Simon 1995) and *Pinus pinaster* (authors, unpubl. data). Among the islands themselves, the resulting clusters were scarcely related to geographical distance or geological island age (Dañobeitia and Canales 2000). However, the clustering can be explained in terms of the area covered by pine stands in each island. Thus, the islands with more extensive pine forests (Tenerife and La Palma) are grouped at a short distance; then joins Gran Canaria, which contained extensive forests in the past, greatly reduced over time. This result is coherent with a high gene flux determined by isozymes (Korol et al. 1999). La Gomera and El Hierro, with their smallest forest groves, seem to differ from the rest regarding their cone morphological traits. In the case of El Hierro, the separation from the rest of the islands and the small population would permit the maintenance of a marked coloniser effect. In La Gomera, the small size of relict stands (less than 2000 individuals) would suggest a strong genetic drift which has diminished cone variability.

Results presented in this paper lead us to consider *Pinus canariensis* a highly variable species in as far as cone morphological traits are concerned. The cone scale polymorphism in *Pinus canariensis* could be compared to that of *Pinus radiata* (Forde 1964, Linhart 1978) without reaching the extremes of *Pinus pseudostrobus* (Farjon and Styles 1997). A possible adaptive significance of high cone variability within populations has been reported for *Pinus radiata* (Farjon and Styles 1997) related to an equilibrium between the energetic cost of producing cones with thicker apophyses and their advantage for protecting seeds from fire and predation. It is significant that some of the pines with the most variable cones are fire-adapted and live naturally in fire-prone areas. The presence of *Pinus canariensis* in the archipelago is documented through fossils records dating back at least as for as the Neogene (Gregor 1980); the long term adaptation to diverse and changing environmental conditions (including fires, recurrent volcanic events, etc.) and the lack of competition with other pines may have tended to maintain high cone variability. Further research must be carried out in order to determine the importance of cone morphology for the species' life history.

This research was supported by the Canary Islands Government (Viceconsejería de Medio Ambiente). Thanks to Esther Perez for apophyses measures and to Leonie Woodin for English Revision.

References

Ager A. A., Stettler R. F. (1982) Local variation in seeds of ponderosa pine. Canad. J. Bot., 61: 1337–1344.

Arco M. J., Perez P. L., Rodriguez O., Sala M., Wildpret W. (1992) Atlas cartogrático de los Pinares de Canarias. Tenerife. Dir. Gral. De Medio Ambiente y Cons. De la naturaleza. Gobierno de Canarias. Sta, Cruz de Tenerife, 228 pp.

Beaufait W. R. (1960) Some effects of high temperatures on the cones and seeds of jack pine. Forest Science 6: 194–199.

Beaulieu J., Simon J. P. (1995) Variation in cone morphology and seed characters in *Pinus strobus* in Quebec. Canad. J. Bot. 73(2): 262–271.

Benkman C. W. (1995) Wind dispersal capacity of pine seeds and the evolution of different seed dispersal modes in pines. Oikos 73: 221–224.

Borghetti M., Giannini R., Menozzo P. (1988) Geographic variation in cones of norway spruce (*Picea abies* L.) Karst. Silvae Genet. 37: 178–184.

Bouroche J., Saporta G. (1983) L' analyse des données. PUF, Paris.

Carlson C. E., Theroux L. J. (1993) Cone and seed morphology of western larch (*Larix occidentials*), alpine larch (*Larix lyallii*), and their hybrids. Canad. J. Forest. Res. 23(7): 1264–1269.

Ceballos L., Ortuño F. (1976) Vegetación y flora forestal de las *Canarias occidentals*. Excmo. Cabildo Insular de Tenerife. Sta Cruz de Tenerife, 433 pp.

Climent J., Gil L., Tuero M. (1996) Regiones de procedencia de *Pinus canariensis Chr*. Sm. ex DC. DGCONA MAPA, Madrid, 49 pp.

Climent J., Ortiz I., Gil L. (2001) Variabiliad morfológica de las piñas en *Pinus canariensis* Chr; Sm., III Congreso Forestal Español. Junta de Andalucia, Granada, Spain, pp. 195–201.

Dañobeitia J. J., Canales J. P. (2000) Magmatic underplatting in the Canary Archipelago. Journal of Volcanology and Geothermal Research 103: 27–41.

Dawson C., Vincent J. F. V., Rocca A. M. (1997) How pine cones open. Nature (London) 390: 668.

Endlicher I. L. (1847) *Synopsis coniferatum*. Sangalli: Scheitlin, Zollikofer.

Everritt B. (1980) Cluster analysis. Halsted, New York.

Farjon A., Burgh J. v. d. (1984) *Pines:* drawings and descriptions of the genus *Pinus*. E. J. Brill, Leiden.

Farjon A., Styles B. T. (1997) *Pinus* (Pinaceae). Flora Neotropica, Monograph 75. The New York Botanical Garden, New York, 291 pp.

Forde M. B. (1964) Variation in natural populations of *Pinus radiata* in California. Part 3. Cone characters. New Zealand Journal of Botany 2: 459–485.

Franco J. M. (1943) Uma nova variedade do pinheiro das Canárias. Anais do Instituto Superior de Agronomia 14: 159–163.

Frankis M. (1999) *Pinus brutia* (Pinaceae). Curtis's Botanical Magazine, 16: 173–184.

Gifi A. (1990) Nonlinear multivariate analysis. Wiley, Chichester.

Greger H. J. (1980) Funde von *Pinus canariensis* Ch. Smith Fossilien aus dem Neogen von La Palma Kanarische Inseln. Vieraea 9: 57–64.

Griffin A. R. (1972) The effects of seed size, germination time and sowing density on seedling development in radiata pine. Austral. Forest Res. 5(4): 25–28.

Hill M. O. (1973) Reciprocal averaging: an eigenvector method of ordination. J. Ecol. 61: 237–249.

Keeley J., Zedler P. (1998) Life history evolution in pines. In: Richardson D. M. (ed.) Cambridge University Press, pp. 219–242.

Khalil M. A. K. (1984) Genetics of cone morphology of black spruce (*Picea mariana* (Mill.) B. S. P.) in Newfoundland, Canada. Silvae Genet. 33: 4–5.

Klaus W. (1982) Ein *Pinus canariensis* Smith-Zapfenfund aus dem Ober-Miozaen Pannon des Wiener Beckens. Ann. Naturhist. Mus. Wien. 84: 79–84.

Klaus W. (1989) Mediterranean pines and their history. Plant Syst. Evol. 162: 133–163.

Korol L., Gil L., Climent J., Zehavi A., Schiller G. (1999) Canary islands pine (*Pinus canariensis* Chr. Sm. ex DC). 2. Gene flow among native populations. Forest Genetics 6: 277–282.

Krupkin A. B., Liston A., Strauss S. H. (1996) Phylogenetic analysis of the hard pines (*Pinus* subgenus *Pinus*, Pinaceae) from chloroplast DNA restriction site analysis. Amer. J. Bot. 83: 4898–98.

Lanner R. M. (1998) Seed dispersal in *Pinus*. In: Richardson D. M. (ed.) Ecology and biogeography of *Pinus*. Cambridge University Press, pp. 281–95.

Linhart Y. B. (1978) Maintenance of variation in cone morphology in California closed-cone pines; the roles of fire, squirrels and seed output. The Southwestern Naturalist 23(1): 29–40.

Liston A., Robinson W., Piñero D., Alvarez-Buylla E. (1999) Phylogenetics of *Pinus* (Pinaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. Molec. Phylog. Evol. 11: 95–109.

Marzol M. V. (1988) La lluvia, un recurso natural para Canarias. S° Publ. Caja Gral. Ahorros de Canarias, Sta. Cruz de Tenerife, 220 pp.

Matziris D. (1998) Genetic variation in cone and seed characteristics in a clonal seed orchard of Aleppo pine grown in Greece. Silvae Genet. 47: 37–41.

Melzack R. N., Grunwald C., Schiller G. (1981) Morphological variation in Aleppo pine (*Pinus halepensis* Mill.) in Israel. Isrel. J. Bot. 30: 199–205.

Mirov N. T. (1967) The genus *Pinus*. Ronald Press C., New York, 602 pp.

Page C. N. (1974) Morphology and affinities of *Pinus canariensis*. Notes Roy. Bot. Gard. Edinburgh, 33(2): 317–323.

Perry J. P. (1991) The pines of Mexico and central America. Timber press, Portland, 231 pp.

Piedra E. (1984) Geographic variation in needles, cones and seeds of *Pinus tecunumanii* in Guatemala. Silvae Genet. 33: 72–79.

Pilger R. (1926) Pinaceae. In: Engler A., Prantl K. (eds.) Phylogenie und Systematik der Coniferae. Die natürlichen Pflanzenfamilien.

Reich P. B., Olekysn J., Tjoelker M. G. (1994) Seed mass effects on germination and growth of diverse European Scots pine populations. Canad. J. Forest. Res. 24: 306–320.

Shaw G. R. (1914) The genus *Pinus*. Publ. Arnold Arbor., 5.

Stockwell W. P. (1939) Cone variation in digger pine. Madroño 5: 72–73.

Strauss S. H., Doerksen A. H. (1990) Restriction fragment analysis of pine phylogeny. Evolution 44: 1081–1096.

Tapias R., Gil L., Fuentes-Ultrilla P., Pardos J. A. (2001) Canopy seed banks in mediterranean pines of Southeastern Spain. A comparison between *Pinus halepensis* Mill., *P. pinaster* Ait., *P. nigra* Arn. and *P.pinea* L. J. Ecol. 89: 629–638.

Vidakovic M. (1991) *Conifers:* Morphology and Variation. Graficki Zavod Hrvatske, Zagreb.

Addresses of authors: L. Gil, J. Climent (e-mail: jcliment@montes.upm.es), N. Nanos, S. Mutke, I. Ortiz, Unit of Plant Anatomy, Physiology and Genetics, ETSI de Montes, UPM, Madrid, Spain. G. Schiller, Agricultural Research Organization, The Volcani Centre, 50250 Bet-Dagan, Israel.