

Functional ecology of fronds in Iberian saxicolous ferns

Andrea Seral¹; Antonio Murciano²; Sonia Molino¹; Pablo de la Fuente¹; José M^a Gabriel y Galán¹

¹ Department of Plant Sciences I, Faculty of Biology, Universidad Complutense. Avda. Jose Antonio Nováis, 12. 28040-Madrid, Spain. "Biodiversity and Taxonomy of Cryptogamic Plants" Research Group, UCM.

² Department of Applied Mathematics (Biomathematics), Faculty of Biology, Universidad Complutense. Avda. Jose Antonio Nováis, 12. 28040-Madrid, Spain. "Neural Plasticity, IdISSC" and "Neuro-computing and Neuro-robotics" Research Groups, UCM.

Correspondencia

J.M. Gabriel y Galán

e-mail: jmgabrie@ucm.es

Recibido: 15 septiembre 2017

Aceptado: 26 octubre 2017

Publicado on-line: diciembre 2017

Abstract

Nowadays, functional traits are widely used to study autoecological aspects in plant species. The analysis of these traits among climatic gradients allows us to know the strategy that plants follow depending on resource availability. Referring to plants, the traits measured in the leaves have a special importance; among these traits we can find SLA (Specific Leaf Area), LDMC (Leaf Dry-Matter Content) and LT (Leaf Thickness). In this work, these traits are measured in four species of the genus *Asplenium*. As extremes of a climatic gradient, we have focused our study in both bioclimatic regions existing in the Iberian Peninsula. Taking into account the differences referring to the resource availability for the species in each region, the main goal of this work is analysing the variations of the three functional traits mentioned in the four species in both locations and associate these variations to macroclimatic variables. To this aim, fronds from the four species were collected in populations of each bioclimatic region to determine the fresh weight, dry weight and the foliar area. From these variables, we calculated the three functional traits. The results have shown that ferns adopt higher productive yields in the Mediterranean region (higher SLA values) and lower LDMC and LT values than in the Eurosiberian region. As to the climate variables effects, the average of the maximum temperatures in the warmest month (TMAX) is the variable that better explains the differences in the productive yield that the plants adopt in each region. On the other hand, annual precipitation seems not to play an important role on any of the functional traits in any of the species. Finally, we didn't find a generalized tendency in the effects of the external variables: frozen period, minimum temperatures in the coolest month and arid period.

Key words: Bioclimatic Region, Ecology, Eurosiberian Region, Fronds, LDMC, LT, Mediterranean Region, SLA.

Resumen

Ecología funcional de las frondas de helechos rupícolas en la Península Ibérica.

En la actualidad los rasgos funcionales son ampliamente utilizados para conocer aspectos autoecológicos de las especies vegetales. El análisis de estos rasgos a través de gradientes climáticos permite conocer la estrategia que siguen los vegetales según cual sea la disponibilidad de recursos. En plantas tienen especial importancia los rasgos medidos en las hojas, entre estos rasgos se encuentran el SLA (Specific Leaf Area), LDMC (Leaf Dry-Matter Content) y LT (Leaf Thickness). En este trabajo estos rasgos son medidos en cuatro especies del género *Asplenium*. Como extremos de un gradiente climático hemos centrado nuestro estudio en las dos regiones bioclimáticas de la Península Ibérica. Teniendo en cuenta las diferencias en cuanto a los recursos que cada región aporta a las especies que en ellas viven, el objetivo principal de este trabajo es analizar las variaciones de los tres rasgos mencionados en las cuatro especies de helechos en las dos

localizaciones y asociar estas variaciones a variables macroclimáticas. Para ello se recolectaron frondas de las cuatro especies en poblaciones de cada región bioclimática para determinar el peso fresco, el peso seco y el área. A partir de estas variables se calcularon los tres rasgos funcionales. Los resultados han mostrado que los helechos adoptan un rendimiento productivo mayor en la región mediterránea (mayores valores de SLA) y menores valores de LDMC y LT que en la región eurosiberiana. En cuanto a los efectos que ejercen las variables climáticas, la temperatura media máxima del mes más cálido (TMAX) se presentan como la variable que mejor explica las diferencias en el rendimiento productivo que las especies adoptan en cada región. Por otro lado, la precipitación anual no parece jugar un papel importante sobre ninguna variable funcional de ninguna de las especies. Finalmente, no encontramos una tendencia generalizada en los efectos de las variables extremas: periodo de heleadas, temperaturas medias mínimas del mes más frío y periodo árido.

Palabras clave: Ecología, Frondas, LDMC, LT, Región Bioclimática, Región Eurosiberiana, Región Mediterránea, SLA.

Introduction

Nowadays, functional traits are widely used as adaptation indicators to environmental conditions in plants. These traits can be defined as morphological, structural, biochemical or physiological characteristics which are considered important not only to understand this adaptation, but also to understand how species influence in ecosystem processes (Violle et al. 2007). It is known that these traits present inter and intraspecific variations, that allow us to extract ecological conclusions of the comparison of those functional traits in different species living under similar environmental conditions (Chen et al. 2011; Vasheka et al. 2017). Also, we can compare these traits in individuals of the same species living under different environmental conditions.

Considering the existence of natural resources gradients (generated by variations of edaphic and climatic conditions among others), the “resource use” axis has been defined, which allows to appreciate functional traits variations in plants following the mentioned gradients (Grime et al. 1997; Wilson et al. 1999). Therefore, this resource use axis allows understanding, by the observation of certain functional traits, the adaptation of plants to rich (non-limiting) or poor (limiting) environments in resources. One of the most interesting aspects in the biological, ecological and biogeographical studies of plants is their relationship with the climate, issue that has called the attention of the botanists since long time ago (Went, 1950). In this point, the variation in climate variables can be appreciated as one important environmental gradient, which affects strongly to vegetal productivity and, allows

detecting the different strategies of exploitation of resources by the different species.

Due to the natural condition of plants, foliar functional traits are greatly important variables for the study of the adaptation to climatic gradients and differences between climatic variables. Among those traits, the specific leaf area (SLA), the leaf dry matter content (LDMC) and the leaf thickness (LT) stand out. Along this axis, ranging for higher to lower resources value, it is expected a decrease of the SLA and an increase of LDMC and LT.

Although the major occurrence of ferns is in tropical areas, they can be easily found in almost all terrestrial temperate communities (Mehltreter et al. 2010). In the Iberian Peninsula ferns are especially important in forests and in saxicolous communities (Given, 1993; Ferrer & Vetaas, 2005; Moreno & Lobo, 2008). Within the saxicolous fern communities, the species of the genus *Asplenium* L. are particularly well represented.

The Iberian Peninsula presents two different biogeographical regions: the Mediterranean Region, which is characterized mainly by its less rainfall and the existence of a more or less prolonged arid period; the Eurosiberian Region, which is characterized in general by its higher rainfall and the absence of arid period (Rivas Martínez, 1987). This great variation in climatic conditions could be seen as extremes of an ecological gradient within a moderately limited geographical area. Thus, considering the climate characteristic of each region exposed here, it can be said that those zones with Mediterranean macroclimate are comparatively more limiting regarding to temperature and water resources (Grime et al. 1997; Wilson et al. 1999). It is expected that the

individuals living under Mediterranean conditions will adopt more conservative characters (higher LDMC and LT values) and lower SLA comparing to those individuals living under Eurosiberian conditions. These assumptions derive from the water resources limitation that plants have to deal with in the Mediterranean locations, as water is usually one of the factors which that most limits production in terrestrial plants (Galmés et al. 2007; Galmés et al. 2013; Joffre et al. 1999) and is also the factor that most often determines character selection in plants (Terradas, 2001). Faced to water stress, vegetal productivity will be limited and plants will adopt strategies to retain the resources.

The main goal of this work is to analyse the variation of frond functional traits in *Asplenium* species under to different macroclimatic conditions. We specifically aim to: a) characterise the functional traits in the selected species b) Explain the expected variations in the foliar functional traits in relation to bioclimatic conditions.

Material and methods

Biological material, area of study and climatic variables

We selected the following species: *Asplenium trichomanes* L., *Asplenium ceterach* L., *Asplenium adiantum-nigrum* L. and *Asplenium billotii* F.W. Schultz (Salvo, 1990) (Figure 1). These species are largely distributed in the Iberian Peninsula and share a series of common ecological characteristics, being able to deal with certain grade of soil drought and high insolation, although *A. billotii* and *A. adiantum-nigrum* present a higher requirement in humidity and substrate depth (Salvo, 1990).

The samples of the selected species were prospected from different populations in the two bioclimatic regions. We sampled 370 fronds of saxicolous ferns (coming from 104 individuals): 204 of those were prospected in population with a Mediterranean location and 166 in populations with Eurosiberian location. Appendix 1 shows the



Figure 1. Species of saxicolous ferns of the genus *Asplenium* selected in this study. **A)** *Asplenium adiantum-nigrum*. **B)** *Asplenium billotii*. **C)** *Asplenium ceterach*. **D)** *Asplenium trichomanes*. **Figura 1.** Especies de helechos rupícolas del género *Asplenium* seleccionadas en este estudio. **A)** *Asplenium adiantum-nigrum*. **B)** *Asplenium billotii*. **C)** *Asplenium ceterach*. **D)** *Asplenium trichomanes*.

Specie	<i>A. adiantum-nigrum</i>	<i>A. billotii</i>	<i>A. ceterach</i>	<i>A. trichomanes</i>
Number of fronds euro	46	23	58	43
Numer of individuals euro	11	7	13	12
Numer of fronds med	19	46	77	58
Numer of individuals med	5	17	18	21

Table 1. Number of fronds and individuals collected of each species in each of both bioclimatic regions. **Tabla 1.** Número de frondas y de individuos recolectados por especie en cada una de las dos regiones bioclimáticas.

sampling locations per species, with basic voucher information. The number of individuals and fronds collected from each species and region is shown in table 1.

As functional traits, we selected SLA, LDMC and LT. Specific leaf area (SLA) is a variable related to the relative rate of growth in plants and is related negatively with its longevity (Poorter & de Jong, 1999; Reich et al. 1997). This variable is related to foliar area and dry weight of leaves, and is expressed in $\text{m}^2\cdot\text{Kg}^{-1}$. The variable LDMC is a trait related to the density of the leaves, and is related to nutrient retention of the plant (Garnier et al., 2004). This variable relates dry weight of the leaves (mg) and fresh weight when the leaves are completely saturated with water (g) and so, is expressed in $\text{mg}\cdot\text{g}^{-1}$. The last variable, LT, refers to leaf thickness (μm), in this work it will be estimated from the other variables mentioned (Vile et al. 2005).

To define each locality, five climatic variables were used. These variables were: TMAX, average of maximum temperatures in the warmest month ($^{\circ}\text{C}$); PA, annual precipitation (mm); TMIN, average of minimum temperatures in the coldest month ($^{\circ}\text{C}$); PAR, arid period (counting those months with $P < 2\text{TMA}$) and PH, frozen period (number of months in which the average of the minimum temperatures $< 0^{\circ}\text{C}$). The RegBio factor was also used, with two levels (Eurosiberian and Mediterranean). The data of the macroclimate variables were taken from SIGA (Information System, Ministerio de Agricultura, Spain) selecting the climatic station closet to the sampling point.

Field and laboratory protocol

The samples were collected in spring and autumn during the last four years. On those localities where the collection was no possible, we used herbarium samples estimating the fresh weight through linear regression from our empiric data. Because the saxicolous ferns distribution is

not random, the collection of the individuals was carried out in a systematic way. In each location, three well-developed fronds from at least three individuals, were randomly selected (fronds non-developed, in state of senescence or sick were never selected). The number of individuals and fronds used is adjusted to the protocol proposed by Pérez et al. (2013).

Considering that the interest of this work is focused in analyse the variations in frond traits related to productivity, sexual structures (sporangia and spores) were removed before weighting.

The measurements of the variables were done following the standard methods previously established (Pérez et al. 2013). In the field, the measurements of fresh weight were done as far as it was possible. If it was not, the frond was placed with a wet paper in plastic bags trying to minimize the possible water lose. In the laboratory, fronds were placed in a fridge no more than 24 hours before carrying out the process. After that, the fronds were scanned in order to obtain its area with the software ImageJ (Rasband, 2007). To carry out the measurements of dry weight, fronds were placed in a heater during 48 hours at 70°C - 80°C . The measurements of dry and fresh weight were done with a scale with 0,001g of precision.

Statistical treatment

In order to carry out the normality analysis of the variables, the test of Lilliefors (Kolmogorov-Smirnov) was used. The functional traits SLA, LDMC and LT for the group of the saxicolous ferns did not follow a normal distribution (p -value $< 0,05$), and they did not present homogeneity of variances either, neither before nor after realizing the pertinent transformations. For this reason, in order to carry out the comparison of the averages in the three functional traits between both regions, the statistical test U Mann-Whitney was used (one for each trait). However, all the variables followed

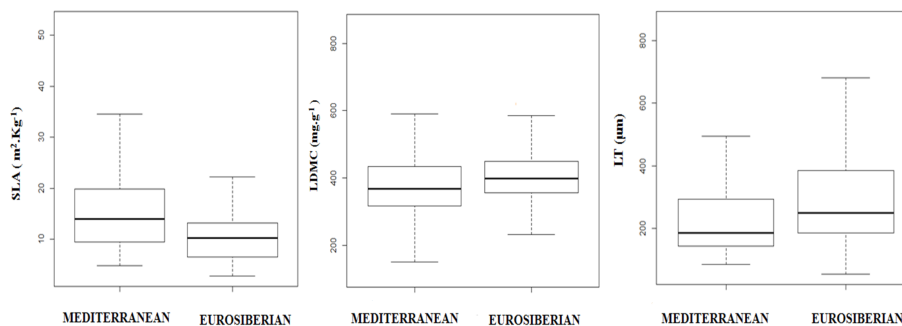


Figure 2. Box-and-whisker plots for SLA Specific leaf area), LDMC (Leaf dry-matter content) and LT (Leaf thickness) of saxicolous ferns' fronds in both Iberian bioclimatic regions. Each graphic show the average value in both regions of one trait. There are significant differences in the three traits between both regions. The levels of significance have been determined by the U test. **Figura 2.** Diagramas de cajas para SLA (Specific leaf area), LDMC (Leaf dry-matter content) y LT (Leaf thickness) de las frondas de helechos rupícolas en las dos regiones bioclimáticas de la Península Ibérica. Cada grafico muestra el valor medio en ambas regiones de un rasgo. Existen diferencias significativas en las tres variables entre las dos regiones. Los niveles de significación han sido determinados mediante el test de la U.

a normal distribution in an intraspecific level, so, to compare the averages of the traits between both regions by the species a T test was used.

To establish the existing relations between the three functional traits and the climatic variables selected, general linear models (GLMs) were carried out with Gaussian's errors distribution and with "identity" vinculum function. These relations were established in a specific level, realizing three GLMs for each species (one for each functional trait selected), so 12 models were generated.

The models were generated as follows: firstly, the effect of the factor RegBio (bioclimatic region) was only taken into account among each of the functional traits in each species. After determining if the effect of that factor was or not significant, five climatic variables were introduced. In some cases, the variable RegBio was not significant, so its effects were now explained by the climatic variables considered. After that, we applied the backward method, so the model was simplified deleting those variables that did not present significant effect in the traits. In some cases (in order to get more parsimonious models) those variables with a significant effect but whose inclusion in the model didn't increase substantially the amount of deviance (D^2), which explains the percentage of the variability in the data that are explained by the model, were eliminated. The statistical analysis was performed in RStudio (R Core Team, 2013).

Results

Functional traits characterization

As it can be observed in figure 2 and table

2, saxicolous ferns in the Mediterranean region presented, as a whole, an average of higher SLA ($15,5 \text{ m}^2\cdot\text{Kg}^{-1}$) than the Eurosiberian populations and, consequently, an average lower values in the other two functional traits associated with more conservative strategies i.e, LDMC and LT, whose average values were, respectively, $361,7 \text{ mg}\cdot\text{g}^{-1}$ and $221,83 \text{ }\mu\text{m}$. The differences between the average values of the three functional traits were significant between regions.

Intraspecific differences of the functional traits between both bioclimatic regions

In Table 3 shows the average and the standard error of the functional traits in each species in each region is shown, as well as the p-value of the differences in the average between both regions. All the species except *A. adiantum-nigrum*, presented a significant higher average of SLA under the Mediterranean macroclimate. All the species showed a higher average LT value under the conditions of Eurosiberian bioclimate. Only *A. billotii* and *A. ceterach* presented significant differences in the average LDMC value between both bioclimatic regions, being the average value of this trait significant higher in the Eurosiberian region in both cases (tab. 3).

Effects of the climatic variables over the functional traits in the fronds of saxicolous ferns of the genus *Asplenium* in the Iberian Peninsula

The models obtained for each of the functional traits in each species, as well as the determination coefficient for each model, are shown in the Appendix II.

Functional trait	Eurosiberian N= 166	Mediterranean N= 204	P-value
SLA (m ² .Kg ⁻¹)	10,9 ± 1	15,67 ± 6,8	< 0,001 (***)
LDMC (mg.g ⁻¹)	416.1± 17,2	361,7 ± 98,1	0,0038 (**)
LT (μm)	296,9 ± 24,4	221,83 ± 96,2	< 0,001(***)

Table 2. Average values and standard error for each functional trait, SLA (Specific leaf area), LDMC (Leaf dry-matter content) y LT (Leaf thickness), of the fronds in saxicolous ferns in each bioclimatic region. N is referring to the number of fronds. It is shown the p-value of the differences in the averages in each functional trait between regions. The levels of significance has been determined by the U test. P-value < 0,05 (*); P-value < 0,01 (**); P-value > 0,001 (***). **Tabla 2.** Valores medios y error estándar para cada rasgo funcional: SLA (Specific leaf area), LDMC (Leaf dry-matter content) y LT (Leaf thickness) de las frondas de helechos con hábito rupícola en cada región bioclimática. N es el número de frondas. Se muestra el p-valor de las diferencias en las medias de cada rasgo funcional entre regiones. Los niveles de significación han sido determinados con el test de la U. P-valor < 0,05 (*); P-valor < 0,01(**); P-valor < 0,001(***).

In the models for the variable SLA of the four species, the variable TMAX presented a significant positive effect (p-value < 0,001***). This implies that an increase on the variable TMAX generates an increase on the variable SLA in every species. The variable TMAX was the only one that exerted positive effects on SLA in three of the four species. In *A. adiantum-nigrum* the variable TMIN also had a significant positive effect (p-value < 0,001***).

Regarding LDMC, some differences in the climatic variables were found, which showed significant effects in each species. In *A. adiantum-nigrum* the PH (frozen period) showed a significant positive effect on LDMC (p-value < 0,001***). Two climatic variables had significant effects on the functional trait LDMC in *A. billotii*, those variables were the bioclimatic region and the TMIN. The first one showed a significant positive effect, and the second one a significant negative effect (p-value RegBio < 0,001***; p-value TMIN < 0,05*). In *A. ceterach*, the variable TMAX was the only one with a significant negative effect on the LDMC and, in *A. trichomanes*, none of the climatic variables had a significant effect on LDMC.

Finally, the variable TMAX had a significant negative effect on the variable LT in *A. adiantum-nigrum* (p-value < 0,001***) and in *A. billotii* (p-value < 0,05*). In *A. trichomanes* the variable PAR (arid period) was the one that had a significant negative effect and, by last, in *A. ceterach* the variable RegBio was the only one with a significant positive effect.

Discussion

The environmental conditions under the Mediterranean macroclimate are more hostile comparing to Eurosiberian macroclimate. The functional traits of the species change under

different environmental conditions and is expected that in those places with more nutrient availability plants will adopt a more productive (Chapin, 2010), it is to say that the present higher SLA values. For that, and considering that ferns are a group of organisms that depend strongly on the environmental and edaphic water availability and humidity (Page, 2002; Tyron, 1964), our initial hypothesis was that under Mediterranean condition ferns would adopt more conservative strategies, because, as it has been commented, the lack of rains during the arid period is one of the factors that affects the most to the production in plants, even a lot of them present as adaptive strategy the foliar abscission, whose trigger is the lack of precipitations (Lasko, 1985).

This pattern has been observed before in other studies of terrestrial plants, observing a decrease in the growth rate and, so that, in the foliar area under water stress conditions (Casper et al. 2001). This statement seems logical, as the lack of water reduces the photosynthetic rate per area unit and, finally, the foliar area (McCree, 1986), variable that is used in the calculation of SLA. However, the pattern observed is the opposite that the one that in first instance thought, this is to say that the saxicolous ferns in the Mediterranean region (in set) show a significant higher SLA average value and significant lower values of LDMC and LT. It is true that the treatment in set of the species generates a lot of variance, considering that the foliar structure affects to the value of the functional traits of the leaves and, those, depends on the species (Poorter & Bongers, 2006). However, the characterization of the fronds in a specific level has shown similar results. All the species except *A. adiantum-nigrum* presented a significant higher SLA value in Mediterranean zones. Likewise, all the species presented an average value significant higher of LT in Eurosiberian zones, although

<i>A. adiantum-nigrum</i>	Eurosiberian N= 46	Mediterranean N=19	P-value
SLA (m ² .Kg ⁻¹)	12,25 ± 0,67	14,75 ± 1,29	0,1 nsig
LDMC (mg.g ⁻¹)	390,77 ± 22,3	367,16 ± 9,10	0,3 nsig
LT (μm)	195,36 ± 10,9	248,47 ± 7,6	< 0,001***
<i>A. billotii</i>	Eurosiberian N= 23	Mediterranean N= 46	P-value
SLA (m ² .Kg ⁻¹)	13,9 ± 2,3	22,6 ± 2,8	< 0,001***
LDMC (mg.g ⁻¹)	589,62 ± 63,6	319,32 ± 35	< 0,001***
LT (μm)	168,7 ± 13,1	150,6 ± 35,2	0,0092**
<i>A. trichomanes</i>	Eurosiberian N= 58	Mediterranean N= 77	P-value
SLA (m ² .Kg ⁻¹)	11,42 ± 0,61	16,7 ± 0,53	< 0,001***
LDMC (mg.g ⁻¹)	423,27 ± 35,62	416,60 ± 40,16	0,62 nsig
LT (μm)	237,7 ± 8,61	155,9 ± 4,01	< 0,001***
<i>A. ceterach</i>	Eurosiberian N= 43	Mediterranean N= 58	P-value
SLA (m ² .Kg ⁻¹)	5,6 ± 0,25	8,79 ± 0,46	< 0,001***
LDMC (mg.g ⁻¹)	395,59 ± 32,4	351,3 ± 36	< 0,05*
LT (μm)	500,19 ± 15,9	356,81 ± 8,4	< 0,001**

Table 3. Average value and standard error in the frond functional traits, SLA (Specific leaf area), LDMC (Leaf dry-matter content) y LT (Leaf thickness), for each species: *Asplenium adiantum-nigrum*, *A. billotii*, *A. trichomanes* y *A. ceterach*, under both macroclimate conditions. N is referring to the number of fronds. The levels of significance have been determined by a T test. P-value < 0,05 (*); P-value > 0,01 (**); P-value 0,001 (***). **Tabla 3.** Valor medio y error estándar de los rasgos funcionales de las frondas: SLA (Specific leaf area), LDMC (Leaf dry-matter content) y LT (Leaf thickness) para cada especie: *Asplenium adiantum-nigrum*, *A. billotii*, *A. trichomanes* y *A. ceterach* bajo las dos condiciones macroclimáticas. N es el número de frondas. Los niveles de significación han sido determinados mediante test de la T. P-valor < 0,05 (*); P-valor < 0,01(**); P-valor < 0,001(***).

only *A. billotii* and *A. ceterach* showed significant differences in the LDMC average value between both regions. Making a comparison between species it is remarkable that LT value in *A. ceterach* is quite superior in both regions above the other species. This could be because of the structures and characteristics of its fronds, which are more coriaceous and thick than the fronds of the rest of the species selected in this study (Salvo, 1990).

The fact that finally these results are the opposite to the established at the beginning could be explained by the physical characteristics of the rock habitat. Many authors have classified these habitats as deserts. This fact is explained by the habitat structural itself (Larson et al. 2005): the verticality of the habitat and, in part as a

consequence of this, the absence of soil. In these habitats the water availability is low, even though the surrounding terrain has high rainfall availability, as it is the case of the Eurosiberian localities in our work (Nuzzo, 1996). This could explain the fact that in this study the variable PA (annual precipitation) did not exerted significant values on any of the functional traits of any of the species. It is true that water is the main limiting in terrestrial plants productions. This habitat is typically more horizontal, and with well-developed soil, where water can accumulate by infiltration and so, the plants have access to this resource during a more prolonged period (Larson et al. 2005; Terradas, 2001). However, it seems that it is not essential in species with a saxicolous way of life, which can be explained by the two

aspects commented previously. This means that, even there are differences in the PA between each region because of the lack of soil and verticality, the differences in the annual precipitation between bioclimatic regions will be annulled in the rock habitats.

When we analyzed the effects of the macroclimatic variables in the functional traits of the fronds, we could see that the TMAX (average of the maximum temperatures in the warmest month) is one of the factors which allows to explain the best the differences in SLA between both regions, which has been significant detected in all the species except *A. adiantum-nigrum*. This result is unexpected, because we started with the previous hypothesis that the average of the maximum temperatures in the warmest month reduced the SLA. In consequence, saxicolous ferns show a foliar strategy routed to an increase of the temperatures, until a certain limit established around 50-60°C (Larson et al. 2005; Terradas, 2001).

Referring to the factors related to cold, although it is an accepted fact that the minimum temperature of the coolest months behaves as limiting in the productivity of all the plant organs (Terradas, 2001), our results doesn't seem to indicate this tendency in general. It only seems to exist a limitation by the TMIN in the cases of *A. adiantum-nigrum* (an increase in the TMIN increases the SLA value) and *A. billotii* (an increase of TMIN decreases the LDMC).

The variable PAR (arid period) doesn't show significant effects in any of the variables except in *A. ceterach* LT, exerting a negative effect on its value. In some studies, it has been observed that the variable SLA increases in some species after the arid period, which can be interpreted as an adaptive strategy of the plants to xeric habitats, which seems to be determined genetically, but we didn't observed these results in this study (Asplémier & Leuchner, 2006).

In some models, the variable LDMC in *A. billotii* and the variable LT of *A. ceterach*, the variable RegBio presented significant effect, this implies that other variables related to the bioclimatic region, which has not been taken into account, exist and would present significant effect over these variables.

Conclusions

Saxicolous ferns exposed to Mediterranean macroclimate conditions show higher SLA values than those in Eurosiberian localities. This

phenomenon is different with other groups of plants and/or habitats.

Saxicolous ferns located in Eurosiberian bioclimatic zones, present higher values in the most conservative traits, especially in which is related to LT.

PA does not affect significantly to the functional traits in saxicolous ferns, this fact could be explained by two fundamental characteristics of the structure in these habitats: the absence of soil and verticality.

Those climate traits related to temperature (especially TMAX) are the ones with a strongest effect on the functional traits in saxicolous ferns, specifically is positively correlated to SLA. This fact could be explained because the species are adapted to deal with high insolation and higher values of temperature.

The rest of the extern climate variables (PAR, PH and TMIN) didn't have significant effects on the functional traits in saxicolous ferns.

To sum up, we can conclude that the foliar strategy in saxicolous ferns seems to be independent from the rainfalls, while the maximum temperature in the warmest month favours the foliar productivity. Because of this, the individuals under Mediterranean conditions take advantage from both circumstances, being able to exhibit a greater foliar productivity.

Appendices

I- List of biological material, ordered by species, province and localities, including basic voucher information.

Asplenium adiantum-nigrum L.

ASTURIAS: Lluarca, *Gabriel y Galán* 03/2015, MACB 107525 (inds. 3-7); Tarna, *Gabriel y Galán* 04/2015, MACB 107547 (inds. 8-12). ÁVILA: Navalperal de Tormes, *Gabriel y Galán* 02/2017, MACB 109651 (ind. 20). CÁCERES: Losar de la Vera, *Gabriel y Galán* 11/2014, MACB 107235 (ind. 1). CANTABRIA: Cóbreces, *Gabriel y Galán* 03/2015, MACB 109661(ind. 2). GUADALAJARA: Corduente, *Gabriel y Galán* 09/2015, MACB 108094 (inds. 13, 14). MADRID: Cercedilla, *Gabriel y Galán et al* 09/2015, MACB 109662 (ind. 15).

Asplenium billotii F.W. Schultz

ALMERÍA: Níjar, *Herrero et al* 06/94, MACB 72179 (ind. 37*); Níjar, *Almaraz & Medina* 04/95, MACB 72162 (ind. 40*). ASTURIAS: Lluarca, *Gabriel y Galán* 03/2015, MACB 108098 (ind. 6). CÁCERES: Losar de la Vera, *Gabriel y Galán* 11/2014, MACB 107236 (ind. 1, 2); Tornavacas, *Gabriel y Galán* 02/2016, MACB 109650 (inds. 34, 35). CÁDIZ: Algeciras, *Herrero, Pangua & Prada* 06/94, MACB 72150 (ind. 38*); Algeciras, *Herrero, Pajarón, Pangua & Prada* 04/95, MACB 72157 (ind.

41*); Algeciras, *Herrero* 03/96, MACB 72151 (ind. 39*); Jerez de la Frontera, *Galiano & Ramos* 05/1989, MACB 32225 (ind. 36*). MADRID: Torrelodones, *Gabriel y Galán* 04/2012, MACB 104525 (inds. 7-9); Torrelodones, *Gabriel y Galán & Gómez Undiano* 11/2014, MACB 107239 (inds. 3-5). PONTEVEDRA: Arousa, *Lavilla* 11/2015, MACB 109663(inds. 28-33).

***Asplenium ceterach* L.**

ALICANTE: Beniarrés, *Ibars et al* 04/91, MACB 43425 (inds. 22, 23). ALMERÍA: entre Félix y Énix, *Castillo* 04/86, MACB 31084 (ind. 19). ASTURIAS: Poncebos, *Gabriel y Galán* 07/2015, MACB 107953 (inds. 4, 5, 8, 9). BARCELONA: Villarana, *Carrillo & Ninot* 03/85, MACB 18866 (ind. 20*). CÁCERES: Villanueva de la Vera, *Gabriel y Galán* 11/2014, MACB 107234 (ind. 1). CANTABRIA: Barros, *Molino* 09/2015, MACB 109664 (inds. 11, 13). CASTELLÓN: Puebla de Benifassar, *Ibars & Balaguer* 09/86, MACB 31078 (ind. 21). GRANADA: Vélez de Benaudalla, *Molero et al* 11/87, MACB 30642 (ind. 25). GUIPÚZCOA: Arantzazu, *García et al* 10/91, MACB 43414 (ind. 31*); Ataun, *Aizpuru & Catalán* 10/87, MACB 31111 (ind. 30*). HUESCA: Bielsa, *Ibars et al* 09/86, MACB 31086 (ind. 28). ISLAS BALEARES (MII): pr Estellencs, *Gabriel y Galán* 02/16, MACB 108370 (ind. 32, 33). LA CORUÑA: La Castellana, *Dalda* 06/68, MACB 1326 (ind. 26). MADRID: Cercedilla, *Sirvent & Seral* 11/2015, MACB 109665 (ind. 10, 18); Montejo de la Sierra, *Molino & de la Fuente* 10/15, MACB 109666 (ind. 17); Torrelodones, *Gabriel y Galán & Undiano* 11/2014, MACB 107240 (ind. 2, 6, 7, 15). NAVARRA: Garaioa, *Rodríguez & Herrera* 09/93, MACB 59106 (ind. 29). ORENSE: Rubiá, *Amigo & Romero* 03/92, MACB 59153 (ind. 27*). VALENCIA: Buñol, *Ibars et al* 10/86, MACB 31145 (ind. 24).

***Asplenium trichomanes* L.**

ASTURIAS: Asiego, *Gabriel y Galán* 07/15, MACB 107950 (ind. 13); Inguanzo, *Gabriel y Galán* 07/15, MACB 107952 (ind. 10); Poncebos, *Gabriel y Galán* 07/15, MACB 107951 (ind. 11, 12); Rioseco, *Gabriel y Galán* 04/15, MACB 107546 (ind. 4-7). ÁVILA: Ortigosa del Tormes, *Gabriel y Galán* 02/17, MACB 109652 (ind. 43). CÁCERES: Tornavacas, *Gabriel y Galán* 02/16, MACB 109649 (ind. 42); Villanueva de la Vera, *Gabriel y Galán* 11/14, MACB 107232 (ind. 1). CANTABRIA: Barros, *Molino* 09/15, MACB 109667 (ind. 20, 21, 35). GUADALAJARA: Torete, *Gabriel y Galán* 09/15, MACB 108095 (ind. 17). LÉRIDA: Arrós, *Gabriel y Galán* 08/15, MACB 107949 (ind. 14-16). MADRID: Cercedilla, *Sirvent & Seral* 09/15, MACB 109668 (ind. 18, 19, 40, 41); Montejo de la Sierra, *Molino & de la Fuente* 10/15, MACB 109669 (ind. 36-39); Torrelodones, *Gómez Undiano & Gabriel y Galán* 11/14, MACB 107238 (ind. 2, 3, 8, 9, 22, 25, 27, 32, 33).

II- Estimated models for the relation between climatic variables and functional variables for each species.

These models show the relation between functional traits (SLA, LDMC and LT) and climate variables (PA,

PAR, PH, TMIN, TMAX and RegBio) in each species. Also, it is shown the determination coefficient D^2 for each model, which represents the percentage of the data variability that the model is able to explain. (*) The model for LDMC in *A. trichomanes* is not shown because any of the variables had significant effects.

Asplenium adiantum-nigrum

SLA = $-10,33 + 0,5126$ TMIN + 0,6 TMAX; $D^2 = 21\%$
LDMC = $251,95 + 19,09$ PH; $D^2 = 31\%$
LT = $453,011 - 8,91$ TMAX; $D^2 = 19\%$

Asplenium billotii

SLA = $-27,22 + 1,56$ TMAX; $D^2 = 41\%$
LDMC = $459,89 + 295,45$ RegiBio- 40,24 TMIN;
 $D^2 = 50\%$
LT = $345,52 - 5,8^*$ TMAX; $D^2 = 10\%$

Asplenium ceterach

SLA = $-6.82120 + 0.51$ TMAX; $D^2 = 48\%$
LDMC = $498.475 - 4,8$ TMAX; $D^2 = 10\%$
LT = $213.44 + 143.38$ Regbio; $D^2 = 40\%$

Asplenium trichomanes

SLA = $-1,74 + 0,58$ TMAX; $D^2 = 21\%$
LDMC = (*)
LT = $249.531 - 34.105$ PAR; $D^2 = 29\%$

References

- Aspelmeier, S. & Leuschner, C. (2006). Genotypic variation in drought response of silver birch (*Betula pendula* Roth): leaf and root morphology and carbon partitioning. *Trees* 20: 42-52.
- Casper, B.B., Forseth, I.N., Kempenich, H., Seltzer, S. & Xavier, K. (2001). Drought prolongs leaf life span in the herbaceous desert perennial *Cryptantha flava*. *Functional Ecology* 15: 740-47.
- Chapin, F.S., Carpenter, S.R., Kofinas, G.P., Folke, C., Abel, N., Clark, W.C., Olsson, P., Stafford, D.M., Smith, B.H., Young, O.R., Berkes, F., Biggs, R., Grove, J.M., Naylor, R.L., Pinkerton, E., Steffen, W. & Swanson, F.J. (2010). Ecosystem stewardship: sustainability strategies for a rapidly changing planet. *Trend in Ecology and Evolution* 25: 241-249.
- Chen, F.M., Niklas, K.J. & Zeng, D.H. (2011). Important foliar traits depend on species-grouping: analysis of a remnant temperate forest at the Keerqin Sandy Lands, China. *Plant and Soil* 340:337-345.
- Ferrer, D. & Vetaas, O.R. (2005). Pteridophyte richness, climate and topography in the Iberian Peninsula: comparing spatial and nonspatial models of richness patterns. *Global Ecology and Biogeography* 14: 155-165.
- Galmés, J., Medrano, H. & Flexas, J. (2007). Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. *New Phytologist* 175: 81-93.
- Galmés, J., Ochogavía, J.M., Gavo, J., Roldán, E. J., Cifre, J. & Conesa, M.A. (2013). Leaf responses to drought stress in Mediterranean accessions of

- Solanum lycopersicum*: anatomical adaptations in relation to gas exchange parameters. *Plant, Cell & Environment* 36: 920-935.
- Garnier, E., Cortez, J., Billès, G., Navas, M.L., Roumet, C., Debussche, M., Laurent, G., Blanchard, A., Aubry, D., Bellmann, A., Neill, C. & Toussaint, J.P. (2004). Plant functional markers capture ecosystem properties during secondary succession. *Ecology* 85: 2630–2637.
- Given, D.R. (1993). Changing Aspects of Endemism and Endangerment in Pteridophyta. *Journal of Biogeography* 20: 293-302.
- Grime, J.P., Thompson, K., Hodgson, J.G., Cornelissen, J.G., Rorison, I.H., Hendry, G.A.F., Ashenden, T.W. & Askew, A.P. (1997). Integrate Screening Validates Primary Axes of Specialisation in Plants. *Nordic Society Oikos* 79: 259-281.
- Joffre, R., Ramal, S. & Damesin, C. (1999). Functional attributes in Mediterranean-type ecosystems. In: F. I. Pugnaire & F. Valladares (eds.), *Handbook of Functional Plant Ecology*: 347–380. New York.
- Lakso, A.N.. (1985). The effects of water stress on physiological processes in fruit crop. *Acta Horticulturae* 171: 275-289.
- Larson, D.W., Matthes, U. & Kelly, P.E. (2005). *Cliff Ecology*. Birks, H.J.B. & Wiens, J.A (Eds.) Cambridge University Press, New York.
- McCree, K.J (1986). Whole plant carbon balance during osmotic adjustment to drought and salinity stress. *Australian Journal of Plant Physiology* 13: 33-44.
- Mehltreter, K., Walker, L.R. & Sharpe, J.M. (2010). *Fern ecology*. Cambridge University Press, Cambridge.
- Moreno, J.C. & Lobo, L.M. (2008). Iberian–Balearic fern regions and their explanatory variables. *Plant Ecology* 198: 149-167.
- Nuzzo, V. (1996). Structure of cliff vegetation on exposed cliffs and the effect of rock climbing. *Canadian Journal of Botany* 74: 607-617.
- Page, C.N. (2002). Ecological strategies in fern evolution: a neopteridological overview. *Review of Palaeobotany and Palynology* 119: 1-33.
- Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, K., Craine, J.M., Gurvich, D.E., Urcelay, C., Veneklaas, E.J., Reich, P.B., Poorter, L., Wright, I.J., Ray, P., Enrico, L., Pausas, J.G., De Vos, A.C., Buchmann, N., Funes, G., Quiétier, F., Hodgson, J.G., Thompson, K., Morgan, H. D, Ter Steege, H., Van De Heijden, M.G.A, Sack, L., Blonder, B., Poschold, P., Vaieretti, M.V., Conti, G., Staver, A.C., Aquino, A. & Cornelissen, J.H.C. (2013). New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* 61: 167-234.
- Poorter, L. & Bongers, F. (2006). Leaf traits are good predictors of plant performance across 53 rain forest species. *Ecology* 87: 1733–1743.
- Poorter, H. & De Jong, R. (1999). A comparison of specific leaf area, chemical composition and leaf construction cost of field plants from 15 habitats differing in productivity. *New Phytologist* 143: 163-76.
- R CORE TEAM (2013-R): *A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Rasband, W.S. (2007). ImageJ, US National Institutes of Health, Bethesda, Maryland, USA.
- Reich, P.B., Walters, M.B. & Ellsworth, D.S. (1997). From tropics to tundra: Global convergence in plant functioning. *Proceedings of the National Academy of Sciences of the United States of America* 94: 13730–13734.
- Rivas-Martinez, S. (1987) Memoria del mapa de Series de Vegetación de España. I.C.O.N.A. Serie Técnica. Publ. Ministerio Agricultura, Pesca y Alimentación. Madrid.
- Salvo, E. (1990). *Guía de helechos de la Península Ibérica y Baleares*. Ediciones pirámide. Madrid.
- Terradas, J. (2001). *Ecología de la vegetación: de la ecofisiología de las plantas a la dinámica de comunidades y paisajes*. Terradas, J. (Ed). Ediciones Omega, Barcelona.
- Tyron, R.M. (1964). Evolution in the leaf of living ferns. *Memoirs of Torrey Botanical Club* 21: 73-82.
- Vasheka, O., Puglèlli, G., Crescente, M.F., Varone, L. & Gratani, L. (2017) Anatomical and morphological leaf traits of three evergreen ferns (*Polystichum setiferum*, *Polypodium interjectum* and *Asplenium scolopendrium*). *American Fern Journal* 4: 258-268.
- Vile, D., Garnier, R., Shipley, B., Laurent, G., Navas, M.L., Roumet, C., Lavorel, S., Díaz, S., Hodgson, J.G, Lloret, F., Midgley, G.F., Poorter, H., Rutherford, M.C., Wilson, P.J., Wright, I.J. (2005). Specific leaf area and dry matter content estimate thickness in laminar leaves. *Annals of Botany* 96: 1129-1136.
- Violle, C., Navas, M.L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. & Garnier, E. (2007). Le concept of trait be funtional! *Oikos* 116: 882-892.
- Wilson, P. J., Thompson, K.E.N. & Hodgson, J.G. (1999). Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. *New Phytologist* 143:155-162.
- Went, F. W. (1950). The response of plants to climate. *Science* 112: 489-494.