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What factors influence the occurrence of the genus *Degelia* (a threatened lichen) in central Spain?



I. MARTÍNEZ*, T. FLORES, G. ARAGÓN, M.A.G. OTÁLORA,
M. RUBIO-SALCEDO

Área de Biodiversidad y Conservación, ESCET, Universidad Rey Juan Carlos, c/ Tulipán s/n, 289337 Móstoles,
Madrid, Spain

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ABSTRACT

The main factors determining the occurrence, abundance, growth and reproductive capacity of threatened lichens need to be known in order to implement conservation initiatives. The main objective of the present study was to evaluate which factors affect the conservation status of *Degelia* species-complex in central Spain. We undertook a non-parametric analysis to evaluate whether population sizes are larger in protected areas. We fitted generalized linear models (GLM) and mixed models (GLMMs) to analyze which variables are driving occurrence and abundance of *Degelia*, the thallus size and reproduction capacity. Results show that population sizes of *Degelia* are very variable, being larger in broad-leaved evergreen forests and inside protected areas. Tree characteristics play an important role in its local dynamics.

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Introduction

Biodiversity conservation is a way to generate a holistic view of nature and so stop perceiving it as a set of individual components (Primack and Ros, 2002). Currently, numerous threats are affecting biodiversity worldwide, but most conservation programs have been directed at birds, mammals or plants, while other organisms are being overlooked (IUCN, 2012). Despite the importance and value of epiphytic lichens in many forest ecosystems (Carroll, 1979; Hayward and Rosentreter, 1994; Petterson et al., 1995), both in terms of species diversity and of ecological function (Longton, 1992; Dix and Webster, 1995), they are often overlooked in determining conservation biology priorities.

One of the major threats to biodiversity is forest fragmentation and forest degradation. It affects the survival and conservation of species worldwide (Debinski and Holt, 2000; Fahrig, 2003), and therefore nowadays is a priority for Conservation Biology (Young and Clarke, 2000). Lichen-rich habitats, such as forests are being destroyed and the number of rare and extinct species has increased in recent decades (Martínez et al., 2012; Scheidegger and Werth, 2009).

Degelia is a threatened genus of epiphytic lichen species in central Spain (Martínez et al., 2003; Otálora et al., 2013). The current taxonomic treatment of *Degelia* species-complex in Europe recognizes four species (Blom and Lindblom, 2010): *Degelia atlantica*, *D. cyanoloma*, *D. ligulata* and *D. plumbea*. In Spain, *D. atlantica* and *D. plumbea* are the two species of this

* Corresponding author. Tel.: +34 914887183; fax: +34916647490.

E-mail address: isabel.martinez@urjc.es (I. Martínez).

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genus which are better represented (Martínez et al., 2003; Otálora et al., 2013), although *D. cyanoloma* has been recently recorded in some western localities (Blom and Lindblom, 2010). *D. plumbea* populations are distributed primarily in Mediterranean-Atlantic regions of Africa, Europe (Portugal to Norway and the Crimea), the Macaronesian islands and North Eastern America (COSEWIC, 2010). *D. plumbea* produces numerous sexual structures (apothecia) while *D. atlantica* seldom forms these structures but forms isidia. In central Spain, the genus *Degelia* is found in a very limited number of localities, specifically in humid valleys and ravines, and within well-preserved forests ranging from 750 to 1 280 m altitude (Carballal et al., 2010). The species are listed as vulnerable in the central Iberian Peninsula (Martínez et al., 2003). The main threats are forest fragmentation, logging and other forest management activities, drought, overgrazing, agricultural, recreational and environmental pollution (Martínez et al., 2003). This genus occurs typically in *Lobarion pulmonariae* Oschner (Barkman, 1958; Rose, 1988) communities, and it is highly susceptible to high incidence of light (Gauslaa and Solhaug, 1996) and the frequency and duration of dehydration events. In addition, *Degelia* distribution can also be limited by the availability of appropriate substrate, pollution and others factors related to dispersal (Gauslaa and Solhaug, 1998; Richardson and Cameron, 2004; COSEWIC, 2010).

In a previous population genetics study in central Spain, Otálora et al. (2013) found high levels of genetic similarity between *D. plumbea* and the inland *D. atlantica*, suggesting that probably *D. atlantica* and *D. plumbea* do not correspond to two distinct species and an urgent study of species boundaries of this species-complex was needed (Otálora et al. in prep.). Thus, the main objective of the present study was to assess the conservation status of populations of the *Degelia* species-complex (named “*Degelia*” elsewhere in the document) and to study the factors affecting its population size as the performance of individuals in central Spain. Our hypothesis was that the occurrence and abundance of *Degelia* would be higher inside protected areas and in more humid places and that the reproductive capacity would be related to thallus size and microclimatic conditions. We examined: (1) which environmental variables determine occurrence and abundance of *Degelia*; (2) which microhabitat factors are affecting size and reproductive capacity of *Degelia*; and (3) differences among population sizes within and outside protected areas in the study area.

Methods

Study area

The study area was located in central Spain and comprised a vast and mountainous region covered with different types of Mediterranean forests (*Quercus faginea*, *Q. ilex* subsp. *ballota*, *Q. pyrenaica*, *Fagus sylvatica*, *Pinus* spp. are the tree species which mainly constitute forests in Central Spain) and scrublands covering an area of 79.409 km², representing almost 15 % of the total Iberian Peninsula. The local climate is continental Mediterranean with an average annual temperature of 14.9 °C and annual rainfall between 400 and 600 mm (Government of

Castilla-La Mancha, www.jccm.es), although in mountainous places climate conditions are characterized by lower temperature and higher precipitation. We have studied the distribution of *Degelia* in this region for more than a decade. All previously reported localities of *Degelia* were visited to delimitate its populations. In addition, some areas with high probability that *Degelia* might be present were also visited (well-preserved broad-leaved evergreen forests and deciduous and semideciduous forests located in mountainous places), although there were not previous records. However, only in two of them did we find *Degelia* individuals.

A total of 15 forest patches were included in this study (Fig 1) between 734 and 1 622 m altitude, most being situated within protected areas such as National Park, Natural Parks and habitat/species management areas (Table 1). Finally, 8 forests of *Q. pyrenaica*, 4 forests of *Q. ilex* subsp. *ballota*, 1 forest of *Arbutus unedo*, and 2 mixed forests, one of *Q. pyrenaica* and *F. sylvatica*, and one with *Q. pyrenaica* and *Q. faginea* (Table 1). *Q. pyrenaica* is a semideciduous oak almost entirely restricted to the Iberian Peninsula with some isolated populations in northern Morocco and on the southwestern tip of France. *Q. ilex* subsp. *ballota* is a large evergreen oak native to the Mediterranean region. *A. unedo* is also a broad-leaved evergreen tree native to the Mediterranean region and Western Europe. *F. sylvatica* is a deciduous tree widespread throughout Western Europe, reaching in central Spain its southern distribution limit in Europe. And *Q. faginea* is a native semideciduous species of Mediterranean forest, growing in areas with Mediterranean climate of North Africa and the Iberian Peninsula.

Experimental design and data collection

In every forest a plot of 3 000 m² (30 × 100 m) was established to determine the abundance of species. Each plot was located at least 100 m from the forest edge to avoid possible effects caused by microclimatic changes associated with edge-interior gradient (Belinchón et al., 2007). To facilitate the field work, the plots were subdivided into 20 small rectangles of 10 m × 15 m. Within each plot, all trees were sampled and all individuals of *Degelia* growing at a maximum height of 2 m tree were considered. Above this height, it is very rare to find individuals of this genus in the study area. A range of abiotic and biotic variables were measured at four levels: stand, plot, tree and lichen.

Variables at the stand level were: (1) Climate variables obtained from climate model estimates for Spain CLIMOEST (Sánchez-Palomares et al., 1999) – total precipitation (mm), winter precipitation (mm), summer precipitation (mm), mean annual temperature (°C), mean maximum temperature of the warmest month (°C), mean minimum temperature of the coldest month (°C), relationship between total precipitation/mean annual temperature (P/T). (2) Geographic and topographic variables, taken *in situ* with GPS – latitude and longitude of each locality in UTM, altitude of each locality (m). (3) Structural variables – Forest type: deciduous (*Q. pyrenaica*, *Q. faginea*, *F. sylvatica*) and broad-leaved evergreen (*Q. ilex* subsp. *ballota*, *A. unedo*). (4) Protection status: 1 – protected areas, 2 – not protected areas.

Variables at the plot level: percentage of trees occupied by *Degelia* in each plot; number of individuals present in each

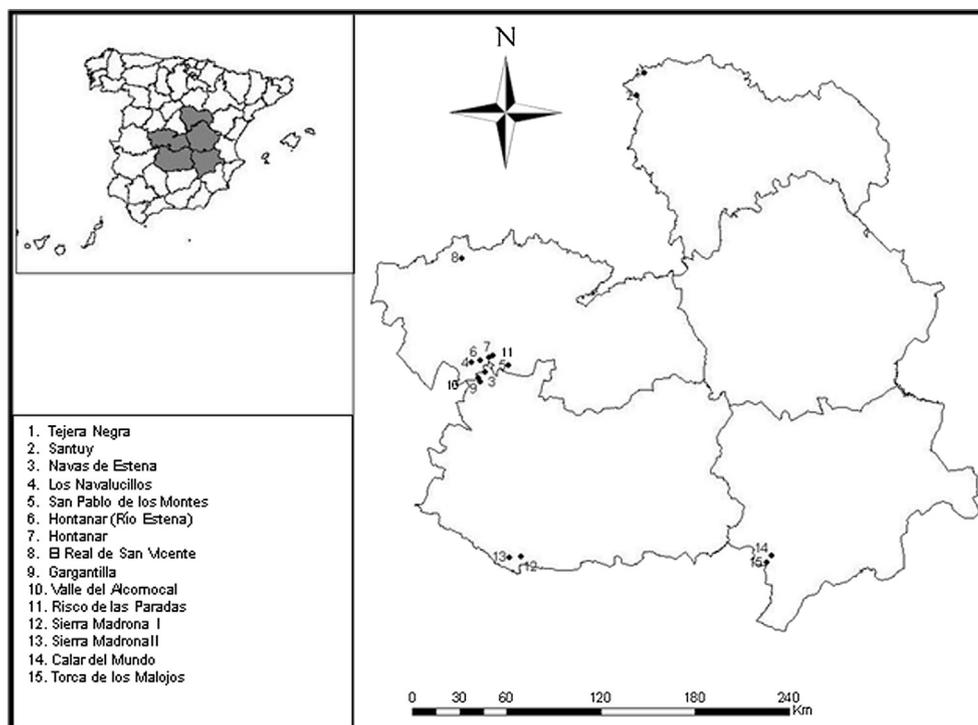


Fig 1 – Geographic situation of studied localities.

plot; mean diameter of all trees in each plot (cm); abundance of trees in each plot (expressed as total number of trees).

Variables at the tree level: presence/absence of individuals in each tree (1–0); abundance of individuals of *Degelia* in each tree (expressed as total number of individuals); diameter of each tree at breast height (cm, measured with a tape measure); inclination of each trunk (degrees, with a level measurement); bark roughness (mm, measured with a caliper).

Variables at the lichen level were: the size of each individual measuring the major diameter with a caliper (mm); presence/absence (1–0) of sexual reproduction (apothecia) and asexual (isidia) reproductive structures. To determine individual microhabitat, two variables were also recorded: height of the tree for each individual (cm); exposure of each individual in the tree ($^{\circ}$). This variable was measured with a compass to show the exact orientation of the individual over the tree. In the analysis, this variable was transformed for a continuous variable using the formula: incident radiation = $-\cos\alpha$, where α is the angle measured from the direction (between 0° and 360°).

Data analysis

In each of the levels considered we carried out a correlation analysis of the mentioned predictors, and excluded those with correlation values above 0.7 to avoid problems of multicollinearity. So, out of 19 predictor variables considered, only 10 were included in the models.

At the plot level, we analyzed which variables are driving percentage of trees occupied by *Degelia* and total abundance of

individuals in each plot. A generalized linear model (GLM) (GENMOD procedure; McCullagh and Nelder, 1989) was fitted using SAS Macro program. The independent or predictor variables in these models were the type of forest, the mean diameter of the trees at plot level (negative correlated to abundance of trees in each plot), and P/T of each locality. For the percentage of occupied trees, a binomial error was used using function “logit”; and for total abundance we normalized the variable by logarithmic function, and used the link function “identity”. At the plot level, we analyzed which variables are driving percentage of trees occupied by *Degelia* and total abundance of individuals in each plot. So, we fitted generalized linear models (GLM) (GENMOD procedure; McCullagh and Nelder, 1989) using SAS Macro program. The independent or predictor variables in these models were the type of forest, the mean diameter of the trees at plot level (negative correlated to abundance of trees in each plot), and P/T of each locality. For the percentage of occupied trees, a binomial error was used binding function “logit”; and for total abundance we normalized the variable by logarithmic function, and used the link function “identity”.

At the tree level, we analyzed the next variables: presence/absence of *Degelia* and number of individuals per tree (abundance). In these cases, generalized linear mixed models were developed (GLMMs) (GLIMMIX procedure; McCullagh and Nelder, 1989), also using SAS Macro program. This modeling approach was chosen because our data had an unbalanced and hierarchical structure with individuals nested within trees and trees nested within plots. A hierarchical data structure implies a correlation between data points at different scales. This correlation inflates degrees of

Table 1 – Geographic, topographic, structural and climatic characteristics of each studied locality. Situation: name of locality and protection status: 1 – protected areas, 2 – not protected areas. Loc: locality; Prov: province; GU: Guadalupe; TO: Toledo; CR: Ciudad Real; AB: Albacete. For: type of forest; 0: broad-leaved evergreen forest; 1: deciduous forest; Arb: tree species; Qp: *Quercus pyrenaica*, Fs: *Fagus sylvatica*, Qr: *Quercus ilex* subsp. *ballota*; Au: *Arbutus unedo*; Qj: *Quercus faginea*. N Arb: number of trees per plot; Dbh: mean diameter of trees; Roug: mean roughness of trees; Inc: mean inclination of trees; P: annual precipitation; Pwin: winter precipitation; Psum: summer precipitation; T: annual mean temperature; Tmax: mean maximum temperature of the warmest month; Tmin: mean minimum temperature of the coldest month; P/T: relationship between total precipitation/mean annual temperature.

Loc.	Prov.	Situation	X	Y	Altitude	For	Arb	N arb	Dbh	Roug	Inc	P	Pwin	Psum	T	Tmax	Tmin	P/T
1	GU	Tejera Negra ¹	468234	4563487	1 622.00	1	Qp y Fs	270	22.93	4.17	82.15	1 039.00	323.00	113.00	6.80	23.40	-3.90	152.79
2	GU	Santuy ¹	463005	4549455	1 285.00	1	Qp	355	16.01	5.96	84.46	867.00	274.00	98.00	9.10	26.30	-2.40	95.27
3	TO	Navas de Estena ¹	367254	4373576	734.00	0	Qr	166	15.20	4.71	71.91	639.00	234.00	53.00	14.20	32.60	0.90	45.00
4	TO	Los Navaluillos ¹	358574	4379559	883.00	0	Qr	261	14.63	3.52	70.30	705.00	267.00	48.00	13.00	30.30	0.30	54.23
5	TO	San Pablo de los Montes ²	381950	4377721	908.00	1	Qp	246	20.11	9.29	82.70	621.00	239.00	48.00	12.80	30.10	0.00	48.52
6	TO	Hontanar (río Estena) ²	363868	4380487	865.00	0	Qr	97	15.29	3.62	80.05	704.00	261.00	59.00	13.60	31.50	0.70	51.76
7	TO	Hontanar ²	372086	4383736	899.00	1	Qp	366	15.92	4.43	84.00	668.00	246.00	49.00	12.90	30.20	0.10	51.78
8	TO	El Real de San Vicente ²	352060	4445771	1 239.00	1	Qp	135	25.64	9.94	84.31	739.00	214.00	58.00	10.50	26.70	-1.00	70.38
9	CR	Gargantilla ¹	363584	4367215	778.00	0	Au	245	11.76	2.03	66.86	652.00	239.00	54.00	14.00	32.40	0.90	46.57
10	CR	Valle del Alcornocal ²	362757	4369364	748.00	1	Qp	224	17.39	9.56	84.43	644.00	236.00	53.00	14.20	32.50	1.00	45.35
11	CR	Risco de las Paradás ²	369194	4382836	1 165.00	1	Qp	391	11.46	9.69	79.36	756.00	286.00	54.00	11.20	27.50	-0.80	67.50
12	CR	Sierra Madrona I ¹	389760	4256065	879.00	1	Qj y Qp	56	14.56	6.63	84.20	690.00	262.00	45.00	14.10	32.20	2.00	48.94
13	CR	Sierra Madrona II ¹	382652	4255571	1 030.00	1	Qp	73	23.01	11.82	85.04	778.00	294.00	49.00	13.30	31.40	1.60	58.50
14	AB	Calar del Mundo ¹	549213	4256923	1 045.00	0	Qr	249	17.17	3.64	80.44	791.00	216.00	51.00	13.60	31.30	-1.60	58.16
15	AB	Torca de los Malojos ²	545875	4252003	1 406.00	1	Qp	46	51.43	13.07	84.85	1 023.00	396.00	77.00	11.60	29.70	0.60	88.19

freedom increasing the chance of making a type I error. To overcome these problems, we analyzed the data using a multilevel approach and, when necessary, considered trees and plot as random factors and applied mixed modeling (Verbeke and Molenberghs, 1997). For these models, we used as explanatory variables: total number of trees, P/T, tree diameter, tree inclination and bark roughness. For the presence of *Degelia*, we fitted GLMM models using the binomial estimation, and “logit” link function, and for number of individuals per tree a Poisson distribution was used, with a link function “log”.

Finally, at the lichen level we also carried out GLMM. These models were developed only with presence data. The dependent variables were: individual size, and presence/absence of sexual and asexual reproductive structures. To analyze which variables influence individual size we included as fixed factors: height and exposure of the individuals on the tree, tree diameter, inclination and tree roughness, and P/T of each plot. To study the presence/absence of sexual and asexual reproductive structures the same independent variables were introduced, excluding bark roughness but thallus size as response variable, since one of the goals was to see if individual size influences reproductive capacity. The response variable presence/absence of individuals in each tree and presence/absence of sexual and asexual reproduction variables were considered as binomial distribution, with a link function “logit”, and the abundance of individuals in each tree and the size of the individuals were defined as variables with a Poisson distribution, with a link function “log” Variables “plot” and “tree” were considered as random factors (Verbeke and Molenberghs, 1997).

The effects of random factors were analyzed using the Wald test Z-statistic, and the fixed factors were examined by the F-test. The significance of each predictor in both procedures was estimated by analyzing the standard deviation (Guisan et al., 2002), and excluded those predictors with a significance >0.05. All analyzes were performed using SAS 9.0. (SAS Institute Inc., 2001).

To determine the probability that a thallus of a given size will develop any reproductive structure, we built generalized linear models (GENMOD SAS v. 9, McCullagh and Nelder, 1989). The significance of each predictor was estimated by a deviance test (Guisan et al., 2002). Binomial error and “logit” function were assumed for the response variable. The developed models were used to estimate probability curves for each one of the two structures considered (isidia and apothecia). The relation between reproductive probability (p) and lichen size was quantified using a logistic regression of reproductive status against lichen size (Wesselingh et al., 1993, 1997) by using the formula $p = 1/(1 + e^{\mu + \alpha x})$, where parameters μ and α of the fitted logistic curve determine the intercept with the x-axis and the slope of the curve, respectively, and can be related to the threshold size of reproduction (Wesselingh et al., 1993; Méndez and Karlsson, 2004).

We compared the population size among protected areas and unprotected ones using a non-parametric Mann–Whitney test. Using the same procedure, we have also evaluated if there are significance differences in environmental variables between protected and unprotected areas.

Results

A total of 15 populations were found in Central Spain. Values of climatic, geographical and topographical variables are listed in Table 1. Besides differences in altitude, also climatic variables showed a large variation, from 621 mm to 1 039 mm of total rainfall, or between 6.80 °C and 14.20 °C of mean annual temperature. A total of 3 180 trees were sampled in the 15 forest patches considered, although number of trees per plot ranged between 46 and 391 trees. Besides, mean tree diameter oscillated between 11.46 cm and 51.43 cm. Bark roughness and tree inclination also showed a considerable range (Table 1).

Of the 3 180 trees included in the study, only 301 had *Degelia* individuals, showing only an occupied percentage of 12.68 % (Table 2). Total number of individuals was 1039, and seven populations had less than 20 individuals. The highest number of individuals was 413, 2 being the minimum number found in two populations. Mean size of *Degelia* individuals was 30.73 mm diameter, although these data varied between populations, the smallest individuals being in locality 7, and the largest ones in locality 8 (Table 2). Height and orientation of individual thalli on trees in Table 2 were divided into three height categories and four different light exposures. The highest number of individuals was at the medium height (height 2), whereas the upper height (height 3) presented a very small number of them (4.81 %). Northern and western exposures were the most favorable to presence of *Degelia*. Regarding the presence of reproductive structures, half of individuals (50.02 %) had apothecia (Table 3). However, isidia were present in a lower percentage (22.71 %). Furthermore, only 53 individuals presented simultaneously both types of reproduction. Therefore, 67.66 % of the individuals had some

type of reproductive structure, the rest being considered juvenile (without apothecia or isidia) (Tables 2 and 3).

Effects of environmental variables on reproduction and thallus size

GLM models explaining percentage of occupied trees and abundance of *Degelia* individuals at the plot level (Table 4) showed that only the type of forest influenced the abundance and percentage of occupied trees. Evergreen broad-leaved forests had a marginally higher abundance of *Degelia* individuals.

Larger and more inclined trees or those with coarser bark had a higher probability of being colonized by *Degelia*. In addition, individuals growing on more inclined trees reached higher sizes while the presence of sexual structures depended on thallus size. Thalli growing higher up a tree had a higher probability of developing isidia (asexual structures).

Climate variables were not significant in any model. The random variable plot only showed a significant effect at the tree level for the variables presence and abundance, and for isidia presence. However, the random variable tree was always significant.

Relationship between size and probability of reproduction

All the logistic models developed to find the relationship between size and reproductive capacity were highly significant (apothecia, size estimator = 2.5494, $p > 0.0001$; isidia, size estimator = 0.8922, $p < 0.0001$). Mean thallus size varied between juvenile individuals and reproductive ones. Mean size and standard deviation of juvenile individuals was 21.05 ± 13.08 mm in diameter, whereas it was

Table 2 – Variables considered at tree and individual levels. Loc: locality; N arb: number of trees occupied by *Degelia plumbea*; % Arb. ocu: percentage of trees with *D. plumbea*; N indiv: number of individuals in each locality; Mean size: mean size \pm standard deviation and minimum and maximum values between parenthesis (mm). Alt 1: no of individuals between 0 and 50 cm; Alt 2: no of individuals between 50 and 150 cm; Alt 3: no of individuals above 150 cm; N: number of individuals in Northern exposure 316°–45°; E: number of individuals in Eastern exposure 46°–135°; S: number of individuals in Southern exposure 136°–225°; O: number of individuals in Western exposure 226°–315°; Apo: number of thalli with apothecia; Isi: number of thalli with isidia; Juve: number of juvenile thalli. *: indicate populations inside a protected area.

Loc.	N arb	%Arb. ocu	N indiv	Mean size	Alt.1	Alt.2	Alt.3	N	E	S	O	Apo	Isi	Juve
1*	33	12.22	140	31.95 \pm 16.34 (2.22 \pm 124.02)	52	71	17	32	14	14	80	86	11	53
2*	1	0.28	37	23.23 \pm 14.98 (6.50 \pm 61.30)	34	3	0	0	0	8	29	0	23	14
3*	44	26.51	106	29.43 \pm 21.64 (8.57 \pm 79.37)	60	45	1	29	26	10	41	56	11	47
4*	87	33.33	413	32.48 \pm 2.17 (7.53 \pm 89.64)	184	220	9	158	157	15	83	265	47	114
5	2	0.81	2	36.61 \pm 16.19 (35.07 \pm 38.14)	1	1	0	2	0	0	0	2	0	0
6*	11	11.34	15	34.39 \pm 12.25 (22.40 \pm 53.02)	7	4	4	14	0	0	1	11	1	3
7	4	1.09	8	19.19 \pm 9.55 (8.78 \pm 42.03)	7	1	0	0	0	6	2	1	1	6
8	2	1.48	2	52.02 \pm 13.74 (40.57 \pm 63.46)	2	0	0	1	0	0	1	1	1	1
9*	39	15.92	108	34.54 \pm 14.94 (6.37 \pm 145.23)	4	94	10	34	42	8	24	50	0	58
10	6	2.68	9	26.68 \pm 8.03 (7.79 \pm 51.27)	1	8	0	3	2	0	4	2	5	2
11	3	0.77	3	38.11 \pm 12.38 (36.18 \pm 41.14)	0	3	0	3	0	0	0	3	1	0
12*	22	39.29	75	22.65 \pm 12.38 (7.14 \pm 77.20)	33	42	2	36	13	7	19	13	75	0
13*	21	28.77	63	27.76 \pm 15.98 (8.71 \pm 70.38)	22	39	2	38	9	5	11	6	56	5
14*	23	9.24	51	28.64 \pm 2.65 (8.34 \pm 79.70)	9	40	5	30	12	3	6	17	1	33
15*	3	6.52	7	31.5 \pm 23.07 (21.74 \pm 42.14)	0	7	0	6	0	0	1	7	3	0
Total	301	12.68	1 039	30.73 \pm 17.65 (2.22–145.23)	416	578	50	386	275	76	302	520	236	336

Table 3 – Percentage and number of juvenile and reproductive individuals.

	Juvenile individuals	Reproductive individuals	With apothecia	With isidia	apothecia + isidia
%	32.34	67.66	50.05	22.71	5.10
n	336	703	520	236	53

26.78 ± 13.74 mm for individuals with isidia, and 39.16 ± 17.02 mm for individuals with apothecia.

The probability that a reproductive structure will be present for a given thallus size is shown in Fig 2. As noted before the probability of having isidia is very low. Thalli of 28.86 mm had a 50 % probability of being found with apothecia and at 89.6 mm in diameter there was 100 % probability that sexual reproductive structures would be found.

Influence of protected areas

Eight of the fifteen populations of *Degelia* were located within Protected Areas. Populations outside of protected areas all had less than 15 individuals per population. Populations 3, 4 and 9 had more than 100 individuals and were located in the Cabañeros National Park, the maximal protection category in Spain. Non-parametric tests revealed significant differences in population size between protected and unprotected areas

Table 4 – Summary of the Generalized Linear Models (GLM) and Generalized Linear Mixed Models (GLMMs) examining effects of environmental factors on the occurrence and abundance of *D. plumbea* at the plot and tree level. and on size, presence of reproductive structures at the individual level. Estimator (ES): coefficient of the variable in the model; F-value: F statistic. p-value: significant value. Type of forest: (0) broad-leaved evergreen. (1) deciduous.

Level	Model	Variable	Fixed factors	Estimator	Chi-square	p-value		
Plot	GENMOD	% Occupied trees	Forest type (0)	2.0133 (1.0610)	3.60	0.0578		
			Forest type (1)	0	–	–		
			Dbh	0.0243 (0.0852)	0.08	0.7751		
			P/T	0.0078 (0.0177)	0.19	0.6594		
			Abundance	Forest type (0)	2.1112 (0.8631)	5.98	0.0144	
		Forest type (1)	0	–	–			
				Dbh	–0.0364 (0.0438)	0.69	0.4051	
				P/T	0.0255 (0.0149)	2.93	0.0869	
				Variable	Fixed factors	Estimator	F-value	p-value
		Tree	GLIMMIX	Occurrence	Total trees	–0.00762 (0.003921)	3.78	0.0757
Dbh	0.04063 (0.008754)				21.55	< 0.0001		
Inclination	–0.07938 (0.005610)				200.24	< 0.0001		
Roughness	0.07071 (0.02006)				12.43	0.0004		
P/T	–0.00619 (0.01513)				0.17	0.6906		
Abundance	Total trees			–0.00485 (0.004330)	1.25	0.2852		
	Dbh			0.03838 (0.007638)	25.25	< 0.0001		
	Inclination			–0.04307 (0.003717)	134.26	< 0.0001		
	Roughness			0.07339 (0.01547)	22.51	< 0.0001		
	P/T			0.003509 (0.01654)	0.05	0.8361		
Individual	GLIMMIX	Size	Dbh	0.006131 (0.003272)	3.51	0.0622		
			Inclination	–0.00431 (0.001521)	8.04	0.0052		
			Roughness	–0.01640 (0.009551)	2.95	0.0895		
			Height	0.000659 (0.000436)	2.28	0.1313		
			Exposure	–0.04814 (0.03094)	2.42	0.1201		
			P/T	0.001342 (0.000949)	2.00	0.1983		
			Apothecia presence	Thallus size	0.08863 (0.01585)	31.26	< 0.0001	
		Dbh		0.002025 (0.02392)	0.01	0.9327		
		Inclination		–0.02013 (0.01285)	2.46	0.1186		
		Isidia presence	Height	–0.00661 (0.004641)	2.03	0.1548		
Exposure	–0.1990 (0.3171)		0.39	0.5304				
P/T	0.01005 (0.01064)		0.89	0.3930				
Thallus size	0.008149 (0.005121)		2.53	0.1119				
Dbh	–0.00885 (0.03430)		0.07	0.7965				
Inclination	0.03337 (0.01757)		3.61	0.0583				
	Height	0.01053 (0.002821)	13.94	0.0002				
	Exposure	0.1301 (0.1658)	0.62	0.4328				
	P/T	–0.01093 (0.03492)	0.10	0.7602				

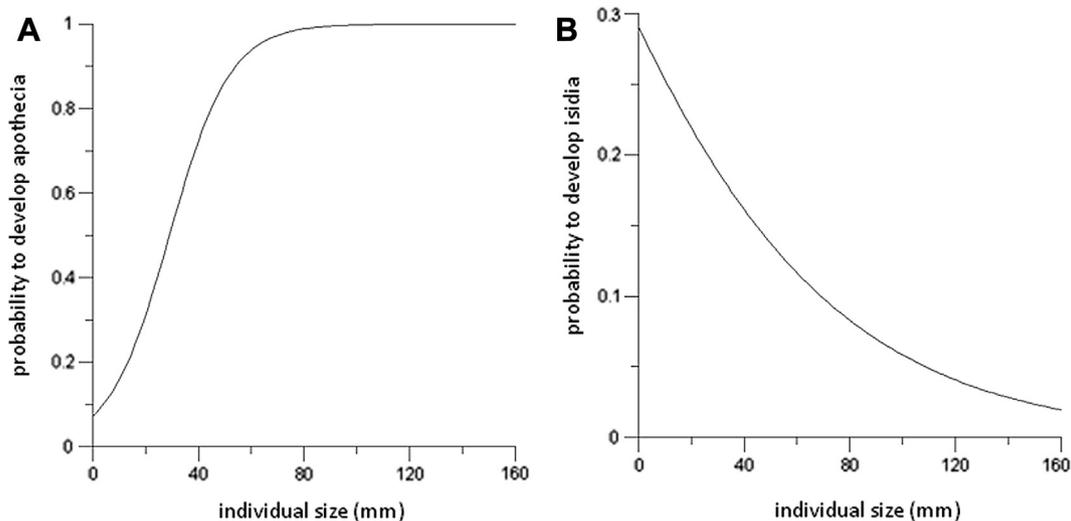


Fig 2 – Probability of *Degelia* having reproductive structures. (A) Apothecia. (B) Isidia.

($p < 0.0001$), population being larger in protected localities. There were no significant differences in mean tree diameter, number of trees per plot and climatic variables between protected and unprotected areas (data not shown).

Discussion

Our results indicate that *Degelia* is very scarce in the study area, especially when considering the size of this region and the number of sites examined. We found only 15 populations after 10 yr of systematic searching and some of them had very few individuals. Besides, *Degelia* density was very variable between locations, ranging from populations with 2 individuals to populations with more than 400 per plot. Our models showed that the only variable at the landscape level influencing the abundance of *Degelia* is type of forest; larger densities being found in broad-leaved evergreen forests. Furthermore, the results suggest that tree characteristics play an important role in the local dynamics of this species-complex.

D. plumbea is a foliose cyanolichen intolerant of high radiation (Gauslaa and Solhaug, 1996) which may cause photo-inhibition mainly in small thalli, because mechanisms to repair this damage are activated only when the thallus is adequately hydrated (Gauslaa and Solhaug, 1998). Moreover, Degelius (1935) demonstrated that individuals of *Degelia*, more exposed to sunlight, died after clearings had been made in the forest. Similar results were also obtained for other species such as *Alectoria sarmentosa* or *Lobaria oregana* (Essen and Renhorn, 1998; Sillet et al., 2000). The Mediterranean region is characterized by a strong summer drought, high temperatures and intense light incidence so the window of time with optimum physiological conditions for active growth is very small. Our results showed that the number of individuals of *Degelia* was higher in broad-leaved evergreen forests than in deciduous ones. Canopy of broad-leaved evergreen forests reduces light levels, the loss of water and heat stress.

Competition with other species may also reduce the presence of *Degelia* in deciduous forests (Armstrong and Welch, 2007).

At the tree level, the selected variables seem to be related to more favorable microclimatic conditions for the colonization and growth of this species-complex. Abundance of *Degelia* is related to tree size, a finding which is related with other variables such as the tree age, which is closely related to tree diameter and forest quality (Dettki and Esseen, 1998; Belinchón et al., 2009; Rubio-Salcedo et al. in prep.). The diameter of the tree also reflects the time available for colonization and represents an increase of different microhabitats more favorable for establishment and colonization by new individuals (Rose, 1992; Kuusinen and Penttinen, 1999; Snäll et al., 2004; Belinchón et al., 2009).

Bark roughness is also linked to tree age. In our study a coarser bark had a positive effect on the presence and abundance of *Degelia*. Deeper fissures may generate a more favorable microhabitat for lichen colonization (Ranius et al., 2008). Young (1938) found that bark of larger trees was more porous and potentially increased water holding capacity, so the conditions for germination would be more favorable in the fissures of the deep bark characteristic of big trees. Furthermore, several studies showed that there are differences in chemical properties between smooth and rough bark (Bates and Brown, 1981), and these differences may affect the species. So, based on our results, bark microtopography has an important influence on the presence of *Degelia*.

Trunk inclination also affects *Degelia* presence, a variable often neglected in the epiphytic studies (Johansson et al., 2009). However, tree inclination can be a very useful indirect measure of the exposure to rain, because these variables are positively related (Ranius et al., 2008). More inclined trunks can cause clear differences in moisture conditions between upper and lower zones of the trunk (Barkman, 1958) which affect the distribution of lichens on the tree (Johansson et al., 2009). In our study, there was more *Degelia* on the most inclined trunks, a result that is consistent with previous ones obtained for other epiphytes such as bryophytes in which

these organisms were dominant on inclined surfaces where water availability is higher (Billings and Drew, 1938; Barkman, 1958; Kenkel and Bradfield, 1986; Bates, 1992; Kuusinen, 1994; Ranius et al., 2008). Our results indicated that the individual size is affected by the characteristics of the tree, particularly by inclination of the trunk. For poikilohydric organisms these factors are very important in the duration of periods of photosynthetic activity (Gauslaa and Solhaug, 1998). *Degelia* presents a thick hypothallus of a filter of rhizinas around the margins, which allows water accumulation during rainy periods and the use of this water to enable photosynthesis during dry periods (Gauslaa and Solhaug, 1998). These authors found that thallus size and hypothallus thickness were relevant traits for the water economy of *D. plumbea* (Gauslaa and Solhaug, 1998). However, not all studies found the same results, leading to some controversy about this factor. For example, Snäll et al. (2005) showed that more inclined trees had a lower probability of colonization by a species of bryophyte, because of the wet conditions or a reduced tree vitality. Johansson et al. (2009) also showed a negative effect of the more inclined trees over the presence of lichen species studied, explaining that this could be due to less habitat available than straighter trunks.

On the other hand, the life cycle of lichen species has three distinct phases: dispersal of propagules, establishment of a new thallus and growth of this thallus (Sillet et al., 2000). Probably, dispersal and establishment are the most critical states for colonization of new habitats (Clobert et al., 2001). Dispersion of lichens depends on their reproductive capacity. Lichens can reproduce sexually, but also they are capable of forming structures of asexual reproduction (Lawrey, 1980). As discussed above *Degelia* may have both types of reproduction, both independently and simultaneously. According to our results, the presence of sexual reproduction, by developing multiple apothecia, is favored when the size of the thallus is bigger, as many other previous studies pointed out for other species (Grime and Hunt, 1975; Walker et al., 1986; Huston and Smith, 1987; Roff, 1992; Stearns, 1992; Martínez et al., 2012), whereas presence of isidia is favored in the higher areas of the tree. Martínez et al. (2012) also found the same results in *L. pulmonaria*.

Conclusions

It is known that many epiphytic lichens are very sensitive to forest management actions because colonization is a slow process and the persistence of the species requires continuity of old trees (McCune, 1993; Kuusinen and Penttinen, 1999; Gu et al., 2001; Kalwij et al., 2005). In fact, for many specialized lichens lack of available substrata probably affects presence and local abundance (Uliezka and Angelstam, 1999). However, our results indicate that the number of trees is not a determining factor in the abundance of *Degelia* as the total number of trees per plot showed no significant influence on these variables.

It seems that microhabitat quality determines the establishment, growth and size of *Degelia* individuals. Therefore, to preserve this endangered species we suggest that conservation plans should include measures that ensure the

maintenance of habitats with favorable microenvironment characteristics. In Mediterranean Spain, *Degelia* seems to require forests with large old trees which can generate adequate levels of humidity within the forest canopy. Forests that meet these requirements should be given special emphasis for protection.

Eight of the fifteen populations of *Degelia* are located within Protected Areas, which guarantees habitat protection because of the favorable environmental conditions and the tightly controlled human activity. *Degelia* populations located outside of protected areas had a much lower density of individuals. The classical metapopulation theory states that the risk of extinction of local populations increases with decreasing size of the population, mainly by environmental events or demographic stochasticity. A local extinction may result from a gradual deterioration of the forest quality, which deterministically decreases the size of the populations to extinction (Harrison and Taylor, 1997).

In conclusion, *Degelia* populations in the central Iberian Peninsula are a matter of great concern. There are several places, mainly not protected, that have a very small density of this lichen and are vulnerable to processes leading to local extinction. The Iberian Peninsula is an example of how persistence of different forms of destruction and degradation linked to over-exploitation have persisted through time and caused the disappearance of most Mediterranean forests (Quézel, 1978) and most of their characteristic biological components. Deforestation in this region has been recognized as a major problem for centuries (Valladares et al., 2004) and is mainly driven by different simultaneous actions, such as timber and firewood extraction, deforestation for expanding agriculture, extensive cattle grazing and hunting management (Thirgood, 1981; Charco, 2002). This situation is even more intense in unprotected areas but there is still a large gap of knowledge on how forest management affects overall biodiversity. Our results point out that a certain degree of protection has allowed the maintenance of larger populations of *Degelia* in Mediterranean Spain. We suggest that in protected areas effective efforts should be made to increase the quality of the forests. Areas where *Degelia* occurs that are outside protection should be subject to recovery plans. Conservation activities should be initiated to prevent local extinctions. The maintenance of this and other rare lichen species not only ensures the maintenance of epiphyte biodiversity but also would benefit a wide variety of other organisms.

The *Degelia*-complex (*D. atlantica* and *D. plumbea*) is listed as vulnerable on the Red List of lichens of central Spain, which means its populations have a small number of individuals and also are in decline. The *Degelia*-complex has a higher probability of having sexual reproductive structures. However, total number of populations found is relatively scarce and population size seems to be very small in almost half of the studied populations. These facts seem to indicate that *Degelia* has some problems in ensuring the establishment of new individuals inside a population or colonization of new habitats. So, further studies are necessary to determine where the bottleneck of this species-complex is.

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REFERENCES

- Armstrong, R.A., Welch, A.R., 2007. Competition in lichens communities. *Symbiosis* 43, 1–12.
- Barkman, J.J., 1958. Phytosociology and Ecology of Cryptogrammicepiphytes. VanGorcum, Assen.
- Bates, J.W., 1992. Influence of chemical and physical factors on *Quercus* and *Fraxinus* epiphytes at Loch Sunart, western Scotland: a multivariate analysis. *Journal of Ecology* 80, 163–179.
- Bates, J.W., Brown, D.H., 1981. Epiphyte differentiation between *Quercus petraea* and *Fraxinus excelsior* in a maritime area of south west England. *Vegetatio* 48, 61–70.
- Belinchón, R., Martínez, I., Aragón, G., Escudero, A., Valladares, F., 2007. Edge effect on epiphytic communities in a Mediterranean *Quercus pyrenaica* forest. *Journal of Vegetation Science* 18, 81–90.
- Belinchón, R., Martínez, I., Otálora, M.A.G., Aragón, G., Dimas, J., Escudero, A., 2009. Fragment quality and matrix affect epiphytic performance in a Mediterranean forest landscape. *American Journal of Botany* 96, 1974–1982.
- Billings, W.D., Drew, W.B., 1938. Bark factors affecting the distribution of corticolous bryophytic communities. *The American Midland Naturalist* 20, 302–330.
- Blom, H.H., Lindblom, L., 2010. *Degelia cyanoloma* (Schaer.) H. H. Blom & L. Lindblom comb. et stat. nov., a distinct species from western Europe. *Lichenologist* 42, 23–37.
- Carballal, R., Paz-Bermúdez, G., López de Silanes, M.E., Pérez-Valcárcel, C., 2010. Flora Liquenológica Ibérica. Pannariaceae, vol. 6. Sociedad Española de Liquenología, SEL, Pontevedra.
- Carroll, G.C., 1979. Forest Canopies: Complex and Independent Subsystems. Oregon State University Press, Corvallis, Oregon.
- Charco, J., 2002. La regeneración natural del bosque mediterráneo en la Península Ibérica. ARBA, Ministerio de Medio Ambiente, Madrid, ES.
- Clobert, J., Danchin, F., Dhondt, A.A., Nichols, J.D., 2001. Dispersal. Oxford University Press, New York.
- COSEWIC, 2010. COSEWIC Assessment and Status Report on the Blue Felt Lichen *Degelia plumbea* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa x + 42 pp.
- Debinski, D.M., Holt, R.D., 2000. A survey and overview of habitat fragmentation experiments. *Conservation Biology* 14, 342–355.
- Degelius, G., 1935. Das ozeanische Element der Strauch- und Laubflechtenflora von Skandinavien. *Acta Phytogeographica Suecica* 7, 1–411.
- Dettki, H., Esseen, P.A., 1998. Epiphytic macrolichens in managed and natural forest landscapes: a comparison at two spatial scales. *Ecography* 21, 613–624.
- Dix, N.J., Webster, J., 1995. Fungal Ecology. Chapman and Hall, London.
- Essen, P.A., Renhorn, K.E., 1998. Edge effects on an epiphytic lichen in fragmented forests. *Conservation Biology* 12, 1307–1317.
- Fahrig, L., 2003. Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology, Evolution and Systematics* 34, 487–515.
- Gauslaa, Y., Solhaug, K.A., 1996. Differences in the susceptibility to light stress between epiphytic lichens of ancient and young boreal forest stands. *Functional Ecology* 10, 334–354.
- Gauslaa, Y., Solhaug, K.A., 1998. High-light damage in air-dry thalli of the old forest lichen *Lobaria pulmonaria* – interactions of irradiance, exposure duration and high temperature. *Journal of Experimental Botany* 50, 697–705.
- Grime, J.P., Hunt, R., 1975. Relative growth rate: its range and adaptive significance in a local flora. *Journal of Ecology* 63, 393–422.
- Gu, W.D., Kuusinen, M., Kontinen, T., Hanski, I., 2001. Spatial pattern in the occurrence of the lichen *Lobaria pulmonaria* in managed and virgin boreal forests. *Ecography* 24, 139–150.
- Guisan, A., Edwards, T.C., Hastie, T., 2002. Generalized linear and generalized additive models in studies of species distributions: setting the scene. *Ecological Modelling* 57, 89–100.
- Harrison, S., Taylor, A.D., 1997. Empirical evidence for metapopulation dynamics. In: Hanski, I., Gilpin, M.E. (Eds.), *Metapopulation Biology. Ecology, Genetics and Evolution*. Academic Press, pp. 27–42.
- Hayward, G.D., Rosentreter, R., 1994. Lichens as nesting material for northern flying squirrels in the northern rocky mountains. *Journal of Mammalogy* 75, 663–673.
- Huston, M., Smith, T., 1987. Plant succession: life history and competition. *American Naturalist* 130, 168–198.
- IUCN, 2012. The IUCN Red List of Threatened Species. Version 2012.2 (accessed 17.08.12) <http://www.iucnredlist.org>.
- Johansson, V., Bergman, K.O., Lättman, H., Milberg, P., 2009. Tree and site quality preferences of six epiphytic lichens growing on oaks in southeastern Sweden. *Annales Botanici Fennici* 46, 496–506.
- Kalwij, J.M., Wagner, H.H., Scheidegger, C., 2005. Effects of stand-level disturbances on the spatial distribution of a lichen indicator. *Ecological Applications* 15, 2015–2024.
- Kenkel, N.C., Bradfield, G.E., 1986. Epiphytic vegetation on *Acer macrophyllum*: a multivariate study of species-habitat relationships. *Vegetatio* 68, 43–53.
- Kuusinen, M., 1994. Epiphytic lichen diversity on *Salix caprea* in old-growth southern and middle boreal forests of Finland. *Annales Botanici Fennici* 31, 77–92.
- Kuusinen, M., Penttinen, A., 1999. Spatial pattern of the threatened epiphytic byrophyte *Neckera pennata* at two scales in a fragmented boreal forest. *Ecography* 22, 729–735.
- Lawrey, J.D., 1980. Sexual and asexual reproductive patterns in *Parmotrema* (Parmeliaceae) that correlate with latitude. *Bryologist* 83, 344–350.
- Longton, R.E., 1992. The role of bryophytes and lichen in terrestrial ecosystems. In: Bates, J.W., Farmer, A.M. (Eds.), *Bryophytes and Lichen in a Changing Environment*. Oxford University Press, New York, pp. 32–76.
- Martínez, I., Aragón, G., Sarrión, F.J., Escudero, A., Burgaz, A., Coppins, B.J., 2003. Threatened lichens in the central Spain (saxicolous species excluded). *Cryptogamie Mycologie* 24, 73–97.
- Martínez, I., Flores, T., Otálora, M.A.G., Belinchón, R., Prieto, M., Aragón, G., Escudero, A., 2012. Multiple scale environmental modulation of lichen reproduction. *Fungal Biology* 116, 1192–1201.
- McCullagh, P., Nelder, J.A., 1989. Generalized Linear Models. In: *Monographs on Statistics and Applied Probability*, second ed, vol. 37. Chapman and Hall/CRC, USA.
- McCune, B., 1993. Gradient in epiphyte biomass in three *Pseudotsuga-Tsuga* forests of different ages in western Oregon and Washington. *Bryologist* 96, 405–411.
- Méndez, M., Karlsson, P.S., 2004. Between-population variation in size-dependent reproduction and reproductive allocation in *Pinguicula vulgaris* (Lentibulariaceae) and its environmental correlates. *Oikos* 104, 59–70.
- Otálora, M.A.G., Salvador, C., Martínez, I., Aragón, G., 2013. Does the reproductive strategy affect the transmission and genetic

- diversity of bionts in cyanolichens? A case study using two closely related species. *Microbial Ecology* 65, 517–530.
- Petterson, R.B., Ball, J.P., Renhorn, K.-A., Essen, P.-A., Sjöberg, K., 1995. Invertebrate communities in boreal forest canopies as influenced by forestry and lichens with implications for passerine birds. *Biological Conservation* 74, 57–63.
- Primack, R.B., Ros, J., 2002. Introducción a la Biología de la Conservación. Ariel, Barcelona.
- Quézel, P., 1978. Analysis of the flora of Mediterranean and Saharan Africa. *Annals of the Missouri Botanical Garden* 65, 479–534.
- Ranius, T., Johansson, P., Niclas, B., Niklasson, M., 2008. The influence of tree age and microhabitat quality on the occurrence of crustose lichens associated with old oaks. *Journal of Vegetation Science* 19, 653–662.
- Richardson, H.S., Cameron, R.P., 2004. Cyanolichens: their response to pollution and possible management strategies for their conservation in Northeastern North America. *Northeastern Naturalist* 11, 1–22.
- Roff, D.A., 1992. *The Evolution of Life Histories. Theory and Analysis*. Chapman and Hall, New York.
- Rose, F., 1988. Phytogeographical and ecological aspects of lobaria communities in Europe. *Botanical Journal of the Linnean Society* 96, 69–79.
- Rose, F., 1992. Temperate forest management: its effects on bryiophyte and lichen floras and habitats. In: Bates, J.W., Farmer, A.D. (Eds.), *Bryophytes and Lichens in a Changing Environment*. Clarendon Press, Oxford.
- Rubio-Salcedo, M., Merinero, M., Martínez, I. Microhabitat selection by the epiphytic lichen *Lobaria pulmonaria*: the host tree species does matter (in preparation).
- Sánchez-Palomares, O., Sánchez-Serrano, F., Carretero-Carrero, M.P., 1999. Modelos y Cartografía de Estaciones Climáticas Termoplumiométricas para la España Peninsular. INIA. Colección Fuera de Serie, España.
- SAS Institute Inc., 2001. *The SAS System, Version 9*. Cary, N.C.
- Scheidegger, C., Werth, S., 2009. Conservation strategies for lichens: insights from population biology. *Fungal Biology Review* 23, 55–66.
- Sillet, S.C., McCune, B., Peck, J.E., Rambo, T.R., 2000. Four years of epiphyte colonization in Douglas-fir forest canopies. *Bryologist* 103, 661–669.
- Snäll, T., Ehrlén, J., Rydin, H., 2005. Colonization-extinction dynamics of and epiphyte metapopulation in a dynamic landscape. *Ecology* 86, 106–115.
- Snäll, T., Hagström, A., Rudolphi, J., Rydin, H., 2004. Distribution pattern of the epiphyte *Neckera pennata* on three spatial scales – importance of past landscape structure, connectivity, and local conditions. *Ecography* 27, 757–766.
- Stearns, S.C., 1992. *The Evolution of Life Histories*. University Press, Oxford.
- Thirgood, J.V., 1981. *Man and Mediterranean Forest*. Academic Press, London, UK.
- Uliezka, H., Angelstam, P., 1999. Occurrence of epiphytic macrolichens in relation to tree species and age in managed boreal forest. *Ecography* 22, 396–405.
- Valladares, F., Camarero, J.J., Pulido, F., Gil-Pelegrín, E., 2004. El bosque mediterráneo, un sistema humanizado y dinámico. In: Valladares, F. (Ed.), *Ecología del bosque mediterráneo en un mundo cambiante*. Ministerio de Medio Ambiente, EGRAF, Madrid, ES, pp. 13–25.
- Verbeke, G., Molenberghs, G., 1997. *Linear mixed models. In: Practice: A SAS Oriented Approach*. Springer-Verlag, New York.
- Walker, L.R., Zasada, J.C., Chapin, F.S., 1986. The role of life history processes in primary succession on an Alaskan floodplain. *Ecology* 67, 1243–1253.
- Wesselingh, R.A., de Jong T.J., Klinkhamer, P.G.L., van Dijk, M.J., Schlatmann, E.G.M., 1993. Geographical variation in threshold size for flowering in *Cynoglossum officinale*. *Acta Botanica Neerlandica* 42, 81–91.
- Wesselingh, R.A., Klinkhamer, P.G.L., de Jong, T.J., Klinkhamer, P.G.L., Boorman, L.A., 1997. Threshold size for flowering in different habitats: effects of size-dependent growth and survival. *Ecology* 78, 2118–2132.
- Young, A.G., Clarke, G.M., 2000. *Genetics, Demography of Fragmented Population*. Cambridge University Press, UK.
- Young, C., 1938. Acidity and moisture in tree bark. *Proceedings Indian Academy Science* 47, 106–115.