



British Mycological
Society promoting fungal science

journal homepage: www.elsevier.com/locate/funbio



Multiple-scale environmental modulation of lichen reproduction

Isabel MARTÍNEZ^{a,*}, Tatiana FLORES^a, Mónica A. G. OTÁLORA^{a,c}, Rocio BELINCHÓN^{a,b},
María PRIETO^{a,c}, Gregorio ARAGÓN^a, Adrián ESCUDERO^a

^aÁrea de Biodiversidad y Conservación, ESCET, Universidad Rey Juan Carlos, C/Tulipán s/n, 28933 Móstoles, Madrid, Spain

^bRoyal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, UK

^cDept. of Cryptogamic Botany, Swedish Museum of Natural History, Stockholm, Sweden

ARTICLE INFO

Article history:

Received 26 April 2012

Received in revised form

18 September 2012

Accepted 24 September 2012

Available online 8 October 2012

Corresponding Editor:

Brenda Diana Wingfield

Keywords:

Asexual reproduction

Climate gradient

Lichens

Sexual reproduction

Size

ABSTRACT

It is necessary to understand how environmental changes affect plant fitness to predict survival of a species, but this knowledge is scarce for lichens and complicated by their formation of sexual and asexual reproductive structures. Are the presence and number of reproductive structures in *Lobaria pulmonaria*, a threatened lichen, dependent on thallus size, and is their formation sequential? Does any size-dependence and sequential formation vary along a climate gradient? Generalized linear mixed models were used to explore the effect of environmental predictors on the size and presence/abundance of each reproductive structure and to determine the probability of a given-sized thallus to develop any reproductive structure. The largest individuals are more likely to develop reproductive structures, and the lichen uses a mixed strategy of early asexual reproduction and late sexual. Macro and microclimatic variables also influenced reproductive capacity. Relationships among climate conditions and lichen size and reproductive capacity can compromise the future viability of the species in the most southern populations of Europe.

© 2012 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.

Introduction

Having good estimates of fitness is a basic requisite for ecological studies at the species level (Young & Clarke 2000; Picó 2002; Milla *et al.* 2009). Understanding plant fitness responses to certain conditions is also critical when assessing the evolutionary impact of environmental heterogeneity, even more so for predicting the viability of the species (Jackson *et al.* 2006; Milla *et al.* 2009). In exploring this broad topic in detail for vascular plants, numerous researchers have developed a conceptual framework for explaining the relationships between size/age and reproduction (e.g., Harper & White 1974; Barot *et al.* 2002) and how the individual adjusts resource allocation between growth and

reproduction (Bazzaz & Reeckie 1985; Shipley & Dion 1992; Méndez 1999). Unfortunately, our knowledge on these critical issues for other biological groups is scarce and especially urgent in the case of certain groups such as lichenized fungi. Among the reasons that have been posed to explain this scarcity are the difficulties in obtaining good fitness estimates due to the 'supposed' great longevity of lichens and to the fact that the stages of their life cycle may not be completely known (Jackson *et al.* 2006). In this sense, we still need detailed demographic research for lichens, urgently for threatened lichen species (Scheidegger & Werth 2009).

Estimating the contribution of various components to the fitness of lichens is especially complex because lichens

* Corresponding author. Tel.: +34 914887183; fax: +34 916647490.

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

1878-6146/\$ – see front matter © 2012 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.

<http://dx.doi.org/10.1016/j.funbio.2012.09.005>

comprise two or more organisms and can reproduce sexually and asexually. Lichens are symbiotic associations between a mycobiont and one or more photobionts, mainly green algae and/or cyanobacteria (Friedl & Büdel 2008). As a consequence, reproduction in lichens is quite complex (Honegger 1998), being able to reproduce sexually, but also to form structures for asexual proliferation. Most lichens form apothecia with sexual fungal spores and symbiotic asexual reproductive structures of various sizes and shapes such as isidia or soredia (Büdel & Scheidegger 2008). The establishment of a new lichen association via sexual reproduction requires the germination of a mycobiont in a suitable microhabitat and rapid contact with a suitable photobiont (Honegger 1996; Seymour et al. 2005), which may be specialist or not (Otálora et al. 2010). Sexual reproduction seems especially suitable for long-distance dispersal, because spores are smaller than asexual propagules (Muñoz et al. 2004). On the other hand, asexual reproduction, which is genetically less variable (Williams 1975; Lawrey 1980), has the advantage of dispersing simultaneously from the photobiont and mycobiont and does not require relichenization (Seymour et al. 2005; Otálora et al. 2010).

Knowing when, where, and how these reproductive structures are developed during the life cycle of lichen species remains a priority. Only Denison (2003) has reported a full life cycle; following five individuals of *Lobaria pulmonaria* (L.) Willd. over a year, founding that apothecia emerge in late spring in this species. Several studies showed that some lichen species have thallus size-dependent survival probably because water retention capacity is higher in large thalli, allowing them to photosynthesize longer than smaller individuals (Hestmark 1997; Gauslaa & Solhaug 1998). Larger thalli could also be more efficient at forming sexual reproductive structures (Hestmark 1992; Ramstad & Hestmark 2001; Pringle et al. 2003). In addition, some studies have shown that some lichen species need to reach a critical mass/size before allocating resources to reproduction (Hestmark 1992; Clayden 1998; Ramstad & Hestmark 2001; Pringle et al. 2003), whereas others found the contrary (Jahns et al. 1978; Tretiach & Carpanelli 1992). Recently, Gauslaa (2006) showed that transplanted lobes of *L. pulmonaria* that had a greater amount of soredia/isidia had growth rates lower than those individuals with few or no asexual propagules, suggesting a trade-off between reproductive and vegetative growth allocation.

Most organisms can be found in a wide range of habitats or environmental conditions and, consequently, substantial intraspecific variation in vital processes such as fecundity should be expected as a reaction to this patchy heterogeneity. Study of the variation in vital processes from lichen to lichen between site/population conditions and particularly in reproductive parameters and structures, is interesting not only from an ecological perspective but also from an evolutionary perspective. Such variation is critical to understand the entire distribution of a species and how its demography adjusts to environmental conditions and, even more importantly, to predict changes in range limits in response to future climate change (Gaston 2003, 2009). Although many studies have been conducted on other groups of organisms to assess this possible relationship (Lennartsson & Oostermeijer 2001; Wilson et al. 2005; Giménez-Benavides et al. 2007; Marrero-Gómez et al. 2007), none have been done for lichens.

To our knowledge, size-dependence of lichen reproduction and between-site variation of reproductive traits have not been addressed together. However, a theoretical framework has been proposed for angiosperms (Freeman et al. 1980; Korpelainen 1998) on the basis of three models: (1) a patchy environment model (Freeman et al. 1980) in which sexual reproduction is mediated by stress conditions, (2) a size advantage model (Warner 1975) in which sexual reproduction is mediated by changes in size, and (3) a mixed model combining models 1 and 2.

To test these theoretical models in lichens, we have chosen as a model species the endangered epiphytic lichen *L. pulmonaria* because it is one of the best-known lichen species from an ecological point of view (Belinchón et al. 2009; Scheidegger & Werth 2009). This epiphytic species forms soredia and/or isidia (isidioid soredia, following Scheidegger (1995)) as asexual reproductive structures and occasionally forms apothecia as sexual reproductive structures (Belinchón et al. 2009; Scheidegger & Werth 2009). Recently, two studies have focused in different aspects related to factors influencing reproduction capacity in *L. pulmonaria*. Hilmo et al. (2011a) in a study about the population characteristics of epiphytic lichens in *Picea abies* plantations, found a correlation between thallus size and probability of reproduction in *L. pulmonaria*. Besides, Mykriukov et al. (2010) developed a study about the influence of certain ecological determinants on the reproduction of *L. pulmonaria* populations in the Urals. They found that total cover of *L. pulmonaria* and type of forest (coniferous or deciduous) influence the reproductive status of the populations.

The study of sexual reproduction in lichens is difficult because lichenized ascomycetes can be either homothallic or heterothallic species, depending on whether the haploid mycelium differentiates gametangia and gametes in the same thallus or on different thalli (Honegger & Scherrer 2008). Unfortunately, for most of lichen species, such information is lacking (Honegger & Scherrer 2008). So, with these difficulties our main goal is to know which are the most important factors determining lichen reproduction. We hypothesized those populations growing in more humid locations will have larger individuals and thus produce more reproductive structures, and that reproductive structures will develop in sequence, first asexual, then sexual. To test our hypothesis we determine different specific objectives: (1) to know when sexual or asexual structures are developed?; (2) to evaluate if there is any kind of hierarchy or sequence between the development of sexual and asexual structures; (3) to know which is the sizes structure of *L. pulmonaria* populations; (4) to evaluate if environmental conditions influence individual size and reproductive capacity.

Materials and methods

We selected 16 locations along a wide bioclimatic gradient that included two biogeographical regions (Atlantic and Mediterranean regions) in Spain (Fig 1; Table 1). Our goal was to include in the study *Lobaria pulmonaria* populations distributed along the Iberian Peninsula, trying to get the highest latitudinal gradient. Besides, populations included in the study had to

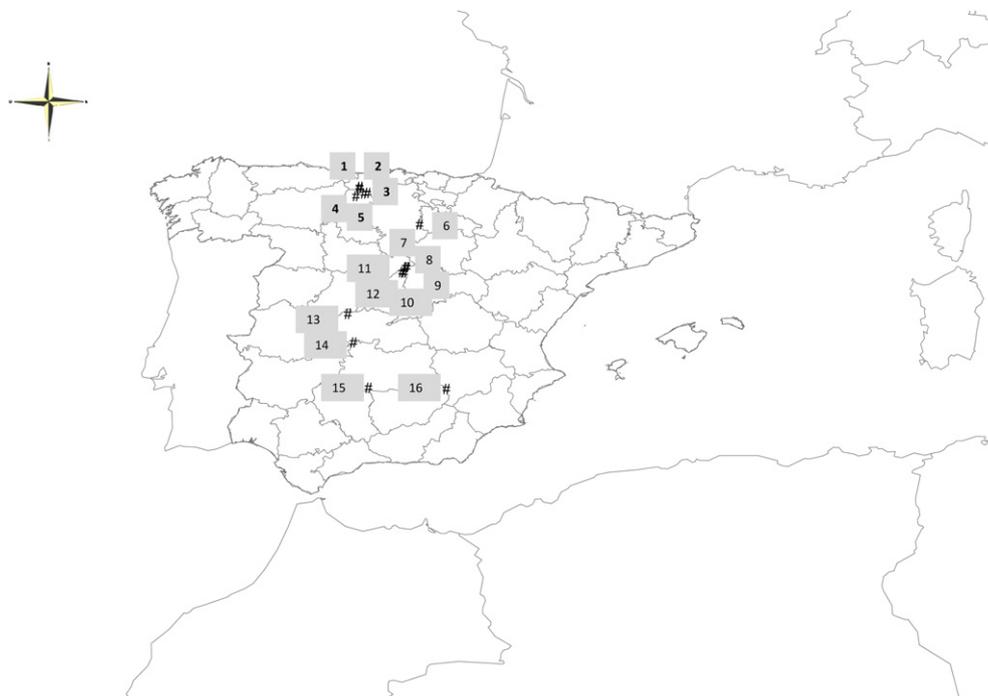


Fig 1 – Map of the Iberian Peninsula showing the different localities studied (number correspond with the number of locality included in Table 1).

have a high number of individuals of *L. pulmonaria* in order to represent well-developed populations and not marginal or impoverishment ones. The sites included ten forest stands of *Quercus pyrenaica* Willd., and six of *Fagus sylvatica* L. (Table 1). Altitude varied between 760 m and 1689 m a.s.l., with the forests at the highest altitudes located in central

Spain and the lowest in the southernmost localities. The ratio between the total rainfall and mean annual temperature (P/T) ranged from 44.6 in the south to 147.63 in the north and 194.62 in the centre of the Iberian Peninsula.

Since the overall objective of this study was to assess the effect of climate and some forest stand features at different

Table 1 – Mean values for different variables at stand and tree levels. Prov: Province; S: Santander; P: Palencia; LR: La Rioja; SG: Segovia; GU: Guadalajara; M: Madrid; TO: Toledo; CR: Ciudad Real; AB: Albacete; Alt.: (altitude, m); For.: forest type (Fs = *Fagus sylvatica*, Qp = *Quercus pyrenaica*); DBH: mean tree diameter; Total trees: total number of trees considered in each locality; P: total rainfall (mm); T: average annual temperature ($^{\circ}$ C); H-1: number of individuals growing below 50 cm; H-2: number of individuals growing between 50 and 150 cm in the tree; H-3: number of individuals growing between 150 cm and 250 cm in the tree; Isi: number of individuals with isidia; Sor: number of individuals with soredia; Apo: number of individuals with apothecia; Juv: number of juvenile individuals; Thallus size: mean value \pm standard deviation.

N ^o	Prov.	Locality	Alt.	For.	DBH	Total trees	P	T	H-1	H-2	H-3	Isi	Sor	Apo	Juv	Thallus size
1	S	Piedrasluengas	1205	Fs	47.1	9	982	8.4	93	187	73	87	148	2	180	23.23 \pm 51.8
2	S	Subida a Piedrasluengas	1354	Fs	32.7	16	1122	7.6	79	141	60	16	93	3	178	19.85 \pm 82.51
3	P	Brañosera	1324	Qp	33.42	10	1100	7.7	162	98	82	43	262	0	76	37.74 \pm 76.83
4	P	Perapertú	1231	Qp	25.7	13	985	8.3	72	103	74	49	140	0	93	28.81 \pm 48.74
5	P	Ruesga	1227	Fs	40.6	10	986	8.3	276	24	0	56	17	0	230	22.48 \pm 58.89
6	LR	Ezcaray	942	Qp	31.28	7	620	10.2	112	328	24	14	16	1	435	32.93 \pm 46.38
7	SG	Puerto de la Quesera	1689	Fs	32.16	10	1265	6.5	85	153	12	3	147	0	100	27.99 \pm 82.77
8	GU	Cantalojas	1617	Fs	40.55	5	1176	6.9	90	95	113	26	135	0	158	7.23 \pm 34.38
9	GU	Cantalojas	1609	Qp	13.1	8	1173	6.9	163	66	49	10	64	0	214	44.98 \pm 48.46
10	GU	San Tui	1312	Qp	30.11	10	867	9.1	166	87	21	78	44	0	196	22.49 \pm 61.50
11	M	Montejo de la Sierra	1284	Qp	29.53	8	825	9.5	177	62	28	74	9	13	184	18.45 \pm 71.11
12	M	Montejo de la Sierra	1278	Fs	24.39	8	827	9.5	248	14	0	24	71	6	189	48.57 \pm 101.58
13	TO	Real de San Vicente	760	Qp	33.84	39	757	13.4	100	72	24	3	27	0	169	50.81 \pm 197.41
14	TO	Navas de Estena	797	Qp	30	8	638	13.6	112	750	62	93	153	50	761	6.88 \pm 13.40
15	CR	Sierra Madrona	887	Qp	21.82	11	620	13.9	147	72	41	100	40	0	124	14.19 \pm 47.14
16	AB	Calar del Mundo	1420	Qp	39.01	8	1148	11.5	32	43	179	133	229	0	2	15.07 \pm 40.05

scales on the populations of *L. pulmonaria*, well-developed and preserved forests were selected in each latitudes to minimize other possible influences on change. *Quercus pyrenaica* is a semideciduous oak almost entirely confined to the Iberian Peninsula, with some isolated populations in northern Morocco and on the southwestern tip of France. It has a wide distribution in the Iberian Peninsula on acid soils (Blanco et al. 2005). In southernmost regions, the species prefer mountainous areas with higher annual precipitation. *Fagus sylvatica* is a deciduous tree widespread throughout Western Europe. In the Iberian Peninsula, it is found mainly in the north, although there are some populations in central Spain, where it reaches its southernmost distribution. Both types of forests are dominated by the corresponding species, but another tree species can also grow, such as *Crataegus monogyna* Jacq., *Ilex aquifolium* L., *Prunus spinosa* L., *Sorbus aucuparia* L., etc.

In each forest stand, we selected trees at random with *L. pulmonaria* individuals located at least 100 m from the edge to avoid possible effects caused by microclimatic changes associated with edge effects (Belinchón et al. 2007). The number of trees considered varied between localities (between five and 39 trees) but guaranteed a survey minimum of 250 individuals of *L. pulmonaria* per locality. To do so, we considered all *L. pulmonaria* individuals present on each main trunk from the base to 2.50 m high.

We know the difficulty to determine size in lichens, as one thallus may be formed by several thalli growing together. This difficulty is even higher in the case of *L. pulmonaria* because this species can form large carpets. We have considered an individual only when we have been able to differentiate one of each other. If we had some doubts, these individuals were not considered in the study. A total of 5253 individuals were surveyed. For each individual thallus, we took the following estimates: (1) size (cm²); *L. pulmonaria* thalli were traced on transparent plastic sheets in the field, and area was later calculated using an image analyzer (ImageJ 1.36b National Institutes of Health, Bethesda, Maryland, USA). As one of our main objectives was not damaged *L. pulmonaria* thalli, we placed the transparent sheets onto moist individuals and adjusted the sheet as possible to the lobes; (2) presence of reproductive (1/0) variables and abundance were always estimated by the same person (% of cover on the thallus surface) of asexual structures (isidia and soredia) and sexual structures (apothecia). In the case of apothecia, we counted all the apothecia present in each thallus.

In addition, the following predictors were measured for each stand: (1) *P* = total rainfall (mm); (2) summer precipitation (mm); (3) winter precipitation (mm); (4) *T* = average annual temperature (°C); (5) average minimum temperature of the coldest month (°C); (6) average maximum temperature of the warmest month (°C); (7) humidity index of Lang ($I_L = P/T$) which is an estimator of the efficiency of precipitation in relation to the temperature; (8) latitude (°); (9) longitude (°); (10) altitude (m a.s.l.); (11) type of forest (beech and oak). Latitude, longitude, and altitude were taken in the field with a GPS (GPSmap 60CSx, Garmin GPS). The first seven climate variables were obtained from CLIMOEST, a climate simulator for the Iberian Peninsula (<http://www2.montes.upm.es/Dptos/DptoSilvopascicultura/Edafologia/aplicaciones/>

[Aplicaciones.htm](#) = Programa Estimaciones Climáticas; Sánchez-Palomares et al. 1999).

We also estimated several variables at the tree level: (12) diameter at breast height (DBH, cm) of the tree on which the lichen was growing; (13) lichen height on tree as < 50 cm, 50–150 cm, or >150 cm, measured from the base of the tree; (14) orientation on the trunk for each individual of *L. pulmonaria*.

Data analysis

Although 14 environmental variables were estimated, only six were included in our statistical models to assess their influence on the size and type of reproduction of *Lobaria pulmonaria* (type and abundance): thallus size, lichen height on tree, lichen orientation in the tree, tree diameter, humidity index of Lang, type of forest. We used a pairwise Pearson correlation between all variables due to their normality and excluded those that had correlation values above 0.7 to avoid problems of multicollinearity. Thallus size was included both as an explanatory predictor in most of the models but also as a response predictor, because one of our goals was to evaluate whether environmental variables may influence the size of the individual. In the other cases, we included thallus size as a covariable to separate out the influence of individual size on the existence of reproductive structures.

To explore the effect of environmental predictors on the size and the presence/abundance of each reproductive structure at each hierarchical spatial scale (i.e., forest stand and tree) (see Belinchón et al. 2009), generalized linear mixed models were used (GLMMs) (Breslow & Clayton 1993). This modelling approach was chosen because our data had an unbalanced and hierarchical structure with trees nested within stands. A hierarchical data structure implies a correlation between data points at different scales, inflating the error degrees of freedom and increasing the chance of making a type I error. To overcome these problems, we analyzed the data using a multilevel approach, and when necessary, considering trees and stands as random factors and applying mixed modelling (Verbeke & Molenberghs 1997), assuming that forest stand and trees finally selected for monitoring purposes are only a subset of trees and forest stands available. The significance of each predictor was estimated by means of an analysis of deviance (Guisan et al. 2002). For the presence and abundance of asexual (isidia and soredia) and sexual (apothecia) reproductive structures, we fit the mixed models using the binomial estimation, a 'logit' link function, and setting the variance to '1-mean'. In the case of thallus size, we used a lognormal distribution, with an 'identity' link function. Effects of random factors were tested using the Wald Z-statistic test. All GLMM computations were performed using SAS Macro program GLIMMIX, which iteratively uses SAS Procedure Mixed until convergence (GLIMMIX ver. 8 for SAS/STAT; available at <http://www.sas.com.techsup/download/stat/>).

To determine the probability of a thallus of a given size will develop any reproductive structure, we built generalized linear models (GENMOD SAS v. 9, McCullagh & Nelder 1989). The significance of each predictor was estimated by means of a deviance test (Guisan et al. 2002). Predictors were excluded from the model when the level of significance was higher than

0.05. Binomial error and logit link function were assumed for the response variable. The developed models were used to estimate probability curves for each one of the three structures considered (soredia, isidia, and apothecia). The relation between reproductive probability (p) and lichen size can be 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 for reproduction (Wesselingh et al. 1993; Méndez & Karlsson 2004).

In order to know the population structure of *L. pulmonaria*, we followed a typical age/size structure population approach in which the vital attributes related to the fertility of each class were considered similar within them. In order to follow this approach, we use the option 'classification of k -means', using the software SPSS v. 17.0. This method is especially indicated those points in the variable that maximizes the differences between groups by minimizing the variability within the groups. This implies the detection or natural points for the size structure. Populations of *L. pulmonaria* are structured in four different classes, and the size threshold of each level is indicated in Table 2.

Results

Descriptive results for forest stands and *Lobaria pulmonaria* thalli

Of the total individuals of *L. pulmonaria* (5253) considered on 180 trees, almost 50 % were found between 50 and 150 cm from the ground, and ca. 40 % were on the first 50 cm of the trunk (Table 1). Interestingly, the two localities with the most thalli at the base of the trees (populations 5 and 12) were those that had no thalli above 150 cm (Table 1). Regarding the presence of reproductive structures, we found that about 30 % of thalli had soredia (1595 individuals), 15 % had isidia (809 individuals), and only 1.4 % (75 individuals) had sexual structures. Furthermore, most of the individuals studied were juvenile, without any reproductive structure (3289 individuals, 62.61 %), and only 0.8 % presented sexual and asexual structures on the same thallus (43 individuals). However, this last value represents almost 50 % of the sexual individuals because we found only 75 individuals with apothecia in all of the studied populations.

The mean size of *L. pulmonaria* individuals is 25.27 ± 77.84 cm², being minimal size of 0.01 cm², and

maximum one of 1989.93 cm². Besides, mean thallus size varies between populations, having the population 14 the smaller mean individual size, whereas the mean individual size of population 13 reaches the highest value (Table 1). Depending on the reproduction structures, mean size of individuals is different: individuals with soredia have a mean size of 59.97 ± 123.09 (0.18–1989.93) cm²; individuals with isidia present a mean size of 65.92 ± 129.46 (0.28–1989.93) cm²; and finally, sexual individuals reach a mean size of 164.76 ± 218.08 (6.11–1524.67) cm².

Predictor effects on reproduction and size

Our results indicated that the size of individuals of *Lobaria pulmonaria* depended exclusively on the relationship between P and T (Table 3); individuals were largest in those populations where this ratio was highest.

Moreover, mixed models also showed that the individual thallus size is the most important predictor explaining the occurrence and abundance of both sexual and asexual reproductive structures. Thus, the likelihood of having reproductive structures increased as the size grew. Moreover, the presence of soredia is higher in those populations growing in areas where the ratio P/T is higher, a rough indicator of less water stress. Presence of apothecia is more common at the tree base (Table 4). In addition, we did not find any influence of the forest type, diameter of the host tree, or orientation of each individual on the trunk on the presence of any reproductive structure.

When modelling the influence of environmental predictors at several scales on the abundance of reproductive structures, the results differed somewhat between the different reproductive structures analyzed. In all cases, thallus size was also decisive (Table 4), but other variables also affected abundance. More asexual structures in general and of soredia in particular were found in populations where the ratio P/T was higher, but these climatic variables did not influence the abundance of isidia or apothecia. For these last structures, some tree level variables had more influence. At the base of trees and on northern exposures, the abundance of apothecia on individual thalli was highest, whereas the number of isidia per thallus reached a maximum on the upper portion of the trunk (Table 4).

Table 2 – Sizes structure of *L. pulmonaria* populations. Mean value in cm², standard deviation, minimum and maximum values are indicated for each size class.

	Class 1	Class 2	Class 3	Class 4
Mean	2.38	23.18	73.84	267.98
Standard deviation	2.62	10.58	17.75	238.63
Minimum	0.01	10.02	48.52	114.11
Maximum	10	48.23	112.94	1989.93

Table 3 – Results of GLMMs.

Response variable	Effect	Coef. (SE)	df	F-ratio	P
Thallus size	Height	0.039 (0.010)	2	12.98	0.069
	Orientation	−0.007 (0.040)	7	0.03	0.859
	Dbh	−0.002 (0.012)	179	0.03	0.871
	P/T	0.002 (0.001)	15	5.64	0.031
	Type of forest	−0.176 (0.495)	1	0.13	0.783

Notes: Coef: variable coefficient in the pattern; SE: standard error; F-ratio: statistical F-ratio; P: level of significance ($P < 0.05$); Height: height of the individual on the tree; Orientation: place of the individual on the tree; Dbh: tree diameter at breast height; P/T : ratio of total rainfall–annual mean temperature; Type of forest: beech forest or oakwood. Significant values are shown in bold.

Table 4 – Results of GLMMs.

Response variables	Effect	Presence				Abundance			
		Coef. (SE)	df	F-ratio	P	Coef. (SE)	df	F-ratio	P
Asexual rep.	Size	1.297 (0.042)	5252	945.49	<0.000	0.570 (0.012)	5252	2196.57	<0.000
	Height	0.002 (0.021)	2	0	0.906	0.007 (0.007)	2	0.99	0.424
	Orientation	-0.083 (0.073)	7	1.3	0.292	0.023 (0.027)	7	0.74	0.418
	Dbh	0.000 (0.009)	179	0	0.979	0.007 (0.004)	179	3.12	0.079
	P/T	0.004 (0.002)	15	3.5	0.081	0.002 (0.001)	15	5.91	0.028
	Type of forest	-1.335 (1.011)	1	1.74	0.413	-0.640 (0.359)	1	3.18	0.325
Isidia	Size	1.124 (0.040)	5252	768.21	<0.000	0.634 (0.020)	5252	936.84	<0.000
	Height	0.077 (0.020)	2	14.25	0.064	0.062 (0.013)	2	22.67	0.041
	Orientation	0.150 (0.075)	7	3.93	0.088	0.079 (0.048)	7	2.68	0.146
	Dbh	0.001 (0.012)	179	0.01	0.904	0.006 (0.008)	179	0.71	0.402
	P/T	-0.003 (0.002)	15	1.54	0.234	-0.001 (0.001)	15	0.92	0.353
	Type of forest	-0.319 (1.196)	1	0.07	0.834	-0.405 (0.783)	1	0.27	0.696
Soredia	Size	1.073 (0.043)	5252	606.37	<0.000	0.541 (0.012)	5252	1815.68	<0.000
	Height	-0.056 (0.023)	2	6.03	0.133	-0.013 (0.007)	2	3.5	0.202
	Orientation	-0.154 (0.082)	7	3.55	0.101	-0.004 (0.028)	7	0.03	0.877
	Dbh	-0.002 (0.009)	179	0.09	0.759	0.007 (0.005)	179	2.18	0.141
	P/T	0.005 (0.002)	15	7.04	0.018	0.003 (0.001)	15	9.7	0.007
	Type of forest	-0.746 (0.873)	1	0.73	0.550	-0.556 (0.477)	1	1.36	0.451
Apothecia	Size	1.551 (0.051)	5252	894.18	<0.000	1.151 (0.026)	5252	1875.19	<0.000
	Height	-0.180 (0.021)	2	69.35	0.014	-0.083 (0.012)	2	43.53	0.022
	Orientation	-0.050 (0.082)	7	0.38	0.557	0.206 (0.055)	7	13.83	0.007
	Dbh	-0.015 (0.039)	179	0.15	0.702	-0.015 (0.033)	179	0.22	0.642
	P/T	-0.012 (0.007)	15	3	0.104	-0.011 (0.006)	15	3.62	0.076
	Type of forest	3.692 (2.945)	1	1.57	0.429	3.136 (2.393)	1	1.72	0.415

Notes: Coef: variable coefficient in the pattern; SE: standard error; F-ratio: statistical F-ratio; P: level of significance ($P < 0.05$); Size: thallus size; Height: height of the individual on the tree; Orientation: place of the individual on the tree; Dbh: tree diameter at breast height; P/T: ratio of total rainfall–annual mean temperature; Type of forest: beech forest or oakwood; Rep.: reproduction. Significant values are shown in bold.

The random variable tree showed significant effects in all of the models, which suggested that undocumented factors for trees may be responsible for the differences in the modelled variables. The same held for the random variable stand, which was also significant in most cases, except for the presence of sexual reproduction, abundance of apothecia, and size of individuals.

Sizes structure of *Lobaria pulmonaria* populations

Sizes structure of the populations studied presents, in most cases, the same pattern, dominating smaller individuals (class 1) and decreasing progressively the number of individuals in the other classes (Table 5). Only in three of the populations studied the number of individuals of class 2 is larger than the class 1 (populations 7, 8, and 16). It is also noteworthy the population 15, which almost not have individuals in classes 3 and 4 (Table 5). Moreover, none of the 16 populations showed a high number of individuals in the biggest classes compared to the smallest ones. In this sense, the number of individuals of the first class represents over 60 % of the total number of individuals, showing a clear negative trend towards the major classes (Table 5).

The smallest individuals belonging to class 1 have almost no apothecia (Table 6), being classes 3 and 4 those where the number of individuals with apothecia is higher. As for asexual reproductive structures, there are a greater number of individuals of class 2 with isidia and/or soredia. However, taking

account the total number of individuals per class, we pointed out that the percentage of individuals with any of the reproductive structure increases with the class, whereas the percentage of juvenile individuals greatly decreases from class 1 to class 4 (Table 6).

Table 5 – Individual number of *L. pulmonaria* of each size class present in each of the studied populations.

Locality	Class 1	Class 2	Class 3	Class 4
1	177	81	11	11
2	171	129	32	21
3	146	128	41	27
4	134	88	17	10
5	243	50	7	0
6	344	79	27	14
7	95	114	24	17
8	102	128	40	28
9	193	41	20	24
10	201	58	9	6
11	192	44	19	12
12	216	32	4	10
13	112	63	16	8
14	729	109	45	40
15	215	44	0	1
16	56	115	62	21
Total	3326	1303	374	250

Table 6 – Total number and percentage of juvenile individuals and individuals with the different reproductive structures in each of the size classes considered.

	Isidia	Soredia	Apothecia	Juvenile	Total individuals
Class 1	178 (5.35 %)	380 (11.42 %)	1 (0.03 %)	2805 (84.33 %)	3326
Class 2	368 (28.24 %)	735 (56.40 %)	17 (1.30 %)	395 (30.31 %)	1303
Class 3	138 (36.89 %)	281 (75.13 %)	25 (6.68 %)	63 (16.84 %)	374
Class 4	125 (50 %)	199 (79.6 %)	32 (12.8 %)	26 (10.4 %)	250

Relationship between size and probability of reproduction

All the logistic models built to find the relationship between size and reproductive capacity were highly significant in all cases (apothecia, size estimator = 0.0047, $P < 0.0001$; isidia, size estimator = 0.0076, $P < 0.0001$; soredia, size estimator = 0.0263, $P < 0.0001$).

The probability that a reproductive structure will be present for a given thallus size is shown in Fig 2. Curves clearly differed for the types of reproductive structures. Individuals of at least 52 cm² reached a 50 % probability of having soredia (254 cm² for 100 % probability), whereas a five-fold higher size (253 cm²) is needed for a 50 % probability of isidia formation (952 cm² for 100 % probability) or 20-fold higher (951 cm²) to reach the same probability of bearing apothecia (2082 cm² for 100 % probability).

Discussion

Importance of size on reproduction

Lobaria pulmonaria seems to need a minimum size to form reproductive structures and uses a mixed strategy of early asexual reproduction and later sexual. As a general rule, larger individuals

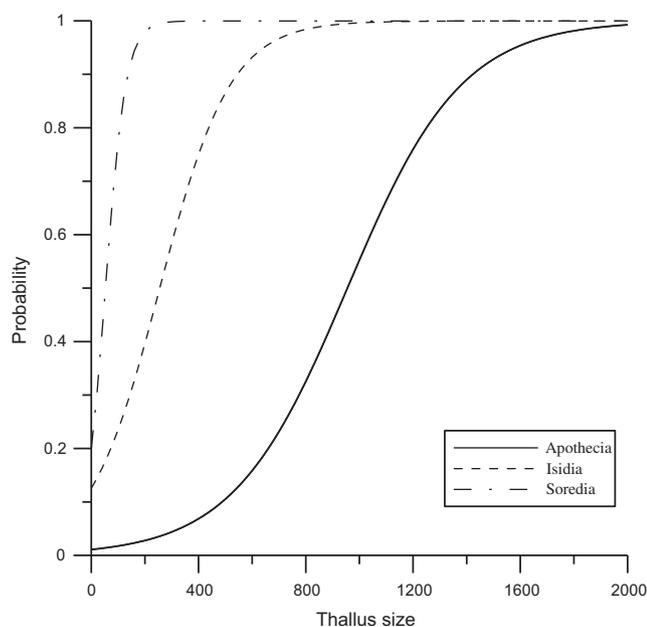


Fig 2 – Reproductive probability of *Lobaria pulmonaria* in relation to thallus size. Axis x: thallus size (cm²); Axis y: reproductive probability.

are more likely to bear any of the three reproductive structures. Moreover, we found that the formation of the reproductive structures along the life of an individual follows a certain sequence as indicated by size, the smallest individuals of the population reproduce by soredia, midsized individuals reproduced also by isidia, and the largest reproduced sexually.

Previous studies about the influence of thallus size on the reproductive capacity in *L. pulmonaria* have been developed, founding similar results although they were based on a lower number of populations and individuals and were located at higher latitudes. Mykriukov et al. (2010) and Hilmo et al. (2011a) found in their respective studies a direct relationship between thallus size of *L. pulmonaria* individuals and their asexual and sexual reproductive capacity. In the same way, Larsson & Gauslaa (2011) pointed out that individuals of *L. pulmonaria* with 985 mm² of size not developed reproductive structures, and they were considered juvenile thalli. These results are according with our analysis which indicates that the mean size of an individual to present soredia with a probability of 50 % is of almost 6000 mm².

On the other hand, the mixed strategy of early asexual proliferation and late sexual reproduction has also been found for other lichens such as *Lasallia pustulata* (Hestmark 1992). In any case, the sole use of diachronic approaches can only suggest a sequence, which can only be confirmed with long-term monitoring of individual thalli. However, our findings clearly showed that thallus size is not the only influence on reproductive behaviour and the ontogenetic sequence of these structures in an individual; some macro (stand level) and microclimatic (tree level) predictors are also involved.

The high number of juvenile individuals, the accompanying increase in asexually proliferating individuals, the almost absolute absence of sexual reproduction in small thalli indicate that size is a good indicator of developmental stage and that there is a strong disposition towards having different modes of reproduction along ontogeny. As we discussed in the introduction, some authors have proposed similar ideas about the relationship between size and sexual reproduction but usually on the basis of far less exhaustive data sets and mainly on field observations (Ramstad & Hestmark 2001; Hestmark et al. 2004; Jackson et al. 2006; Hilmo et al. 2011a).

Size distribution of individuals in our studied populations was strongly skewed; most were small, whereas far fewer individuals were large. This structure has also been found in populations of other lichens (Hestmark 1992; Hestmark et al. 2004). For instance, Hestmark (1992) pointed out that in the case of *Lasallia pustulata*, this demographic structure should indicate that the populations were expanding and had not yet exhausted the local available, suitable habitat. If this idea were true, all of our populations would be expanding.

However, the idea needs to be qualified since, as Gauslaa (2006) pointed out, lichen size depends not only on external factors such as the amount of light received during the hydrated state, but also on various internal factors, of which the investment in soredia plays a significant role. In that case, individuals that invest in soredia would be decreasing growth; thus, the populations might not always represent young populations, given their small mean size and the individuals that are investing in asexual reproduction by soredia. Moreover, Hilmo *et al.* (2011b) in an experiment about the establishment success of diaspores of *L. pulmonaria* and *Lobaria scrobiculata* found that this establishment success also depended on species-specific differences in morphological and physiological traits of reproductive structures. So, the probability of establishment may influence the size distribution of a population and the ratio between juvenile and reproductive individuals. In summary, we think that this demographic structure could be a result of high number of factors, where high mortality of thalli when they reach a specific size must be also included in our hypothesis. High mortality could be caused by different factors, such as e.g., competition, instability of the substrate or insect attack. So, further long-monitoring studies must be developed in order to elucidate all the factors involved in the population structure of lichen species.

Environmental impact on reproduction

Reproductive capacity in *Lobaria pulmonaria* not only depends on thallus size, but also on environmental conditions. Macroclimatic variables determine the abundance of asexual reproduction by soredia. However, the presence and abundance of isidia seems to be related to some microclimate/tree level factors. Isidia structures were more frequent in trunk areas farther from the ground where they were exposed to greater microclimate stress (higher radiation and temperatures, lower humidity). In this sense, Larsson & Gauslaa (2011) already pointed out that it is important not to exclude the influence of environmental conditions in the reproductive capacity of *L. pulmonaria* and mainly in the change between a juvenile and an asexual reproductive stage.

Furthermore, microclimatic variables also affected sexual reproduction because the abundance of apothecia is higher at the tree base and northern exposures, which could be considered the wettest surfaces of trees at these latitudes. Several authors have also found the influence on microclimatic variables on sexual reproduction in lichens (Monte 1993; Jackson *et al.* 2006; Tolpysheva & Timofeeva 2008; Mykriukov *et al.* 2010), although with different conclusions. For instance, Monte (1993) and Tolpysheva & Timofeeva (2008) found that production of apothecia increased when the microclimatic conditions were better. Contrarily, Seymour *et al.* (2005) indicated that in hostile environments many lichens produce sexual structures, often in abundance. In vascular plants, similar controversial results in relation to the effect of increasing environmental stress on reproduction have been reported (Saikkonen *et al.* 1998; Sandvik 2001; Aragón *et al.* 2009). This is an interesting point to tackle in the future to elucidate the reproductive costs to lichen species under contrasting environmental stresses.

And finally, we have also found that these trends are rather similar in the two types of forests considered because we did not find any effect of this habitat variable on any of the modelled variables. This lack of effect is especially relevant because habitat differences were critical to the performance of this lichen in Mediterranean forests (Belinchón *et al.* 2009, 2011). However, Mykriukov *et al.* (2010) found that the proportion of fertile individuals of *L. pulmonaria* in the Urals depended on tree species, being higher in deciduous than on coniferous trees.

Conclusions

In the future, if the size of individuals and their reproductive capacity depend on the precipitation and temperature (i.e., drought stress), climate changes may present a major problem for the species. Currently, we know that sexual reproduction in this species is very rare in Spain; only six populations had at least one individual with apothecia. Because current climate models predict a sharp decrease in rainfall and an increase in temperature, these conditions may affect the population structure by diminishing the average thallus size, further compromising the ability to reproduce sexually and the capability to adapt with this reduced genetic load.

Studies on vascular plants to assess their responses to climate changes are providing valuable information about the ability of these plants to respond to these changes (Tøtland 2001; Freeman *et al.* 2003; Molau *et al.* 2005; Giménez-Benavides *et al.* 2007; Marrero-Gómez *et al.* 2007). Although the mechanisms for adapting to climate changes are not yet known for lichens, the ability of *Lobaria pulmonaria* populations in more stressful areas to accommodate such changes should most likely determine the likelihood that they can locally persist, especially if their ability to reproduce sexually is linked to a minimal size.

Moreover, current habitat fragmentation can reduce the ability of species to quickly respond to climate change by colonizing new habitats or migrating simultaneously with their habitat shifts in altitude or latitude (Travis 2003). In the case of lichen species, their capacity to migrate to suitable habitats in response to climate change would depend on their type of reproduction. In this sense, different studies have already shown that differences in the capacity of lichen species to disperse within and between patches of habitat depend on whether they reproduce sexually or asexually (Hedenås *et al.* 2003; Löbel *et al.* 2006a, b; Scheidegger & Werth 2009). The additive effect of climate change and habitat fragmentation can accentuate the difficulties of lichen species to respond to this new scenario, especially for species that proliferate asexually and are thus limited in their dispersal. There are also negative effects from genetic isolation, which could reduce the potential to adapt to climate change *in situ* (Brooker 2006).

Acknowledgments

This research was supported by the Ministerio de Educación y Ciencia of Spain (project BIOFRAG, CGL2007-66066-C04-04/BOS), Comunidad Autónoma Castilla-La Mancha (POII09-0286-4849), Ministerio de Ciencia e Innovación (project

BIOCON, CGL2010-22049/BOS), and Comunidad Autónoma de Madrid (REMEDINAL2, P2009/AMB-1783). We thank Dr Méndez and Dr Albert (Spain), and Dr Gauslaa (Norway) for their valuable comments and Dr Beth E. Hazen (USA) for English revision and comments.

REFERENCES

- Aragón CF, Méndez M, Escudero A, 2009. Survival costs of reproduction in a short-lived perennial plant: live hard, die young. *American Journal of Botany* **96**: 904–911.
- Barot S, Gignoux J, Legendre S, 2002. Stage-classified matrix models and age estimates. *Oikos* **96**: 56–61.
- Bazzaz FA, Reekie EG, 1985. The meaning and measurement of reproductive effort in plants. In: White J (ed.), *Studies on Plant Demography: a festschrift for John L. Harper*. Academic Press, London, pp. 373–387.
- 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 MAG, 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.
- Belinchón R, Martínez I, Aragón G, de la Cruz M, Escudero A, 2011. Fine spatial pattern of an epiphytic lichen species is affected by habitat conditions in two forest-types in the Mediterranean region of the Iberian Peninsula. *Fungal Biology* **115**: 1270–1278.
- Blanco E, Casado MA, Costa M, Escribano R, García M, Génova M, Gómez A, et al., 2005. *Los Bosques Ibéricos*. Planeta, Barcelona.
- Breslow NE, Clayton DG, 1993. Approximate inference in generalized linear mixed models. *Journal of the American Statistical Association* **88**: 9–25.
- Brooker RW, 2006. Plant–plant interactions and environmental change. *New Phytologist* **171**: 271–284.
- Büdel B, Scheidegger C, 2008. Thallus morphology and anatomy. In: Nash III T (ed.), *Lichen Biology*. Cambridge University Press, Cambridge, pp. 40–68.
- Clayden SR, 1998. Thallus initiation and development in the lichen *Rhizocarpon lecanorinum*. *New Phytology* **139**: 685–695.
- Denison WC, 2003. Apothecia and ascospores of *Lobaria oregana* and *Lobaria pulmonaria* investigated. *Mycologia* **95**: 513–518.
- Freeman DC, Harper KT, Charnov EL, 1980. Sex change in plants: old and new observations and new hypothesis. *Oecologia* **47**: 222–232.
- Freeman RS, Brody AK, Neefus CD, 2003. Flowering phenology and compensation for herbivory in *Ipomopsis aggregata*. *Oecologia* **136**: 394–401.
- Friedl T, Büdel B, 2008. Photobionts. In: Nash III T (ed.), *Lichen Biology*. Cambridge University Press, Cambridge, pp. 9–26.
- Gaston KJ, 2003. *The Structure and Dynamics of Geographic Ranges*. Oxford University Press, Oxford.
- Gaston KJ, 2009. Geographic range limits: achieving synthesis. *Proceedings of the Royal Society B* **276**: 1395–1406.
- Gauslaa Y, 2006. Trade-off between reproduction and growth in the foliose old forest lichen *Lobaria pulmonaria*. *Basic and Applied Ecology* **7**: 455–460.
- Gauslaa Y, Solhaug KA, 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.
- Giménez-Benavides L, Escudero A, Iriondo JM, 2007. Reproductive limits of a late-flowering high-mountain Mediterranean plant along an elevational climate gradient. *New Phytologist* **173**: 367–382.
- Guisan A, Edwards TC, Hastie T, 2002. Generalized linear and generalized additive models in studies of species distributions: setting the scene. *Ecological Modelling* **157**: 89–100.
- Harper JL, White J, 1974. The demography of plants. *Annual Review of Ecology and Systematics* **5**: 419–463.
- Hedenäs H, Bolyukh VO, Jonsson BG, 2003. Spatial distribution of epiphytes on *Populus tremula* in relation to dispersal mode. *Journal Vegetation Science* **14**: 233–242.
- Hestmark G, 1992. Sex, size, competition and escape – strategies of reproduction and dispersal in *Lasallia pustulata* (Ascomycetes). *Oecologia* **92**: 305–312.
- Hestmark G, 1997. Competitive behaviour of umbilicate lichen – an experimental approach. *Oecologia* **111**: 523–528.
- Hestmark G, Skogedal O, Skullerud Ø, 2004. Growth, reproduction, and population structure in four alpine lichens during 240 years of primary colonization. *Canadian Journal of Botany* **82**: 1356–1362.
- Hilmo O, Ely-Aastrup H, Hytteborn H, Holien H, 2011a. Population characteristics of old forest associated epiphytic lichens in *Picea abies* plantations in the boreal rainforest of Central Norway. *Canadian Journal of Forest Research* **41**: 1743–1753.
- Hilmo O, Rocha L, Holien H, Gauslaa Y, 2011b. Establishment success of lichen diaspores in young and old boreal rainforests: a comparison between *Lobaria pulmonaria* and *L. scrobiculata*. *Lichenologist* **43**: 241–255.
- Honegger R, 1996. Experimental studies of growth and regenerative capacity in the foliose lichen *Xanthoria parietina*. *New Phytologist* **133**: 573–581.
- Honegger R, 1998. The lichen symbiosis: what is so spectacular about it? *Lichenologist* **30**: 193–212.
- Honegger R, Scherrer S, 2008. Sexual reproduction in lichen-forming ascomycetes. In: Nash III T (ed.), *Lichen Biology*. Cambridge University Press, Cambridge, pp. 94–103.
- Jackson HB, Clair L, Egget D, 2006. Size is not a reliable measure of sexual fecundity in two species of lichenized fungi. *The Bryologist* **109**: 157–165.
- Jahns HM, Herold K, Beltman HA, 1978. Chronological sequence, synchronization and induction of the development of fruit bodies in *Cladonia furcata* var. *furcata* (Huds.) Schrad. *Nova Hedwigia* **30**: 469–525.
- Korpelainen H, 1998. Labile sex expression in plants. *Botanical Review* **73**: 157–180.
- Larsson P, Gauslaa Y, 2011. Rapid juvenile development in old forest lichens. *Botany* **89**: 65–72.
- Lawrey JD, 1980. Sexual and asexual reproductive patterns in *Parmotrema* (Parmeliaceae) that correlate with latitude. *The Bryologist* **83**: 344–350.
- Lennartsson T, Oostermeijer JGB, 2001. Demographic variation and population viability in *Gentianella campestris*: effects of grassland management and environmental stochasticity. *Journal of Ecology* **89**: 451–463.
- Löbel S, Snäll T, Rydin H, 2006a. Metapopulation processes in epiphytes inferred from patterns of regional distribution and local abundance in fragmented forest landscapes. *Journal of Ecology* **94**: 856–868.
- Löbel S, Snäll T, Rydin H, 2006b. Species richness patterns and metapopulation processes – evidence from epiphyte communities in boreo-nemoral forests. *Ecography* **29**: 169–182.
- Marrero-Gómez MV, Oostermeijer JGB, Carqué-Álamo E, Bañares-Baudet A, 2007. Population viability of the narrow endemic *Helianthemum juliae* (Cistaceae) in relation to climate variability. *Biological Conservation* **136**: 552–562.
- McCullagh P, Nelder JA, 1989. *Monographs on Statistics and Applied Probability*. Generalized Linear Models, 2nd edn., vol. 37, Chapman and Hall/CRC.
- Méndez M, 1999. Effects of sexual reproduction on growth and vegetative propagation in the perennial geophytes *Arum italicum* (Araceae). *Plant Biology* **1**: 115–120.

- Méndez M, Karlsson PS, 2004. Between-population variation in size-dependent reproduction and reproductive allocation in *Pinguicula vulgaris* (Lentibulariaceae) and its environmental correlates. *Oikos* **104**: 59–70.
- Milla R, Escudero A, Iriondo JM, 2009. Inherited variability in multiple traits determines fitness in populations of an annual legume from contrasting latitudinal origins. *Annals of Botany* **103**: 1279–1289.
- Molau U, Nordenhall U, Eriksen B, 2005. Onset of flowering and climate variability in an alpine landscape: a 10-year study from Swedish Lapland. *American Journal of Botany* **92**: 422–431.
- Monte M, 1993. The influence of environmental conditions on the reproduction and distribution of epilithic lichens. *Aerobiologia* **9**: 169–179.
- Muñoz J, Felicísimo AM, Cabezas F, Burgaz AR, Martínez I, 2004. Wind as a long-distance dispersal vehicle in the southern hemisphere. *Science* **304**: 1144–1147.
- Mykriukov VS, Mikhailova IN, Scheidegger C, 2010. Reproductive parameters of *Lobaria pulmonaria* (L.) Hoffm. in the Urals. *Russian Journal of Ecology* **41**: 475–479.
- Otálora MAG, Martínez I, O'Brien H, Molina MC, Aragón G, Lutzoni F, 2010. Multiple origins of high reciprocal symbiotic specificity at an intercontinental spatial scale among gelatinous lichens (Collemaataceae, Lecanoromycetes). *Molecular Phylogenetics and Evolution* **56**: 1089–1095.
- Picó FX, 2002/3. Desarrollo, análisis e interpretación de los modelos matriciales para la Biología de la Conservación. Ecosistemas URL: <http://www.aeet.org/ecosistemas/023/investigacion2.htm>
- Pringle A, Chen D, Taylor JW, 2003. Sexual fecundity is correlated to size in the lichenized fungus *Xanthoparmelia cumberlandia*. *Bryologist* **106**: 221–225.
- Ramstad S, Hestmark G, 2001. Population structure and size-dependent reproductive effort in *Umbilicaria spodochoera*. *Mycolgia* **93**: 453–458.
- Saikkonen K, Faeth SH, Helander M, Sullivan TJ, 1998. Fungal endophytes: a continuum of interactions with host plants. *Annual Review of Ecology and Systematics* **29**: 319–343.
- Sánchez-Palomares O, Sánchez-Serrano F, Carretero-Carrero MP, 1999. *Modelos y Cartografía de Estaciones Climáticas Termopluriométricas para la España Peninsular*. INIA, Madrid.
- Sandvik SM, 2001. Somatic and demographic costs under different temperature regimes in the late-flowering alpine perennial herb *Saxifraga stellaris* (Saxifragaceae). *Oikos* **93**: 303–311.
- Seymour FA, Crittenden PD, Dyer PS, 2005. Sex in the extremes: lichen-forming fungi. *Mycologist* **19**: 51–58.
- Scheidegger Ch, 1995. Early development of transplanted isidioid soredia of *Lobaria pulmonaria* in an endangered population. *Lichenologist* **27**: 361–374.
- Scheidegger Ch, Werth S, 2009. Conservation strategies for lichens: insights from population biology. *Fungal Biology* **23**: 55–66.
- Shipley B, Dion J, 1992. The allometry of seed production in herbaceous angiosperms. *The American Naturalist* **139**: 467–483.
- Tolpysheva TY, Timofeeva AK, 2008. The effect of the substrate on the growth and reproduction of the lichens *Cladonia rangiferina* and *C. mitis*. *Moscow University Biological Sciences Bulletin* **63**: 170–177.
- Tøtland O, 2001. Environment-dependent pollen limitation and selection on floral traits in an alpine species. *Ecology* **82**: 2233–2244.
- Travis MJM, 2003. Climate change and habitat destruction: a deadly anthropogenic cocktail. *Proceedings of the Royal Society of London* **270**: 467–473.
- Tretiac M, Carpanelli A, 1992. Chlorophyll content and morphology as factors influencing the photosynthetic rate of *Parmelia caperata*. *Lichenologist* **24**: 81–90.
- Verbeke G, Molenberghs G, 1997. *Linear Mixed Models in Practice: a SAS-oriented approach* Lecture Notes in Statistics 126. Springer, New York.
- Warner RR, 1975. The adaptive significance of sequential hermaphroditism in animals. *American Naturalist* **109**: 61–82.
- Wesselingh RA, Jong TJ de, Klinkhamer PGL, van Dijk MJ, Schlatmann EGM, 1993. Geographical variation in threshold size for flowering in *Cynoglossum officinale*. *Acta Botanica Neerlandica* **42**: 81–91.
- Wesselingh RA, Klinkhamer PGL, Jong TJ de, Klinkhamer PGL, Boorman LA, 1997. Threshold size for flowering in different habitats: effects of size-dependent growth and survival. *Ecology* **78**: 2118–2132.
- Williams GC, 1975. *Sex and Evolution*. Princeton University Press, New Jersey.
- Wilson RJ, Gutiérrez D, Gutiérrez J, Martínez D, Agudo R, Monserrat VJ, 2005. Changes to the elevational limits and extent of species ranges associated with climate change. *Ecology Letters* **8**: 1138–1146.
- Young AG, Clarke GM, 2000. *Genetics, Demography and Viability of Fragmented Populations*. Cambridge University Press, Cambridge.