Global Ecology and Conservation 17 (2019) e00563

Contents lists available at ScienceDirect

Global Ecology and Conservation

journal homepage: http://www.elsevier.com/locate/gecco

Original Research Article

Optimal census method to estimate population sizes of species growing on rock walls: The case of mature *Primula pedemontana*

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ARTICLE INFO

Article history:
Received 17 December 2018
Received in revised form 11 February 2019
Accepted 11 February 2019

Keywords:
Minimal effort
Census optimization
Rupicolous flora
Monitoring
Inaccessibility

ABSTRACT

This study calculates the minimum effort needed to survey the species *Primula pedemontana* Thomas ex. Gaudin on the vertical walls of Aguja de Pastel in the Curavacas Massif (Spain). The short and variable period of flowering that this plant has, the inaccessibility and the physiognomy of the place in which it grows, the resources needed, as well as the meteorological harshness linked to the time of the year in which the censuses are carried out make it a priority to optimize the time spent on fieldwork. For this reason, we designed a method to reduce the sampling load. This method allows us to reduce the time spent and the material, methodological and human resources used in censuses, while maintaining the highest possible precision.
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1. Introduction

Primula pedemontana Thomas ex Gaudin is a European high mountain plant belonging to the *Primulaceae* family, which has a singular alpine-Cantabrian disjunction. It is distributed throughout the western French and Italian Alps, as well as the Cantabrian mountain range (Palencia, Spain) where it appears only in an extremely reduced area of the Curavacas Massif (Valentine and Kress, 1972; Kress, 1997). The Cantabrian Mountains mark the western and southern limits for the species under study in the Curavacas Massif. It shelters in rock fissures, in fresh exposures and oozing environments (Ruiz de Gopegui et al., 2010), which represent an extreme habitat (Alfaro-Saiz, 2016). Migration of propagules or "rescue effect" (Hanski and Michael, 1997), from the rest of conspecific populations, is null or negligible in this species (Alfaro-Saiz et al., 2017). This is probably due to the colonization of new individuals which is greater when the distance between the fragments is smaller and because they depend on the characteristics of the terrain and the species involved (Tellería, 2012).

Given that the allopatry is considered as the main mechanism for alpine speciation components of the *Primulaceae* family, Boucher et al. (2015) highlight the role of geographical separation in the diversification of the *Auricula* section. Thus, the taxa of the Spanish subpopulation were initially described as *P. pedemontana* subsp. *iberica* (Losa and Montserrat, 1952), although this range has not been recognized in subsequent studies based on molecular analysis (Kress, 1997; Zhang and Kadereit, 2004). Morphological differences can be attributed to local adaptations related to geographic isolation (Slatkin, 1987),

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https://doi.org/10.1016/j.gecco.2019.e00563
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which may be indicative of phenomena related to peripatric speciation, which is why it is twice as interesting to conserve the genetic heritage of the Spanish sub-population. In addition, evidence of climate change in ecosystems and in the distribution of alpine flora change (Markham, 1996; Pauli et al., 1996; Guisan and Theurillat, 2000; Stanisci et al., 2005; Walther et al. 2005; Franzén and Molander, 2012) could endanger the status of this species.

This species is considered as being very rare in the Cantabrian Mountain Range (Llamas et al., 2007) and it is among the priority plants for conservation in the area (Jiménez-Alfaro, 2008). From the Alfaro-Saiz et al. (2017) method, the regional threat-status assessment of the Spanish sub-population of *P. pedemontana* following the guidelines of the International Union for Conservation of Nature and Natural Resources −IUCN- (IUCN, 2001, 2003) is "Critically Endangered". A restricted geographical distribution, the existence of a single regional isolated sub-population and a continuous deterioration in habitat quality are sufficient to assign them to this category. This plant is not included in Spanish legislation, although it is protected at regional level in Castilla y León (JCYL, 2007) under the "Vulnerable" category. At present, this sub-population is not being monitored with public resources as its conservation is not considered a priority by the authorities.

Judging from above, we consider it appropriate to track the Spanish subpopulation, in which *Primula pedemontana* has a peripheral behaviour. The results are directly applicable in cataloguing and managing the correct measures to prevent and avoid its disappearance. Because of the inaccessibility of the place in which it grows *P. pedemontana* and the short and unpredictable flowering period an annual census is not feasible. The inherent inaccessibility has identified a number of changes to the usual census methods and monitoring the installation of scaffolding or development of climbing techniques and rappelling applied to taxa of the vascular flora threatened. However, due to the high cost in human and economic terms which make up sampling campaigns, it is necessary to optimize the method and reduce the minimum effort required to meet the proposed objective. Besides, it is necessary to consider the meteorological harshness and the instability, associated with them, which exist in the mountain areas. For example, during the 2012 fieldwork, strong storms prevented the census from being completed when only about half the face walls had been sampled. Therefore, optimizing the time and methodology is an essential task to be able to improve the monitoring of this plant in Spain. The publication of censuses of threatened plant species is not common. However, it is usual - and even periodic - the publication of censuses of threatened animal species and the discussion of the data and methods used to obtain them (García-Baquero et al., 2002).

The aim of this study is to propose a method of estimating the number of mature reproducing *Primula pedemontana* individuals to help fieldwork, optimize materials, methodological, economic and human resources deployed in conducting censuses, and facilitate the regular monitoring of the Spanish subpopulation.

2. Methods

The fieldwork was carried out from the months of May to July in 2013 and in 2014. For the annual monitoring it was necessary to prepare at least 2 people for about 15−20 h work in a single day or 4 people for about 10−13 h work which included access to the study area. For the census preparation, an adaptation of the proposed methodology for rupicolous species (species growing in rock cracks) based on the counting of "visual units" was carried out using optical devices (García et al., 2002, Goñi et al., 2006). The visual counting unit was the floral scape as it is the only part of the plant susceptible to be individualized and whose number corresponds to that of mature individuals. Two sectors were then selected, one of them formed part the east wall and the other part of north wall of the "Aguja del Pastel" peak. We defined as sectors those homogeneous units differentiated according to geomorphological and topographic criteria (walls, channels and other formations) named according to place names. The two sectors contain more than 60% of the "visual units" counted in previous censuses (Alfaro-Saiz et al., 2017). Both are vertical walls, accessible and relatively close to each other, which allows the census to be carried out in a single field day. Once the target sectors of the monitoring were selected, a photograph was taken of each one, looking for the maximum perpendicularity of the wall. Fig. 1c was compiled by merging three photographs taken at regular distances to minimize the spatial deformation. The image was measured using a Geographic Information System (GIS) in the ArcGIS-10 (ESRI, 2010) software and adapting the picture so the dimensions correspond to the actual distance, following the method developed by Goñi et al. (2006). A 10 × 10 m grid (Fig. 1) was then added to the image, assigning an individual identification code to each of the grid-cells. In the days prior to the census, several visits were made by the census team in order to determine the beginning of flowering and to calculate the optimum time of the census (i.e. days in which the maximum flowering individuals could be seen). The short and quick flowering period of the plant (which lasts about 3−4 days) allows us to establish the moment of maximum flowering of individuals in flower, as long as it was possible to carry out the previous surveys. The census was carried out by locating the boundaries of the squares and counting the "visual units" coinciding with maximum flowering present in each one, later obtaining all mature individuals of each sector by adding the results of each one. The grid-cells of the extremities, which showed a very small percentage of rock in the photograph, were eliminated to avoid the edge effect (note the absence of taxon in all of them).

The Correction Factor −CF- measures the relationship between the number of individuals observed through the use of optical devices and the actual count in accessible areas (García et al., 2002; Goñi et al., 2006). The inability to find enough accessible areas, a necessary requirement to calculate this CF, has led us to the need to adapt this calculation to our particular conditions. To do so, 30 and 40 easily delimited areas were selected in which the observers carried out two counts: one with the lens with which the census was developed (20x) and the other with a higher magnification lens, which allowed individuals to count high precision (60x), however too slow to apply such an increase covering the entire wall. Once the data were obtained, the quotient between the counts made with greater precision (60x) and the number of "visual units" counted

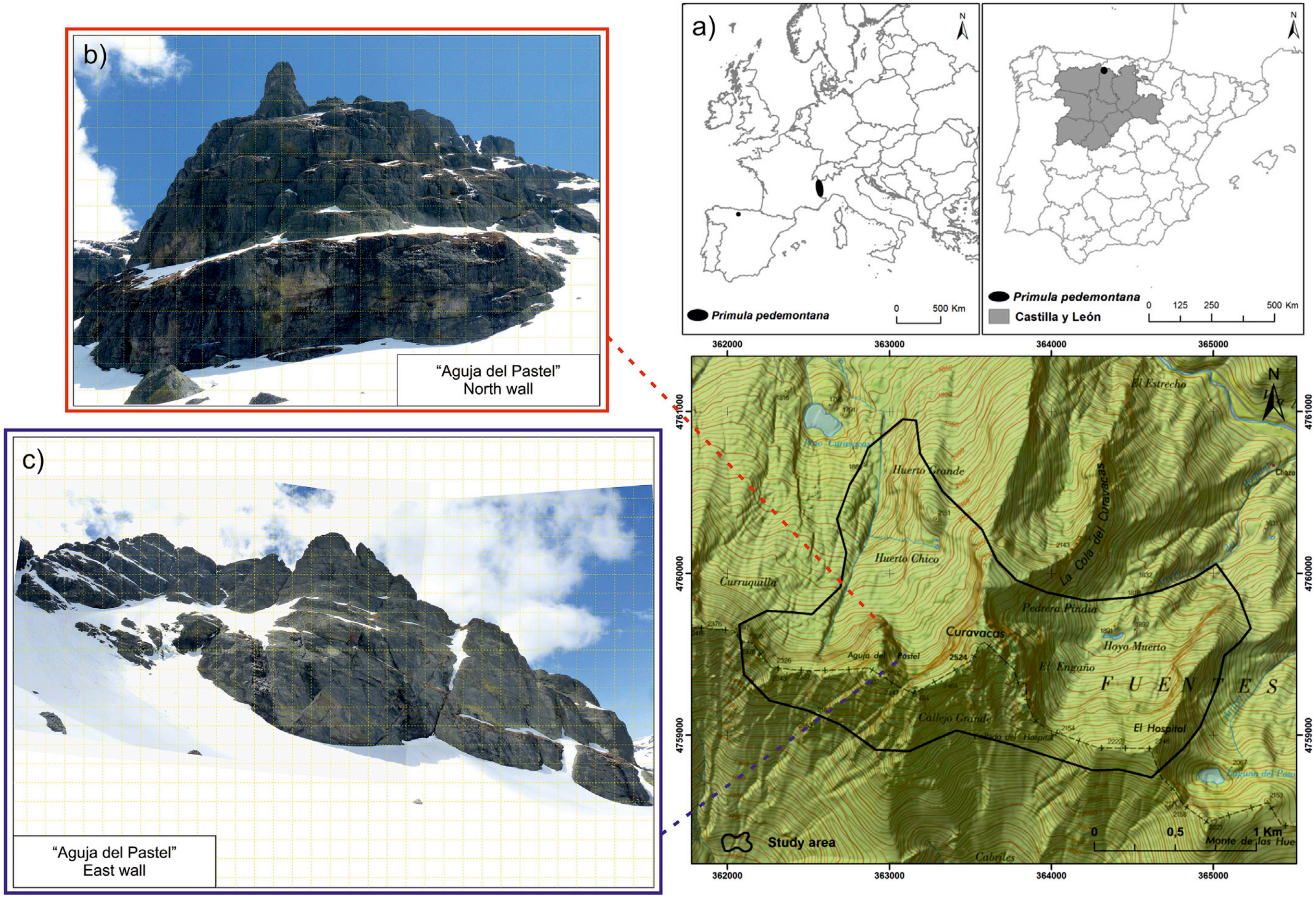

Fig. 1. a) Distribution map of *Primula pedemontana*, b) Sector "North Wall of the Aguja del Pastel", c) Sector "East Wall of the Aguja del Pastel".

using the optics with which the census were carried out (20x) was calculated. The data were adjusted to a normal distribution with the statistical software *R* (R Core Team, 2015). The average was multiplied by the total of "visual units" registered for the whole. This procedure was repeated for each observer and for the different sampling years. A total of 376 plots were included in the analysis: 229 on the east wall and 147 on the north wall. The CF obtained were 0.88 in 2013 and 1.1 in 2014.

Once the complete results of the censuses of the two vertical walls were obtained by adding the individual results of each of the grid-cells, data were analysed using *MASS* package (Venables and Ripley, 2002) and *fitdistrplus* package (Delignette-Muller and Dutang, 2015) from statistical software *R* (R Core Team, 2015). The first step was to determine the best adjustment of the data to possible distributions (Geometric, Poisson and Negative Binomial), comparing for this the frequencies observed with the theoretical frequencies by graphical analysis and through goodness of fit *Chi-square* (χ^2) test. It was then proposed as a hypothesis (H_0 the observed frequencies did not significantly differ from the expected frequencies) and therefore the data were adjusted to the relevant distribution in each case. H_0 was rejected if the *p-value* obtained was less than the chosen significance level for the test (0.05).

In order to evaluate numerically the model that showed a better adjustment, indices -AIC- *Aikake's Information Criterion* (Aikake, 1987) and *Bayesian Information Criterion* -BIC- (Schwarz, 1978) were analized. It is considered a better fit for those models that had a lower value of these indices. The graphical evaluation was carried out by analysing the cumulative distribution function *[Cumulative Distribution Function* (CDF)], *PP Plot* and *QQ Plot* graphics. *PP Plot* graphics were built from the empirical distribution function of the sample (x) and designed so as to represent each empirical observation, versus the expected value thus a straight line was obtained. *QQ Plot* graphics, which represent empirical quantiles obtained in the sample versus the corresponding quantile of the distribution, were also obtained. Taking into account that the final result (result of the complete census of the two walls) was known, we proceed to determine the smallest sample size we could stipulate in order to obtain a reliable measure of population size, thus determining the minimum effort required to carry out the census. In order to do so, the total data were adjusted to the distribution which presented the best adjustment and the parameters of this distribution were thus obtained. Subsequently, different data subsets were created, gradually reducing the sample sizes and then calculating the estimated population number to compare it with the real number and also the confidence intervals (CI). The percentage of plots sampled was reduced by 5% each time, starting at 90% and ending at 5%, always selecting plots at random. CI values were calculated using two methods: *"Maximum Likelihood"* (ML) and "*Simple Bootstrap*" (SB). ML is an approximate to the normal logarithm transformation of the variable. SB is a method to calculate CI in which the initial sample (the percentage of grid-cells sampled) was resampled 10,000 times with replacement. The process was repeated 25 times for each of the plots sampled. The results are represented by a series of box plots *(Box and whisker plot)* in which CI values (mean, maximum and minimum) appear as well as the population estimated number calculated for 25 repetitions. Finally, the number of breeding individuals recorded in the censuses, after applying the correction factor, was plotted on a graph. The results obtained for the years 2007 and 2010 calculated in previous years (Alfaro-Saiz, 2016) were added.

3. Results

The numerical results obtained after the application of the χ^2 adjustment test are shown in Table 1. The analysis shows that the best adjustment of the real data to a theoretical distribution was obtained using the Binomial Negative distribution model, as indicated by the AIC and BIC indexes, which, in all cases, indicated lower values for this distribution (which indicated a best adjustment). The significance level was found to be greater than 0.05 in the case of the Negative Binomial distribution data from the "Aguja del Pastel North Wall" sector, so in the case of this sector, we cannot reject H_0 assuming that there are no

Table 1
Results obtained by applying goodness test of *Chi-square* of fit for the years 2013 and 2014 for different distributions studied.

	2013					
	East wall			North Wall		
	Poisson	Negative Binomial	Geometric	Poisson	Negative Binomial	Geometric
χ^2 statistics	–	19.0	2330.9	3.1	11.0	336.1
Degrees of freedom (df)	7	6	7	8	7	8
χ^2 *p-value*	0	0.004	0	0	0.138	$8{,}221\ 10^{-68}$
Aikake's Information Criterion (AIC)	38670.4	1969,4	2429.7	15730.7	1375,	1486.3
Bayesian Information Criterion (BIC)	38673.8	1976,2	2433.1	15733.7	1381.4	1489.3

	2014					
	East wall			North Wall		
	Poisson	Negative Binomial	Geometric	Poisson	Negative Binomial	Geometric
χ^2 statistics	–	18.9	1430.8	–	2.9	225.0
Degrees of freedom (df)	6	5	6	6	5	6
χ^2 *p-value*	0	0.002	$5{,}312 \times 10^{-306}$	0	0.719	$8{,}822\ 10^{-46}$
Aikake's Information Criterion (AIC)	16300.3	1475.6	1967,4	3377.1	817.0	958.6
Bayesian Information Criterion (BIC)	16303.0	1482.4	1970,8	3380.1	822.9	961.6

significant differences between the expected and observed frequencies and that the sample data are adjust to the theoretical distribution. The analysis shows that the χ^2 *p-value* obtained a significance level less than 0.05 in the data obtained in the sector "Aguja del Pastel East Wall" for all distributions studied, which allowed us to reject the H_0 and therefore, the theoretical distribution studied (Table 1). However, the Negative Binomial was again the theoretical distribution that best result showed when the data from the "Aguja del Pastel East Wall" sector were modelled.

Fig. 2 shows the cumulative distribution function or empirical and theoretical CDF for all the distributions considered as well as for the years and sectors studied. As we can see from the analysis chart, the theoretical curve that best fits the observed data is that which represents the Negative Binomial distribution model, coinciding with the result obtained using the χ^2 goodness of fit test. Appendix S1 and Appendix S2 show PP Plot and QQ Plot, respectively. Again, the interpretation of the graphs confirms the best fit for this theoretical distribution.

Appendix S3 shows the probability density when the data have already been adjusted to a Negative Binomial function and the graphs that represent the empirical and theoretical cumulative distribution function (CDF) for the years 2013 and 2014 in both sectors under study.

The results obtained from calculating CI from the data subsets for each sampling percentage (5–100%) are shown in Fig. 3. The box plot diagrams allow us to visually determine that smaller intervals were obtained using "*Simple Bootstrap*" (BS) than using "*Maximum Likelihood*" (ML)" method and also how CI are reduced as the sample rate is increased.

The number of mature individuals obtained from the censuses conducted, including the results obtained by Alfaro-Saiz et al., (2017) (after applying the correction factor) are shown in Appendix S4. The year in which the highest number of mature individuals was recorded was 2013 with a total of 27,140 individuals, while the year that fewer individuals were counted was 2010 with a total of 8808.

4. Discussion

The monitoring of *Primula pedemontana* in the Curavacas Massif is the key to recognizing the evolution of this peripheral taxon in Spain, nestled in the rocky walls of fresh orientations and oozing environments. Because of their structure, habitats and species associated with rocky outcrops and vertical walls pose an additional challenge for managers and researchers (Goñi et al., 2006; Olea and Mateo-Tomás, 2013). The inherent inaccessibility has identified a number of changes to the usual census methods and monitoring the installation of scaffolding or development of climbing techniques and rappelling applied to taxa of the vascular flora threatened (Garcia et al. 2002, 2007 . Goñi et al. 2006). However, due to the high cost in human and economic terms which make up sampling campaigns, it is necessary to optimize the method and reduce the minimum effort required to meet the proposed objective (Pozo et al., 2005). This has promoted studies, such as this one, adapting methods based on the use of tools of a digital nature related to teledetection systems and GIS (Goñi et al., 2006) in order to reduce the

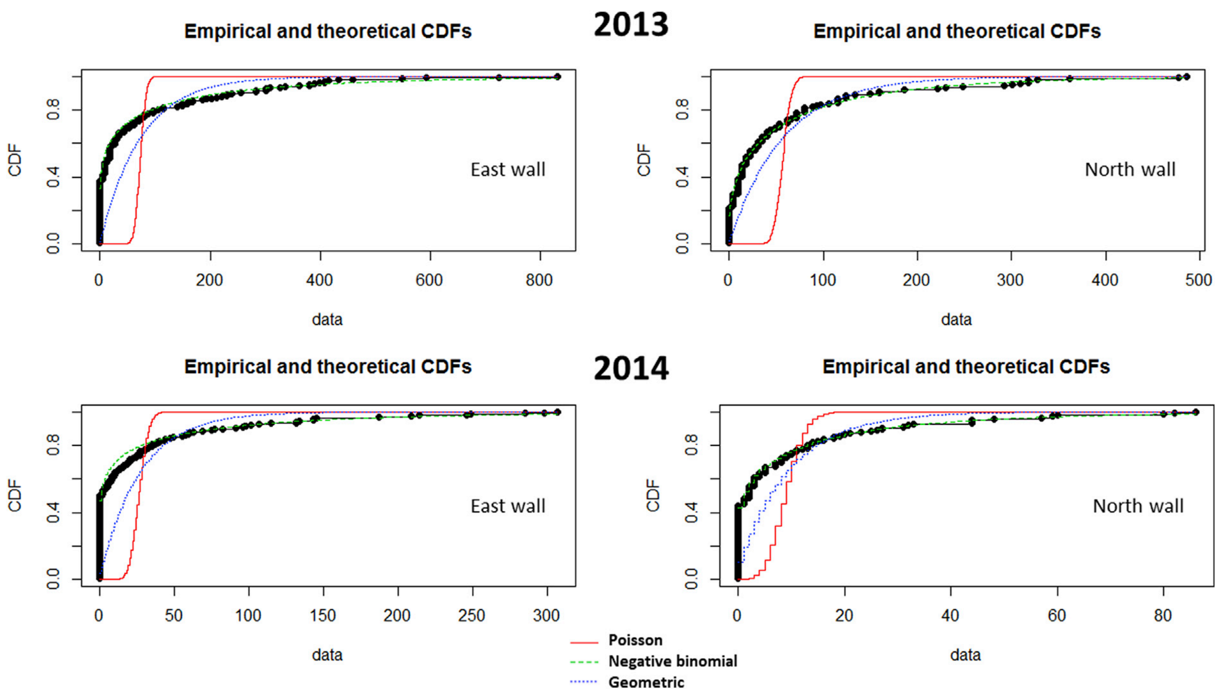

Fig. 2. Cumulative distribution function (CDF) empirical and theoretical for the years 2013 and 2014 in both studied sectors.

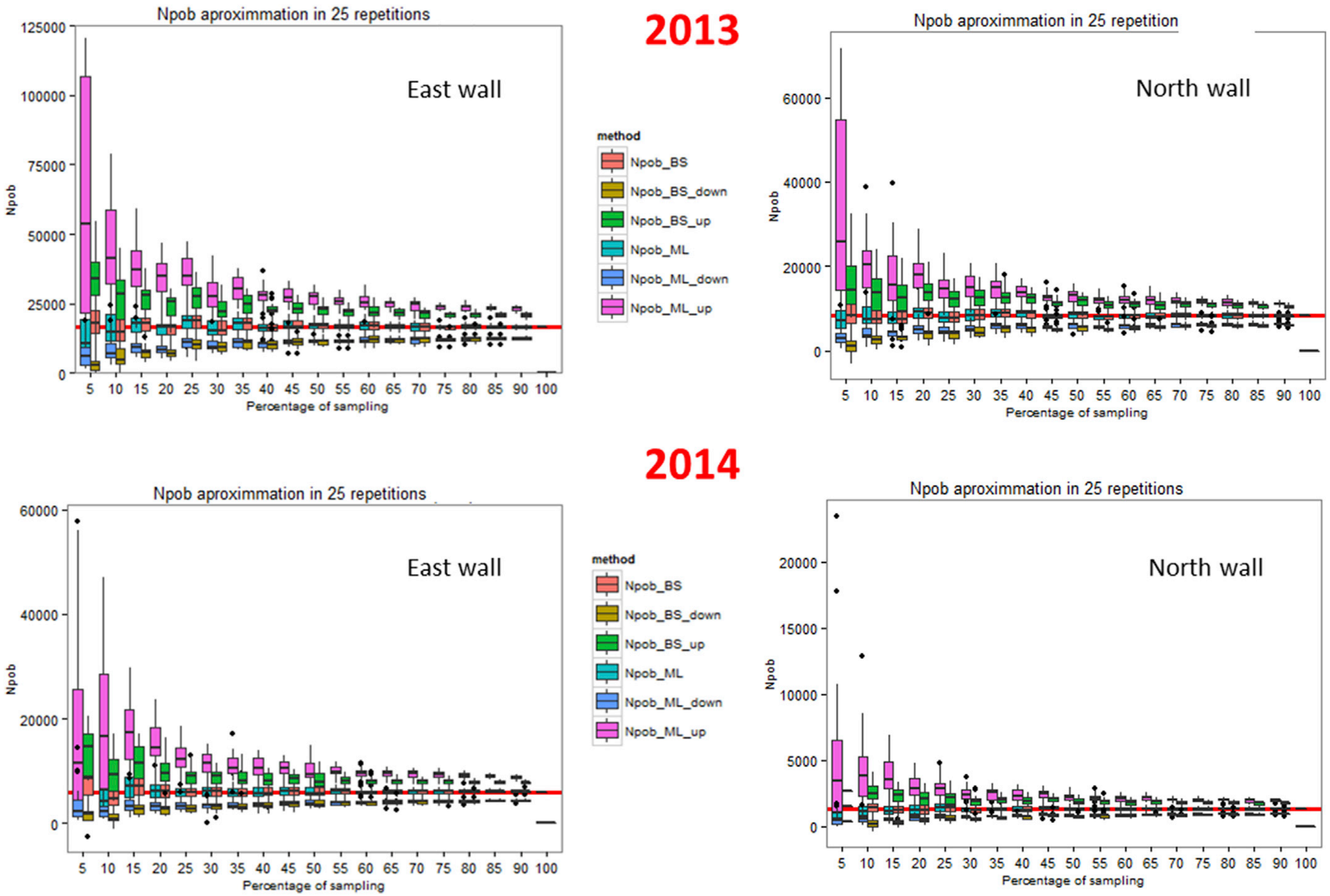

Fig. 3. Confidence Intervals (CI) calculated for each percentage of random sampling for the years 2013 and 2014 using two methods: "*Simple Bootstrap*" (BS) and "Maximum Likelihood" (ML). The red line represents the total population size. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

resources associated to fieldwork, a fact that has been evaluated in terms of economic savings (Olea and Mateo-Tomás, 2013; Gaston et al., 2014).

In this paper, we developed a method that allows us to optimize the time and materials, methodological and human resources used in the census the number of mature individuals of this species. The proposed method, as well as involving a reduction of the resources used in the census, allows for high flexibility and adaptability in the data collection. A minimum percentage of sampling units is established which can be reduced or increased depending on the weather conditions or the time available, which allows us to regulate the effort used if, for example, the meteorological conditions are inadequate and in this case evaluating the effort used against loss of accuracy. This optimized method could be applied to the study of other organisms that develop in extreme environments.

The complete census of the two walls in the years 2013 and 2014 has allowed us to make accurate calculations to validate this method. By knowing the number of mature individuals in advance, we were able to compare the results and calculate the smallest sample size, which allowed us to stipulate and obtain a reliable measure of the number of breeding individuals present in the sectors studied, thus obtaining the minimum effort necessary for census elaboration. It is the reason to recommend at least a census of the full wall.

This method could be adapted to the study of other species with similar requirements grow on inaccessible rocky cliffs. However, working with *Primula pedemontana* could be advantageous. This species has a short flowering cycle and most individuals were in bloom at the time of the census. In addition, confusing them with other species is very difficult. However, the application of the CF proposed by other authors makes it possible to estimate the total number of individuals from the number of visual units, even when these are in a different phenological state. For the calculation of the CF a sampling including at least 30 accessible sites is recommended. This ensures that the method is statistically consistent and that the parameters needed, such as its normal distribution, range or mean could be calculated correctly.

The theoretical distribution function having a best adjustment to the actual distribution of *P. pedemontana* in the vertical walls is the Negative Binomial. After studying the results, we consider this distribution to be the most adequate to adjust the data obtained from both Sectors. The close relationship which shows *Primula pedemontana* with this distribution seems to be typical of other similar species, which have an added or contagious spatial behaviour (García-Baquero et al. 2002) as it takes into account the effect of clustering or grouping of the individuals in each sample unit by using a dispersion parameter. This pattern is repeated in different plant biotypes which develop in very different habitats. This is the case of most arborescent palms studied in an area in Bolivia for which it was shown that the probability of finding a conspecific of one palm tree close to another was relatively high (Cabrera and Wallace, 2007). In the case of the cactus *Lophophora diffusa* (Croizat) Bravo in the subtropical desert in Mexico, which also had a contagious distribution in this environment, its presence is related to the nurse effect exerted by the bushy vegetation (Zúñiga et al., 2005).

Due to its greater accuracy, the method that obtained the best results in the calculation of CI was "Simple *Bootstrap*". By applying the proposed method, the number of sampled grid-cells could be reduced by 65–50%. From this percentage, we can observe that the CI begin to stabilize in all cases and the final estimate is not significantly affected so that the mean and the calculated CIs in this way would prove to be reliable and contrasted data. The minimum effort considered would be about 35% of sampled grid-cells and the recommended range would be to sample at least 40–50% of the grid-cells. If we consider a reduction of 50–65% of the grid-cells sampled in the fieldwork, with the reduction of the sampling time involved, the method significantly streamlines the census, facilitating a constant monitoring over a period of time without any significant loss of accuracy.

The individualization of the grid-cells also allows us to control their oscillation over a period of time through the labelling of "permanent plots" in which they can be constantly monitored (Loayza et al., 2015), regardless of whether these plots are randomly indicated in an annual follow-up.

Fluctuations in the number of mature individuals (Appendix S4) could be related to different factors, such as climatology in previous months and during the flowering period. We consider that the monitoring of this species will allow us to predict its evolution and to take adequate conservation measures, if necessary. The fact that there are years in which a low number of mature individuals are reported, either because the plants develop less floral scapes or because the storms cause the corollas to fall, can be related to a reduction in reproductive success mainly due to the reproductive biology of these plants. *Primula* genus presents distyly, polymorphism characterized by the presence of mutual herkogamy and self-incompatibility system between similar morphs (Solbrig, 1976; Bell, 1995; Taisma and Wolfang, 2005). In addition, fecundation depends to a large extent on the success of the pollinator. The fall of the corollas affects the visit of the pollinators, which can no longer be attracted by the intense violet colour of the same corollas. The annual follow-up of the number of mature individuals is, therefore, an indicator of the state of evolution and the future fitness of this plant.

Finally, it must be taken into account that in order to calculate the real areas and to be able to determine values such as the density of individuals at some future stage GIS techniques in 3D could be applied. Some of them are being developed, such as GIS tools which have already integrated the possibility of creating three-dimensional maps (although they are only available in some cities) and will allow for the calculation of areas by incorporating the slope of the terrain, thus correcting the effect of the relief. Another technique which could be useful in monitoring this species is the analysis of high - resolution photographs carried out on a small scale, unmanned vehicles, RPAS (Remotely Piloted Aircraft System), which have already shown a high applicability in studies related in natural environments (George et al., 2006; Hardin and Hardin, 2010) and conservation biology (Sandbrook, 2015), which has established itself as a very efficient and low-cost tool in some applications (Koh and Wich, 2012). It was also revealed as a powerful revolution in design methods applied to non-invasive monitoring wildlife study (Vermeulen et al., 2013) or biodiversity assessment (Getzin et al., 2012). The proposed method could be very useful in the experimental design of these methodologies.

Acknowledgments

We wish to thank Sonia Oreca, Tino García and David Ríos for their help and company in carrying out the censuses. We thank Donal Savage and Félix Llamas for their suggestions with the text translation. The authors are grateful with the reviewers for their comments and suggestions that have improved the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gecco.2019.e00563.

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