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1 **Census and contemporary effective population size of two populations**
2 **of the protected Spanish Moon Moth (*Graellsia isabellae*)**

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18 Short title: N and N_e of a protected moth.

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21

22 **Abstract**

23 1. *Graellsia isabellae* is a protected lepidopteran both in France and Spain. However,
24 there has been considerable debate over its conservation status. Recent literature
25 emphasised the need of monitoring population size in the different mountain ranges
26 where this iconic species occurs.

27 2. We used mark-capture-recapture and genotypes of nine molecular microsatellite
28 markers to estimate the census (N) and contemporary effective population size (N_e) of
29 two Spanish populations extending over similar size areas (10-15 km²): Puebla (Eastern
30 Spain) and Ordesa (Western Pyrenees). Only adult males were captured and analysed,
31 as sampling was based on the use of the synthesized female sex pheromone.

32 3. Estimates of N were rather different in the two populations: 3,398 males in Puebla
33 (95% CI = 2,875–4,145) and 1,500 in Ordesa (95% CI = 1,229–1,932), although the
34 area occupied by the populations was larger and more densely forested in Ordesa than
35 in Puebla.

36 4. Several lines of evidence pointed to a moderate-large contemporary N_e at Puebla
37 (173-178 individuals) and a one-order of magnitude lower N_e at Ordesa (27-49). Thus,
38 N_e/N ratios were very low (0.026 and 0.01, respectively).

39 5. We recommend *G. isabellae* to be classified as of Least Concern under the IUCN
40 criteria. However, the high temporal fragmentation index and the very low values of the
41 N_e/N ratios obtained for this species, as compared with those recorded for most others,
42 are usually taken as indicators of actual threat for their conservation. As a cautionary
43 measure, managers should aim at maintaining gene flow by ensuring connectivity of
44 *Pinus sylvestris* in these areas.

45

46 **Keywords:** capture-mark-recapture, CMR, conservation, microsatellites, inbreeding N_e ,

47 variance N_e , insect, phased emergence.

48

49 **Introduction**

50 The Spanish Moon Moth, *Graellsia isabellae* (Graells, 1849) (Lepidoptera,
51 Saturniidae), is a protected species by the Bern Convention (Appendix III; Council of
52 Europe, 1979) and the Habitats Directive of the European Union (Annexes II and V;
53 Council Directive, 1992). This univoltine moth flies at dusk from mid March to early
54 July in mountains of the eastern half of Spain, Pyrenees, and the Western Alps. Its
55 larvae exclusively feed on *Pinus sylvestris* in the Central Iberian System, Pyrenees, and
56 Alps. However, this spectacular insect inhabits forests of *P. nigra* in the southern Betic
57 Mountains and uses both *P. sylvestris* and *P. nigra* in the Eastern Iberian System, where
58 it also displays the highest genetic diversity (Marí-Mena *et al.*, 2016).

59 In the last decades, there has been considerable debate over the conservation
60 status of *G. isabellae*. Some authors warned against the scarce numbers of the Alpine
61 population (Auger-Rozenberg *et al.*, 1998), the decline of the populations of Central
62 Spain caused by the use of pesticides against *Lymmantria monacha* in the 1950s, and
63 the more recent threat posed by the use of difluorobenzon against *Thaumetopoea*
64 *pityocampa*, namely in the Western Pyrenees (Soria *et al.*, 1986). Other authors
65 suggested that the populations of *G. isabellae* from the Eastern Iberian System are
66 abundant and actually expanding (de Arce Crespo *et al.*, 2010 and references therein).
67 This seeming contradiction likely caused not only the change from Vulnerable to Data
68 Deficient at the International Union for Conservation of Nature (IUCN) Red List
69 (World Conservation Monitoring Centre, 1996), but also the removal of *G. isabellae*
70 from the Spanish Catalogue of Endangered Species (Ministerio de Medio Ambiente,
71 2000).

72 At present, the Spanish Moon Moth is protected both in France (Arrêté
73 interministériel, 2007) and Spain (Ministerio de Medio Ambiente, 2011). Romo *et al.*

74 (2012) warned about the lack of any knowledge of the size of populations of this
75 protected lepidopteran and emphasised the need of monitoring the census size of at least
76 one population per mountain range.

77 Population size constitutes the most fundamental data to be obtained when
78 evaluating conservation priorities for a species (IUCN, 2012a). However, conservation
79 biology is not only concerned by census size, usually defined as the number of
80 mature/adult individuals (N), but also by the effective population size. In classical
81 population genetics, the effective population size (N_e) is the size of an idealised
82 population that would be expected to display the rate of inbreeding or the rate of change
83 in variance of gene frequencies found in the study population. Factors such as
84 fluctuations in population size, variance in family size, stage of life cycle surveyed,
85 taxonomic group, and unequal sex ratio can cause N_e to differ from N (Frankham,
86 1995). Both parameters are strongly correlated with population viability, as census size
87 is the outcome of demographic processes which strongly affect its survival probability
88 when subject to hard selection challenges, whereas effective population size reflects
89 standing genetic variation, and thus the capacity to respond to soft selection forces and
90 so adapt to environmental changes (reviewed by Waples (2013). Effective population
91 size can be estimated at different time (long-term vs. contemporary) and spatial
92 (isolated populations vs. gene flow scenarios) scales, under various inheritance modes
93 (e.g. sex-linked genes, haplo-diploid species), and demography (e.g. polygyny,
94 overlapping generations). Congruently, a number of different approaches have been
95 developed to estimate effective size from multilocus genotypes, such as heterozygosity
96 excess, linkage disequilibrium (LD), temporal changes in allele frequency, and
97 relatedness (reviewed by Wang *et al.* (2016)).

98 In this study, we estimated census size and effective size of two Spanish

99 populations of *G. isabellae*. The first study area is an isolated and panmictic population
100 in the Eastern Iberian System. The second one is a set of Western Pyrenean localities
101 presumably interconnected by gene flow. We used traditional mark-release-recapture
102 (MRR) to calculate two estimators of the census size: the super-population and seasonal
103 adult sizes. Super-population size refers to the total number of individuals ever present
104 in the experiment, whereas seasonal adult size is the total number of individuals
105 occurring in one generation. We focused on the contemporary effective population size
106 (the one of the sampled generation or just a few generations in the past) because it is the
107 most relevant for conservation genetics. Multilocus genotypes of 195 individuals
108 sampled in 2008-2010 were used to calculate inbreeding and variance N_e using single-
109 sample and temporal methods, respectively.

110

111 **Materials and methods**

112 *G. isabellae* is a univoltine macro-moth that flies from dusk to midnight, for
113 approximately two months each year (end of April-early June) in the eastern Pyrenees
114 (Ylla i Ullastre & Sarto i Monteys, 1993). The sedentary and non-gregarious caterpillar
115 develops in five instars, and dwells from June to August in pine forests before pupating.
116 Larvae are narrowly oligophagous, foraging on *Pinus sylvestris* and *P. nigra*. Adults do
117 not feed. Females reared in captivity (average lifespan = 8.4 days) barely move from the
118 pine branches where they emerge, so males (average lifespan in captivity = 5.87 days)
119 are expected to be the dispersing sex (Ylla i Ullastre, 1997; Collectif OPIE, 1998).
120 Indeed, adult males are able to fly up to 2 km per night (Data S2).

121

122 *Study areas*

123 We estimated adult census and contemporary effective population sizes of *G.*
124 *isabellae* in two protected forests of *Pinus sylvestris*: “Puebla” stands for Natural Park
125 of *Puebla de San Miguel* (Mediterranean region), whereas “Ordesa” refers to one
126 locality within the National Park of *Ordesa y Monte Perdido* (Eurosiberian region) (Fig.
127 1). Puebla belongs to the so-called “EI” (Eastern Iberian) genetic cluster of *G. isabellae*,
128 whereas Ordesa is part of the “WP” (Western Pyrenees) one. The populations forming
129 the EI cluster show the highest levels of mitochondrial and nuclear diversity of the
130 whole distribution area. In addition, the EI cluster is the most likely origin of the
131 Pyrenean and Alpine populations (Marí-Mena *et al.*, 2016).

132

133 FIGURE 1

134

135 Puebla represents an isolated unstructured population (predominantly panmictic
136 with no apparent geographic substructuring) of *G. isabellae* feeding on *P. sylvestris*.
137 Indeed, no *G. isabellae* was collected by light trapping in the surrounding forests of *P.*
138 *nigra* and *Juniperus thurifera* (Baixeras, J. & Marí-Mena, N., personal observation). By
139 contrast, Ordesa is surrounded by pine woodland where *G. isabellae* occurs. We
140 obtained genetic data from *G. isabellae* sampled in four of these interconnected forest
141 patches: one within the National Park (Cotatuero), and three others within the National
142 Park buffer zone (Línea, Bujaruelo, and Diazas) (Fig. 2). Significant population
143 differentiation has been reported between Ordesa and the easternmost part of the
144 National Park (La Sarra site, ca. 23 km apart, pairwise $F_{ST} = 0.087$, Marí-Mena *et al.*,
145 2016). However, at present it is unknown whether Ordesa and these four surrounding
146 localities are part of a same panmictic population or gene flow is restricted to a certain
147 level. The 107 individuals sampled in 2010 in these five Pyrenean localities were
148 pooled together for some analyses, where they are referred as “National Park”.

149

150 FIGURE 2

151

152 *Mark-release-recapture*

153 Mark-release-recapture (MRR) (a.k.a. capture–mark–recapture, CMR) was
154 performed in a single adult generation for each population (3/6/2009-14/6/2009 at
155 Puebla, 29/05/2010-23/6/2010 at Ordesa). Given the pine patch-structure found in
156 Ordesa, we performed the MRR experiment where we observed it was the centre of the

157 distribution of the moth taking into account the continuity of the habitat (Fig. 2). We
158 used the synthesized female sex pheromone of *G. isabellae* (Millar *et al.*, 2010) to
159 sample individuals, and thus only adult males were included in this study. The sampling
160 design was adapted not only to the life cycle of *G. isabellae*, but also to the forest
161 structure of each study location and the human resources available (slight variations in
162 the sampling design used at Puebla and Ordesa are detailed in Data S1). The pheromone
163 dose needed to attract males from approximately 500 m was previously calculated using
164 individuals reared in captivity (Data S2). On this basis, on each sampling occasion a
165 new rubber septum (Sigma-Aldrich Co.) impregnated with 100 µg of sex pheromone
166 was used as a lure.

167 We are confident that all of the males flying during the experiment were
168 effectively exposed to being sampled sooner or later. This conclusion is based on the
169 dispersal capacity of adult males as well as the localisation of the sampling sites within
170 the relatively small areas occupied by both populations (approximately 10 km² for
171 Puebla and 15 km² for Ordesa, Fig. 2).

172

173 *Estimation of population parameters from MRR data*


174 Full capture and recapture data were entered into a matrix (Table S1) for
175 analysis with program MARK 2.1 (White & Burnham, 1999). Capture-recapture data
176 were analysed according to the Jolly-Seber (JS) model (Schwarz, 2001), with the goal
177 of estimating parameters related to abundance, daily survival and capture probability of
178 adult males. In the JS model, the proportion of marks in a sample must be an unbiased
179 estimate of the proportion of marks in the population. Average lifespan was derived
180 from daily survival estimates as in Nowicki *et al.* (2005).

181 Prior to MRR analyses, data were explored to detect heterogeneity in capture
182 results by means of a goodness-of-fit test (GOF), revealed as mandatory by previous
183 MRR analyses with Lepidoptera (Marschalek & Klein Sr, 2010). U-CARE 2.2.2
184 (Choquet *et al.*, 2005) includes a specific test for transience (Test 3.SR), which assesses
185 whether survival soon after marking is different from survival thereafter, as well as for
186 trap-dependence (Test 2.CT), which tests whether recapture probability at time i
187 depends on the past capture history. We performed the GOF tests assuming full time
188 variation for all parameters (the Cormack-Jolly-Seber (CJS) model) to detect
189 heterogeneity of capture.

190 In order to estimate population size, we applied an open-population Jolly-Seber
191 model to the mark-recapture data using the POPAN option implemented in MARK.
192 This “open” option refers to the possibility of newly emerged adults to be incorporated
193 to the super-population, not to entry by migration from neighbouring areas. For t
194 capture occasions the model provides $t-1$ estimates of θ (apparent survival), t estimates
195 of p (capture probability given the animal is alive and available for capture), $t-1$
196 estimates of β (probability of entry into the population per occasion), and N (super-
197 population size, i.e. every individual that was exposed to sampling during the course of
198 the experiment). Models were fitted using the *logit* link function for θ and \hat{p} , the
199 identity link function for \hat{N} , and the multinomial *logit* link function to constrain the set
200 of $\hat{\beta}$ parameters to ≤ 1 .

201 The selection of the most appropriate model for each dataset was based on
202 Akaike’s Information Criterion corrected for small samples size (AICc; Burnham &
203 Anderson, 1998), together with likelihood ratio tests (LRT) based on the chi-square
204 approximation. Models were scored according to the differences in AICc values
205 (Δ AICc) and relative support of each model (AICc weight). Models with the

206 significantly lowest AICc value were retained as the best compromise between a high
207 proportion of deviance explained and a low number of parameters in the model.

208 We estimated the seasonal population size of males, i.e. the number of
209 individuals occurring in one generation (Nowicki *et al.* 2005), as (flight period * super-
210 population size) / MRR experiment duration. This formula assumes that all of the males
211 flying during the experiment in the study areas were effectively exposed to being
212 sampled. We used a flight period of 60 days. We could not strictly use the approach
213 described by Nowicki *et al.* (2005), who developed a simplified method based on
214 checking the presence of flying adults at the beginning and end of the flight period, as
215 we were not sure whether the peak population size () was included in our data.

216

217 *Genetic diversity and population structure*

218 Estimates of N_e were based on the multilocus genotypes of the following
219 samples. Two temporally separated samples of individuals were taken in both areas:
220 Puebla (2008, n = 29 males; 2009, n = 32 males) and Ordesa (2009, n = 27 males; 2010,
221 n = 31 males). By doing so, we were able to apply single-sample and temporal methods
222 to estimate N_e . In addition, in 2010 we also obtained tissue from 76 males collected in
223 four other patches of the National Park and its buffer zone (Fig. 2). The 107 Pyrenean
224 males collected in 2010 are referred as “National Park”. All individuals were marked in
225 their wings as previously described and non-lethally sampled by clipping a fragment of
226 the right hind-wing tail (Vila *et al.*, 2009). Tissue sampling was performed in between
227 MRR working days. This procedure revealed the exchange of some migrants among the
228 five sites: one individual from Ordesa moved to Bujaruelo, one individual from
229 Bujaruelo and another one from Ordesa were collected at Cotatuero, and three

230 individuals from Ordesa were found at Diazas. Each wing piece was dry-stored in an
231 individual envelope and frozen at -20°C upon arrival to the lab. Genomic DNA was
232 extracted using a commercial kit (High Pure PCR Template Preparation Kit, Roche)
233 following the manufacturer’s instructions. We screened the 195 sampled males with a
234 set of nine polymorphic microsatellite loci and the protocol described by Vila *et al.*
235 (2010). Ten percent of the samples of the project were randomly re-genotyped. PCR
236 products (1.2 µL) were mixed with 16 µL formamide containing GENESCAN-500
237 (ROX) Size Standard (Applied Biosystems, ABI) and the allele size of PCR products
238 was determined on a 96-capillary 3730xl DNA Analyzer (ABI). Two researchers
239 independently scored allele peaks. Both of them used GENEMAPPER 4.0 (ABI).

240 We ran STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) to assess any clustering
241 pattern of the 107 Pyrenean individuals collected in 2010 (“National Park”).
242 Simulations were run assuming the admixture ancestry model, correlated allele
243 frequencies, and the LOCPRIOR option. We set up a burn-in of 100,000 iterations
244 followed by 500,000 iterations for parameter estimation. Each simulation was run 10
245 times, exploring values for K ranging from one to six. We inspected the values of $L(K)$
246 and determined the biologically meaningful partitions of the data using STRUCTURE
247 HARVESTER (Earl & vonHoldt, 2012).

248 Number of segregating alleles, observed and expected heterozygosities, F_{IS} -
249 analogue, and tests for departures of Hardy-Weinberg equilibrium and gametic
250 disequilibrium were calculated with FSTAT 2.9.3.2 (Goudet, 1995). Allelic richness
251 averaged over loci was obtained using rarefaction (averaging across subsamples of five
252 diploid individuals) as implemented in HP-RARE (Kalinowski, 2005). For the five
253 Pyrenean sites sampled in 2010 (“National Park”, $n = 107$), we also used FSTAT to
254 obtain the unbiased estimators of pairwise $F_{ST}(\theta)$ and their significance after 10,000

255 permutations. The frequency of null alleles was estimated using the EM algorithm as
256 implemented in FREENA (Chapuis & Estoup, 2007). We applied the F_{ST} outlier
257 method (*fdist*) to test the neutrality of the alleles at the nine loci, as implemented in
258 LOSITAN (Antao *et al.*, 2008). A total of 95,000 simulations were computed under the
259 Stepwise Mutation Model (SMM) with the options ‘neutral mean F_{ST} ’ and ‘force mean
260 F_{ST} ’.

261

262 *Effective population size*

263 *Single sample estimators.* Contemporary N_e was calculated using a bias-corrected
264 version of the linkage-disequilibrium (LD) moment method by Waples & Do (2008) as
265 implemented in NeESTIMATOR v2.01 (Do *et al.*, 2014). Parametric confidence
266 intervals were calculated. The minimum frequency of observed alleles was set at 0.05.
267 We also used the approximate Bayesian computation implemented in ONeSAMP 1.1
268 (Tallmon *et al.*, 2008) to estimate N_e by comparing eight summary statistics (including
269 LD). ONeSAMP has been recommended for moderate-large populations (Saarinen *et*
270 *al.*, 2010), although it may overestimate small N_e (Gilbert & Whitlock, 2015). We
271 established upper and lower bounds on the N_e prior considering the results of N in the
272 MRR study.

273 *Temporal methods.* We used the program MLNe (Wang & Whitlock, 2003) to calculate
274 a maximum likelihood (Wang, 2001) and a moment estimate of N_e over time for the
275 following data: Puebla: 2008-2009, Ordesa: 2009-2010, and Ordesa 2009-National Park
276 2010). For the three cases, we assumed an isolated population that is not at mutation-
277 drift equilibrium and a maximum N_e value allowed of 1500, as from our MRR study. In
278 the absence of migration, the moment estimator implemented in MLNe is equivalent to

279 the one developed by Nei & Tajima (1981) (Wang & Whitlock, 2003). In addition, we
280 used NeESTIMATOR v2.01 for computing moment estimates of N_e (Waples, 1989)
281 with two options for computing the standardised variance in allele frequency: F_e (Nei &
282 Tajima, 1981) and F_s (Jorde & Ryman, 2007). We selected Plan I (Waples, 2005), so an
283 estimate of 1500 as census size was provided. Again, the lowest allele frequency used
284 was 0.05 and parametric confidence intervals were calculated.

285 We followed Waples & Do (2010) in order to combine the different estimates of
286 effective size obtained for Puebla and Ordesa. We firstly used the single-sample
287 estimates obtained from the LD and ONeSAMP methods and calculated the harmonic
288 mean single-sample estimate, weighted by sample size and number of allelic
289 comparisons. Second, we applied “strategy 2” in order combine the temporal estimates:
290 the two-moment estimators were combined before including the ML estimate in the
291 calculations, i.e. the harmonic mean of the two moment-based estimates is then used
292 with the ML estimate to calculate an unweighted harmonic mean. Thirdly, we combined
293 temporal and single-sample estimates. The rationale for this calculation is that we were
294 mostly interested in an overall estimate of N_e that is expected to fluctuate moderately
295 around a mean value. The combined value is $1/[(\text{weight of the single-sample}$
296 $\text{method}/\text{combined estimate for single-sample method})+(\text{weight of the temporal}$
297 $\text{method}/\text{combined estimate for temporal method})]$. We considered five values of the true
298 unknown N_e (25, 50, 100, 500 and 1000) and included the maximum likelihood
299 estimates. For further details and the calculation procedure, the reader is referred to
300 Waples & Do (2010) and Data S3, respectively.

301 In an organism with discrete generations and that is non-lethally sampled, single
302 sample (inbreeding-based) N_e estimates will apply to the previous generation, whereas

303 temporal (variance N_e) estimators will apply to the period from sampling point 0 to
304 sampling point $t-1$ generations (Waples, 2005). This means that there is a mismatch
305 between the temporal frame of our estimates of N (e.g., 2009 in Puebla) and the
306 different estimates of N_e (e.g., temporal estimators obtained from Puebla will apply to
307 2008, but single-sample estimators using data from Puebla/2008 will apply to 2007). In
308 addition, the multiple-source method implemented in ONeSAMP generated N_e
309 estimates with finite 95% CIs. These estimates do not have an explicit temporal
310 reference point (Wang, 2016). Therefore, our combined estimate aims at an overall
311 estimate of N_e that is expected to fluctuate only moderately around a mean value in the
312 near past (Waples & Do, 2010).
313

314 **Results**

315 *MRR and N*

316 As many as 303 adult males of *G. isabellae* were caught and marked during the
317 nine sampling occasions in Puebla. We recaptured 23.4% of the marked individuals. In
318 Ordesa, 322 adult males were marked and 24.2% were recaptured along the twelve
319 sampling occasions.

320 The general model fits the data well in both sampling locations and we did not
321 find any significant transient effect (Puebla: $\chi^2 = 12.008$, $P = 0.10031$; Ordesa: $\chi^2 =$
322 6.0534 ; $P = 0.735$), or trap-dependence (Puebla: $\chi^2 = 6.5999$, $P = 0.35944$; Ordesa: $\chi^2 =$
323 2.4877 ; $P = 0.93$).

324 Using the POPAN open-population Jolly-Seber model structure, we obtained a
325 model with time-dependent probability of entry into the population $\theta(\cdot) p(\cdot) b(t) N$ in
326 the case of Puebla, and a top-ranked fully time-dependent model $\theta(t) p(t) b(t) N$ for
327 Ordesa (Table S2). Under the POPAN approximation, the estimated super-population
328 size is interpreted as the total number of individuals ever present in the experiment and
329 does not represent the number present at any particular point in time. From these
330 models, the super-population estimates were 623 males (95% CI = 527 to 760) in
331 Puebla; and 625 males (95% CI = 512 to 805) in Ordesa. The sex-ratio at imago
332 emergence is 1:1 (Ylla i Ullastre, 1997; Goussard, F. & Lopez-Vaamonde, C., personal
333 observation). Should that unbiased ratio be maintained during the whole adult lifespan,
334 the average adult super-population sizes of Puebla and Ordesa would be very similar,
335 1246 (1054–1520) and 1250 (1024–1610), respectively.

336 The daily male survival estimates produced by POPAN were higher for Ordesa,
337 0.700 (0.6543–0.7419), than for Puebla, 0.603 (0.5436–0.6601). Correspondingly, the
338 average lifespan of males was nearly 50% longer at Ordesa (2.83, 2.39–3.37) than at
339 Puebla (2.02, 1.69–2.44), and substantially lower than the scores reported for males of
340 this species in captivity (see Material and Methods). Assuming a flight period of two
341 months for both populations, the temporal fragmentation index (flight period / lifespan)
342 reached values of 21.2 in Ordesa and 29.7 in Puebla. This result indicates a very strong
343 temporal structuring of the seasonal population. In agreement with this prediction,
344 several peaks were observed in the adult capture curves (Fig. 3).

345

346 FIGURE 3

347

348 Our super-population estimates correspond to different proportions of the flight
349 period in the two populations. The MRR experiment took place during 11 days in
350 Puebla, but during 25 in Ordesa (Table S1). Taking this difference into account, and
351 assuming that the population dynamics were similar for the whole flight period, the
352 seasonal population sizes would be 3,000 (2,458–3,864) individuals for Ordesa, and
353 6,796 (5,749–8,291) for Puebla.

354

355 *Genetic variability and population structure*

356 Overall, the number of alleles per locus ranged between one (locus *GI23*,
357 Pyrenean localities) and 21 (*GIII*, both generations sampled at Puebla). Locus *GI23*
358 was removed from all the analyses concerning the Pyrenean sites because of its

359 monomorphism (allele 163). According to F_{IS} values, two out of 18 combinations of
360 locus x population showed a significant homozygote excess at the 5% level (after
361 Bonferroni correction) in both generations of Puebla for locus *GII8*. This result was
362 mostly likely due to the high proportion of segregating null alleles in Puebla (Table S3).
363 Therefore, locus *GII8* was excluded from further analyses concerning Puebla. No
364 significant genetic linkage disequilibrium was observed for any pair of loci per site after
365 Bonferroni correction ($P > 0.0009$ for Puebla; $P > 0.0003$ for the Pyrenean sites).
366 Selective neutrality for all microsatellite markers was confirmed by LOSITAN in both
367 sampling sites (Fig. S1).

368 Both generations sampled in Puebla showed higher genetic diversity than those
369 from National Park (Table 1). There was no significant differentiation among the five
370 Pyrenean sites (National Park) based on pairwise F_{ST} distances (range = 0-0.032, all $P \geq$
371 0.005, adjusted nominal level (5%) for multiple comparisons). Accordingly, $K = 1$ was
372 the partition of the data with the highest $L(K)$ value as revealed by the Bayesian
373 clustering algorithm implemented in STRUCTURE.

374

375 TABLE 1

376

377 *Effective population size*

378 Point estimates of N_e obtained with single-sample methods ranged from 6.31
379 (Cotatuero, ONESAMP, Fig. 4) to infinite (most localities analysed with the LD and H_e
380 excess methods). Actually, these two methods produced infinite upper bounds for all
381 but one (Diazas) of the 95% confidence intervals (CI). By contrast, most of the 95% CI

382 estimated by the temporal methods and all those calculated by ONESAMP were finite
383 (Tables 2 and 3).

384 FIGURE 4

385 TABLE 2

386 TABLE 3

387 Focusing on samples with more than 25 individuals, the only significant
388 difference in N_e (i.e. non overlapping 95% CI) was the larger N_e at Puebla than at
389 Ordesa detected by Jorde & Ryman's temporal method (Table 3). With regard to
390 temporal methods, it should be highlighted that the moment-based estimates of N_e for
391 Ordesa produced very similar results regardless of using Ordesa/2010 or
392 NationalPark/2010 as the second generation. Estimates produced by the maximum
393 likelihood method were slightly, but not significantly, higher when using
394 NationalPark/2010 as the second generation. Combined estimates from the single-
395 sample ($N_{e(SS)}$), temporal ($N_{e(T)}$) and across methods ($N_{e(SS+T)}$) resulted in a one-order of
396 magnitude larger contemporary N_e at Puebla than at Ordesa (Table 4; average across-
397 methods is 176 for Puebla and 29 for Ordesa).

398

399 TABLE 4

400

401 **Discussion**

402 Estimation of population size in moths by means of MRR dates back to the very
403 beginning of population genetics, when an English population of *Callimorpha dominula*
404 surveyed between 1941 and 1946 was estimated to harbour from 1000 adults (1943) to
405 6000-8000 (1946) (Fisher & Ford, 1947). Since then, MRR has been mostly used in
406 moths to estimate mobility and dispersal, particularly of pest species (e.g.
407 Margaritopoulos *et al.*, 2012). MRR based estimates of adult population size are
408 scarcer, likely due to constraints posed by the life history of many moths, i.e. only
409 males being conspicuous, rapid individual turnover, phased emergence, and nocturnal
410 activity (Gibson & New, 2007; Nowicki *et al.*, 2008). However, estimates of population
411 size are particularly timely in the light of the decrease in the abundance and distribution
412 of macro-moths reported from several European countries (reviewed by Fox (2013)). In
413 light of this, our results will be useful to assess the conservation status of the protected
414 Spanish Moon Moth. The two different populations showed different seasonal adult
415 sizes, but in the opposite direction to predictions based on the area they occupy and the
416 density of pines. The genetic results also indicated a larger N_e at Puebla (Mediterranean
417 region) than at Ordesa (Eurosiberian).

418

419 *Adult population size*

420 According to the adult population sizes estimated for both Puebla and Ordesa, *G.*
421 *isabellae* should be currently classified as Least Concern under the IUCN criteria
422 (IUCN, 2012b). Just these two populations of Puebla and Ordesa most likely sum more
423 than 10,000 adults, which is the minimum required for a species to be classified as
424 Vulnerable. Therefore, it is reasonable to think that the whole distribution area hosts far

425 more than this number. Indeed, a population of the Yellow-banded Skipper *Pyrgus*
426 *sidae*, a VU D2 (*Very restricted distribution and plausibility and immediacy of threat*)
427 butterfly in Spain, was notably smaller (569 ± 83 adults; Hernández-Roldán *et al.*,
428 2009). With regard to moths, each of our estimates was roughly double that obtained for
429 one population of the Australian Golden Sun Moth *Synemon plana* (Richter *et al.*,
430 2013), whose conservation status is also under debate (New, 2014). Lastly, our
431 estimates are also more than double the estimates obtained for each of the three German
432 populations of the Burnet *Zygaena carniolica*, a calcareous-grassland specialist,
433 surveyed by Nowicki *et al.* (2005).

434 We estimated a very similar number of adult males in both super-populations of
435 *G. isabellae* (623, 625), which include every individual that was exposed to sampling
436 during the course of the experiments. We are confident that this result is not an artefact
437 caused by sampling design or effort. Firstly, every night we captured as many males as
438 possible within their active flight period. Secondly, the number of sampling occasions
439 and stations was not exactly the same in Puebla and Ordesa. As a matter of fact, the
440 different sampling design applied in the two forests was precisely oriented to
441 counterbalance the obvious differences between them in the density and distribution of
442 pines. In that respect, our approach seems to have been extremely successful. Since the
443 lifespan of adult males is much shorter than the length of the flight period, once the
444 distinct duration of the MRR experiments in each locality is taken into account the
445 estimates of the seasonal population size happens to be twice higher at Puebla (6796)
446 than at Ordesa (3000).

447 Many forest lepidopterans are known to experience periodic fluctuations of
448 population size (Klemola *et al.*, 2006). For instance, four species of moths whose larvae
449 fed on needles of *Pinus sylvestris* strikingly coincided in their cyclical density

450 fluctuations in the same German area over 60 years (Varley, 1949). From the spatial
451 perspective, the fluctuating behaviour of disjunct populations can be asynchronous,
452 synchronous or with a temporal lag in spatial synchrony (reviewed by Tenow *et al.*
453 (2007) and Haynes *et al.* (2013)). Thus, the adult seasonal population sizes reported for
454 Puebla and Ordesa should be taken with caution, as a one-year MRR survey is merely a
455 snapshot of population dynamic cycles. On the other hand, adult population size may
456 not be as relevant for the ecology of *G. isabealle* as population density (e.g. Reilly &
457 Hajek, 2008; Nowicki *et al.*, 2009; Tamburini *et al.*, 2013). Considering all of the
458 suitable areas for *Graellsia* around Puebla and Ordesa and the average lifespan
459 estimated for adult males in each case, the density would be 11.7 males/night/km²
460 (9.92–14.30) and 3.34 males/night/km² (2.73–4.3), respectively. Since the density of *P.*
461 *sylvestris* was evidently much lower at Puebla than at Ordesa (Figs. 1e and 1f), the
462 difference between these two sites would be even more pronounced if the density was
463 expressed in terms of individuals per host tree. However, confirmation of a larger
464 density of *G. isabellae* in Puebla than in Ordesa will require knowledge about the fine-
465 scale species' distribution in time and space, movement patterns before and after
466 encountering the pheromone lure, as well as the average probability of capture for all
467 individuals in a trapping area of known size (reviewed by Adams *et al.* (2017)).

468

469 *Phased emergence*

470 To the best of our knowledge, this is the first report of phased (a.k.a. polymodal)
471 emergence in *G. isabellae*. In fact, the Spanish Moon Moth may be classified as a type
472 A polymodal species, i.e. diapause does not extend over more than one winter and
473 exhibit a bi/trimodal emergence curve, as some other Saturniidae moths (Waldbauer,

474 1978). We cannot rule out a more rapid pace of emergence in Ordesa. Indeed, phased
475 emergence has shown interpopulation variability in other Lepidoptera (Waldbauer &
476 Sternburg, 1985; Goulson, 1993 and references therein), as expected from a
477 phenomenon in which genetic and environmental factors interplay (reviewed by Tuskes
478 *et al.* (1996)). Phased emergence has been interpreted as an adaptive strategy to increase
479 reproductive success by escaping detrimental and unpredictable environmental factors.
480 This argument is supported by the latitudinal changes in modality of emergence of
481 several insects: unimodal in higher and more stable latitudes, whilst polymodal in lower
482 and warmer latitudes (reviewed by Goulson (1993)).

483 Phased emergence together with short adult lifespans produce a temporal
484 fragmentation of butterfly populations, whose impact on their risk of extinction can be
485 even higher than the effects of spatial subdivision (Bubová *et al.*, 2016). Our estimates
486 of the index of temporal fragmentation for *G. isabellae* are extremely high, due to both
487 its quite short adult life expectancy and quite long flight period. Regarding this last
488 variable, it should be noted that we have no direct records from neither of the sites
489 studied in this work. We are assuming for these localities the same length of the flight
490 period (two months) observed in a study carried out by others in a large area of *P.*
491 *sylvestris* located in the Eastern Pyrenees, under similar conditions of temperature and
492 relative humidity to those recorded during our experiments (Ylla i Ullaster & Sarto i
493 Monteys, 1993). In this respect, we are likely to be overestimating the real value of the
494 fragmentation index. But even if the true length of the flight period in our localities is
495 50% shorter, the values of the temporal fragmentation index would be in the range 10–
496 15, clearly within the category of "conservation concern" established by Bubová *et al.*
497 (2016) for other European butterflies.

498

500 Our results show that Puebla is not a small population, as its combined N_e is
501 larger than 100. The combined N_e of undifferentiated Pyrenean localities we named
502 “National Park” points to a small population ($N_e < 100$). We obtained different
503 estimates of contemporary N_e for each locality, as expected after using several
504 estimators with different assumptions (e.g. Pérez-Figueroa *et al.*, 2016; Martínez *et al.*,
505 2018). Nevertheless, several lines of evidence pointed to a larger N_e at Puebla than at
506 the Pyrenean site of Ordesa. On the one hand, both the single-sample (inbreeding N_e)
507 and the temporal (variance N_e) algorithms indicated a larger N_e at Puebla than at Ordesa,
508 although only Jorde & Nyman’s moment estimator provided a significant difference. On
509 the other hand, when the Pyrenean individuals sampled in 2010 were pooled (i.e.
510 “National Park”), the point estimate resulting from the LD method was almost twice the
511 one obtained for Puebla/2008, although such a difference was not significant and
512 ONeSAMP did not reveal such a pattern. A putative low N_e at the Pyrenean sites is also
513 compromised by the finding of infinite estimates: five out of nine of the point estimates
514 of inbreeding N_e from a single source were infinite and all but one of the 95% CI
515 (Diazas, LD method) had infinite as upper bound. This result obtained from a typical
516 dataset ($n \geq 25$ per site, 9 loci) points to moderate or large population size ($N_e > 500$), as
517 the precision of genetic methods for estimating contemporary N_e is expected to be poor
518 unless vast amounts of data are gathered. Even when such datasets are available,
519 obtaining a finite upper bound for the 95% CI may not be possible for large populations.
520 Actually, the finite lower bound can provide useful information about plausible limits of
521 N_e (Waples & Do, 2010). According to such finite lower bounds (149.2, 109.8; Table
522 2), the Pyrenean sites might also be a moderate-size population.

523 We are aware that different sources of noise may affect estimates of N_e in

524 moderate-large populations: data errors, slight departures from random sampling, and
525 violations of underlying model assumptions (Waples & Do, 2010). Firstly, we expect
526 data errors to be negligible, as two researchers independently performed the allele
527 calling and 10% of the samples of the project were randomly re-genotyped. Secondly,
528 our studied localities did not show any substantial deviation from random mating, but
529 for Puebla/2008 and Bujaruelo/2010. Despite having excluded locus *GII8* from
530 analyses involving Puebla, we cannot rule out a slight effect of null alleles, a well-
531 known phenomenon in Lepidoptera (e.g. Song *et al.*, 2017) in our results (see *GI6* at
532 Puebla/2008 and *GII7* at Bujaruelo/2010, Table S3). Nevertheless, our conclusion of
533 moderate-large N_e for Puebla is not jeopardised by this possibility, as null alleles bias
534 toward low N_e when estimated with the LD method (Sved *et al.*, 2013). Thirdly, most
535 single-sample approaches to estimate contemporary N_e assume a closed population
536 (reviewed by Wang (2016)), which is definitely not the case of the Pyrenean sites
537 surveyed in the present study. Still, the LD method is fairly robust to migration, as long
538 as equilibrium migration rate is below 10% and population size is ≤ 500 (Waples &
539 England, 2011; Gilbert & Whitlock, 2015). Thus, the only local precise estimate
540 obtained by the LD method, Diazas/2010, may be the result of lower migration rate
541 and/or lower N_e than the other surveyed Pyrenean sites: a lower N_e probably meant that
542 we sampled at least 10% of its N_e , as suggested by Palstra & Ruzzante (2008). Lastly,
543 the temporal methods provided more precise estimates than the single-sample
544 estimators of inbreeding N_e , as well as a more marked difference between the
545 contemporary N_e of Puebla and the Pyrenean sites. An even better performance would
546 probably be obtained by using a wider sampling interval (Wang *et al.*, 2016), which was
547 not possible due to logistical reasons. Anyhow, the better precision of the temporal-
548 based estimates we obtained seems a logic consequence of the fewer assumptions of

549 these kinds of methods and their higher robustness in real scenarios (Wang *et al.*, 2016).

550 To the best of our knowledge, only Franklin *et al.* (2014) estimated
551 contemporary N_e of local populations of a moth. They applied the same three temporal
552 methods that we used to estimate the variance N_e in five Canadian locations of the
553 Western Tent Caterpillar (*Malacosoma californicum pluviale*). Their results showed
554 little population structure and a slightly higher genetic diversity than ours (but for
555 Galiano/2011 and Puebla/2008 with same H_E). Their study sites were sampled during
556 high-density peaks, although they showed $N_e < 200$. Multiple paternity (i.e. increased
557 variance in male reproductive success) was advocated to account for the low N_e
558 obtained. Males of *G. isabellae* can also mate with several females (Ylla i Ullastre,
559 1997; Vila *et al.*, 2009), so any putative downward bias of our estimates because of
560 multiple paternity would additionally support a moderate-large N_e of Puebla. On the
561 other hand, the temporal fragmentation of *G. isabellae* seasonal populations, higher at
562 Puebla than at Ordesa, should be expected to impact more on our estimates of N_e in
563 Puebla, and therefore cannot account for the lower N_e obtained for Ordesa. Franklin *et*
564 *al.* (2014) also found that maximum likelihood estimates assuming no migration were
565 notoriously higher than the moment estimates. Interestingly, their maximum likelihood
566 estimates dramatically decreased when assuming migration. This result raises concerns
567 about how much migration may be biasing the maximum likelihood estimates we
568 obtained at the Pyrenean sites, which were also higher than the moment-based ones.
569 Because of migration, the Pyrenean N_e may be underestimated when inferred from the
570 moment-based methods (Tajima & Nei, Jorde & Ryman), but overestimated if
571 calculated with the maximum likelihood method (Gilbert & Whitlock 2015). It is
572 tempting to postulate that the true N_e of the Pyrenean samples lies between the putative
573 overestimation and underestimation of the likelihood and moment-based estimates,

574 respectively. However, a more refined knowledge of the genetic structure of *G.*
575 *isabellae* in the National Park of Ordesa y Monteperdido and its surrounding area is
576 needed before drawing further conclusions.

577

578 *Implications for conservation*

579 Frankham *et al.* (2014) recently argued that retention of long-term evolutionary
580 potential requires N_e over 1000 and avoiding of a high increase in inbreeding rate
581 requires N_e over 100. According to this, the moderate-large estimated N_e suggests a
582 positive prospect of *G. isabellae* in Puebla. With regard to Ordesa/National Park, even
583 if the low combined estimate of contemporary N_e is proved true by future studies that
584 more accurately consider the role of migration in the estimation, this Pyrenean
585 population is not necessarily at risk of losing genetic diversity as long as it is connected
586 to other populations by gene flow (Waples, 2010). Managers should, therefore, aim to
587 maintain the connection of patches of *P. sylvestris* in that Pyrenean area. This is because
588 the actual risk of genetic erosion will most likely arise from fragmentation and isolation
589 of a population that historically was connected by migration (Waples, 2010).

590 N_e is generally much lower than N in natural populations, namely because of
591 unequal sex ratio, high variance in reproductive success, and fluctuations in census size
592 (Frankham, 1995). The N_e/N ratio has been the subject of a great bulk of research
593 aiming at disentangling the relative risks that demographic, environmental, and genetic
594 factors might pose for population persistence (reviewed by Palstra & Fraser (2012)).
595 These authors suggested the incorporation of uncertainty into N_e/N estimates. To do so,
596 one has to assume both N_e and N to be constant in the recent past, as we did for the
597 combined estimates of N_e . Using $N_{e(SS)}$ and $N_{e(T)}$ (Table 4) and following Belmar-Lucero
598 *et al.* (2012) we obtain that the N_e/N ratio is 1.1 to 5.4 times higher in Puebla (0.025,

599 0.033) than in the National Park (0.024, 0.006). This finding warns against the
600 extrapolation of N_e/N ratios, as different populations of the same species are likely to
601 experience differences in the aforementioned factors. Ours are likely the first N_e/N
602 values reported in any moth and are quite similar to the ones published for wild
603 populations of endangered butterflies by Saarinen *et al.* (2010) and Turlure *et al.*
604 (2014).

605 Lastly, we are aware that our results should be taken with caution. To be
606 validated, future studies will have to investigate whether populations of *G. isabellae*
607 experience fluctuations of population size and, if so, if oscillations are (i) cyclic, (ii)
608 synchronous among different populations, and (ii) density-dependent. Also, the role of
609 migration in the estimates of contemporary N_e of the Pyrenean sites deserves further
610 investigation. In the present study, lack of fine-scale knowledge of *G. isabeallae* in that
611 area prevented us from evaluating whether breeding and sampling windows
612 mismatched, one of the possible reasons for the overwhelming amount of studies
613 reporting a ratio between effective and census population size (N_e/N) below 1 (Neel *et*
614 *al.*, 2013). Lastly, future research will likely provide better ways to combine results
615 from different estimators and to compute confidence intervals for a combined estimate
616 of contemporary N_e (Waples, 2016).

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628

629 **Supporting Information**

630 Additional Supporting Information may be found in the online version of this article
631 under the DOI reference: **XXXXXXXX**.

632 **Data S1.** Details on the MRR procedures performed in 2009 (Puebla) and 2010
633 (Ordesa).

634 **Data S2.** Details on the attraction range assessment of the sexual pheromone of *G.*
635 *isabellae*.

636 **Data S3.** Details on the calculation of combined estimates of N_e across single
637 sample (SS) and temporal (T) methods following Waples & Do (2010).

638 **Figure S1.** Results from the LOSITAN F_{ST} outlier analysis.

639 **Table S1.** MRR: Dates, conditions, and capture matrix for each sampling location

640 **Table S2.** MRR: top-ranked models from the capture matrix.

641 **Table S3.** Summary statistics for each microsatellite locus and sampled site of
642 *Graellsia isabellae*. NA: number of segregating alleles; null: percentage of null alleles ;
643 H_E : unbiased expected heterozygosity; F_{IS} : inbreeding coefficient. Significant
644 deviations from Hardy-Weinberg expectations are shown in bold (global test based on
645 360 (Puebla) and 960 (all Pyrenean samples) randomisations, adjusted nominal level
646 (5%) = 0.00278 for Puebla and 0.00104 for the Pyrenean localities).

647

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- 903

904 **Tables**

905 **Table 1.** Genetic variation in each sample of *G. isabellae*. Calculations based on eight
 906 microsatellite loci: all but *GI18* (strongly affected by null alleles) for Puebla and all but
 907 *GI23* (monomorphic) for the Pyrenean sites. Missing data are displayed as number of
 908 individuals failing at a given locus (in parentheses). *AR* = allelic richness was calculated
 909 using a sample of five diploid individuals and averaged over loci. *H_O* = observed
 910 heterozygosity. *H_E* = unbiased expected heterozygosity. Significant deviations from
 911 Hardy-Weinberg expectations are shown in bold (test based on 320 (Puebla) and 960
 912 (all Pyrenean samples) randomisations, adjusted nominal level (5%) = 0.00313 for
 913 Puebla and 0.00104 for the Pyrenean localities).

914

Site/year	<i>n</i>	Missing data	<i>AR</i>	<i>H_O</i>	<i>H_E</i>	<i>F_{IS}</i>	
Puebla/2008	29	1(<i>GI6</i>), 1(<i>GI11</i> , <i>GI25</i>)	4.94	0.657	0.756	0.131	
Puebla/2009	32		0	4.76	0.699	0.735	0.049
Ordesa/2009	27		0	3.15	0.537	0.61	0.119
Ordesa/2010	31	1(<i>GI18</i>)	3.21	0.619	0.606	-0.021	
Línea/2010	11		0	3.11	0.614	0.636	0.036
Bujaruelo/2010	27	1(<i>GI17</i>)	3.16	0.519	0.602	0.137	
Cotatuero/2010	6	1(<i>GI15</i> , <i>GI25</i>)	3.36	0.579	0.643	0.099	
Diazas/2010	32	1(<i>GI6</i> , <i>GI17</i> , <i>GI21</i> , <i>GI26</i>)	3.14	0.557	0.595	0.064	

915

916 **Table 2.** Estimates of inbreeding effective population size obtained using the LD
 917 method implemented in NeESTIMATOR.

Locality/year	n	N_e	95% CI
Puebla/2008	29	233.5	49.7- ∞
Puebla/2009	32	∞	95.1- ∞
Ordesa/2009	27	67.3	19.7- ∞
Ordesa/2010	31	∞	149.2- ∞
Línea/2010	11	∞	8.7- ∞
Bujaruelo/2010	27	∞	78.0- ∞
Cotatuero/2010	6	∞	2.1- ∞
Diazas/2010	32	32.0	13.9-173.2
National Park/2010	107	451.1	109.8- ∞

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920 **Table 3.** Estimates of variance effective populations size obtained using the maximum likelihood and moment methods implemented in MLNe as
921 well as two moment-based methods (Tajima & Nei, Jorde & Ryman) implemented in NeESTIMATOR.

Site/years	Maximum Likelihood (Wang) Isolated population			Moment-based (Waples) Moment-based (Wang) Isolated population		Moment-based (Waples) F_e (Nei & Tajima) F_s (Jorde & Ryman)		
	n	N_e	95% CI	N_e	95% CI	N_e	95% CI	95% CI
Puebla/2008-2009	61	173.85	50.54- >1500	193.31	275.3	20.8- ∞	915.2	554.4- 1363.4
Ordesa/2009-2010	58	29.82	15.77- 111.67	11.45	14.7	5.2- 64.3	11.4	6-18.5
Ordesa/2009-NationalPark/2010	134	48.94	32.36- 92.16	14.15	12.9	5.3- 32.9	11.0	5.8- 17.8

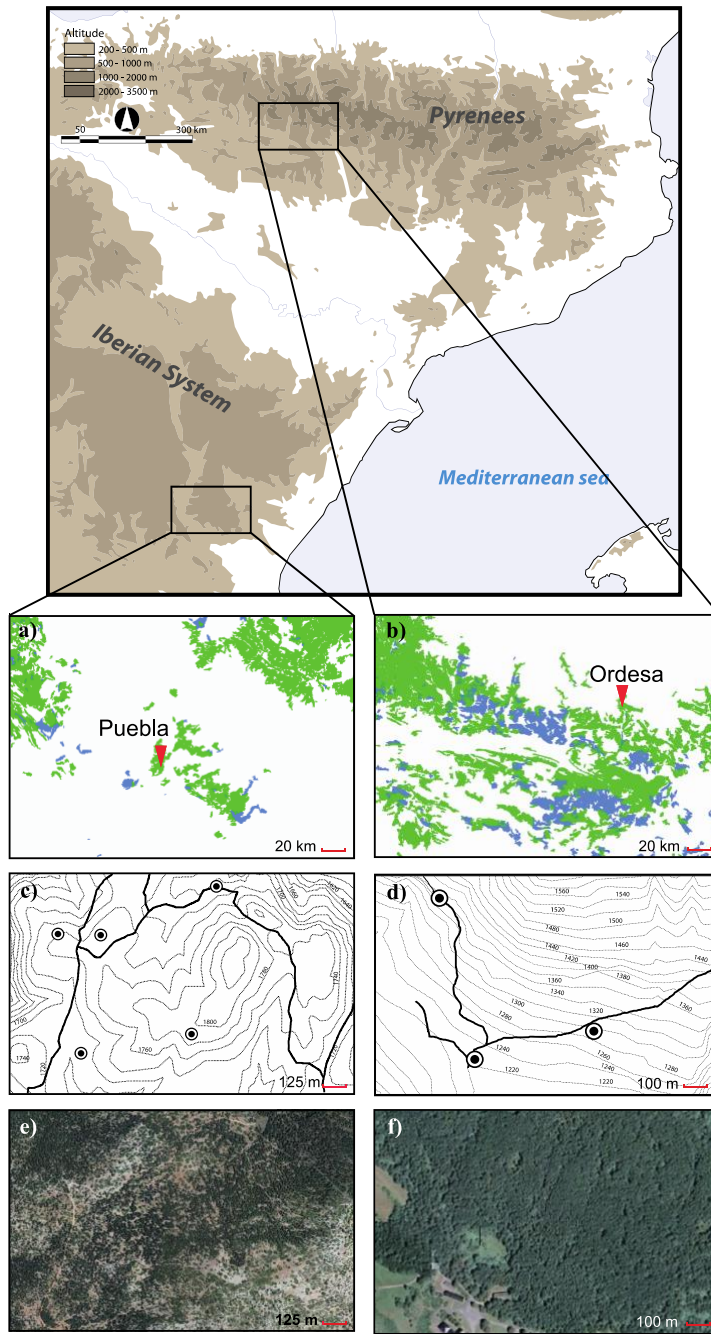
923 **Table 4.** Combining estimates of effective population size within (single-sample or temporal) and across methods following Waples & Do
 924 (2010). Single-sample calculations used the results obtained with ONeSAMP for the 6-1500 prior. $N_{e(SS+T)}$ shows the results obtained for five
 925 possible values of the true (unknown) N_e , from 25 to 1000. $N_{e(SS+T)}$ moved closer to the temporal estimate for larger population size, as previously
 926 found by Waples & Do (2010). Readers are referred to Data S3 for further details on all these calculations.
 927

Site/years	Single-sampled	Temporal	Across-methods: $N_{e(SS+T)}$ with ML				
	$N_{e(SS)}$	$N_{e(T)}$	$N_e = 25$	$N_e = 50$	$N_e = 100$	$N_e = 500$	$N_e = 1000$
Puebla/2008-2009	168.8	220.96	173.25	174.79	176.21	178.02	178.31
Ordesa/2009-10	53.01	17.46	33.48	30.69	28.81	26.97	26.72
Ordesa/2009-NationalPark/2010	92.32	19.97	48.69	42.77	38.50	34.22	33.62

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931 **Figure legends**

932 **Figure 1.** Geographic location of the two studied populations of *Graellsia isabellae*:
933 Puebla (left) and Ordesa (right); a), b): *P. sylvestris* forest patches in the region as from
934 GIS-FOREST ([https://sites.google.com/site/sigtreforestspeciesenglis/home/mapas-de-](https://sites.google.com/site/sigtreforestspeciesenglis/home/mapas-de-especies)
935 [especies](https://sites.google.com/site/sigtreforestspeciesenglis/home/mapas-de-especies)). Red arrow: sampling site, green area: autochthonous woodland, blue area:
936 reforested); c) and d): sampling design corresponding to each locality (pheromone
937 trapping locations are represented by dark circles); e), f): aerial images of sampling
938 areas produced by SIGPAC 3.3 (<http://sigpac.mapa.es/fega/visor/>).



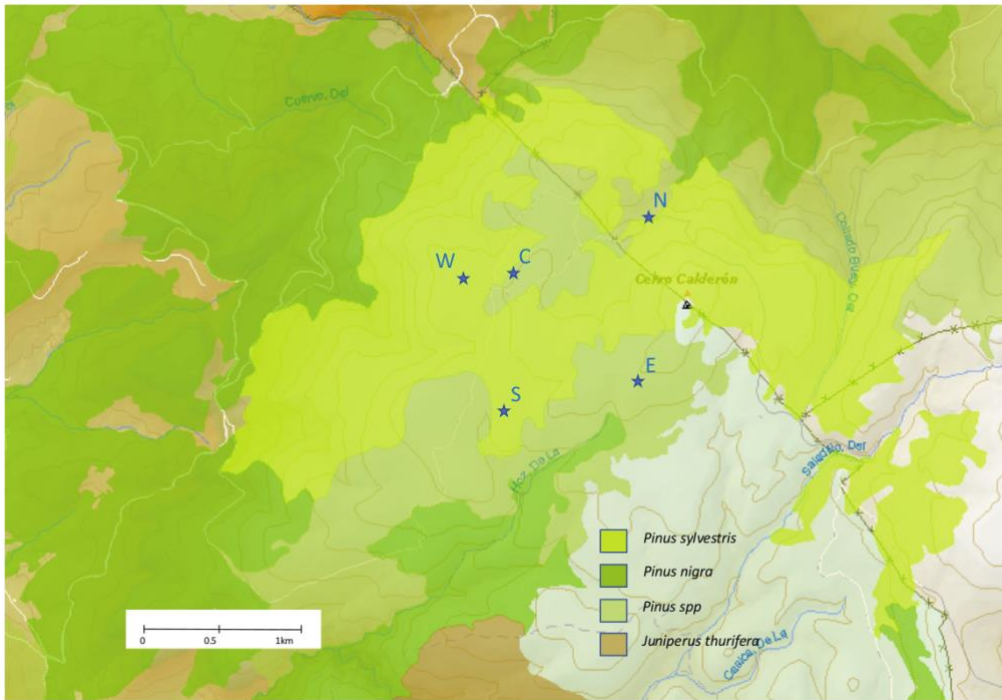
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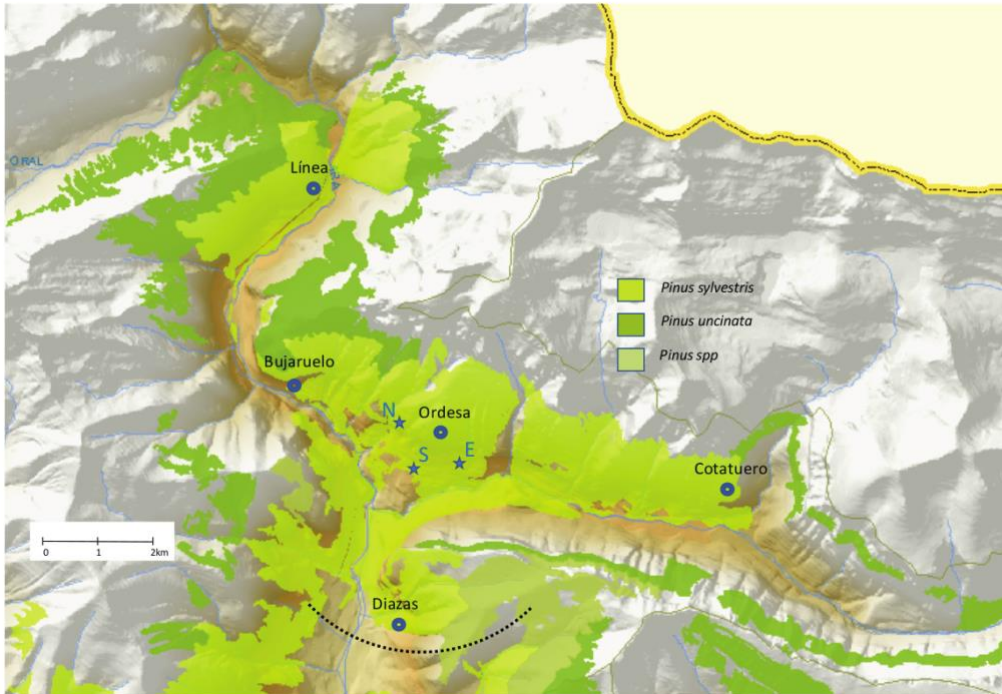
941 **Figure 2.** Geographic locations of the study sites at the Natural Park of *Puebla de San*
942 *Miguel* (a) and at the National Park of *Ordesa y Monteperdido* (b). Maps were produced
943 by the Geo Portal of the Spanish *Ministerio de Agricultura y Pesca, Alimentación y*
944 *Medio Ambiente* (<http://sig.mapama.es/geoportal/>), using the BDN database (*Banco*
945 *de Datos de la Naturaleza*), last updated for the studied regions in 2006. According to
946 Geo Portal “*Pinus* spp.” stands for a mixture of autochthonous pines. Letters identify
947 trapping locations (C, central sampling location; N, E, S, and W, cardinally oriented
948 sampling locations; see further details in Data S1). The dashed line separates the study
949 population from other populations of *G. isabellae* according to genetic clustering

950 algorithms (Marí-Mena, 2013).

a)



b)

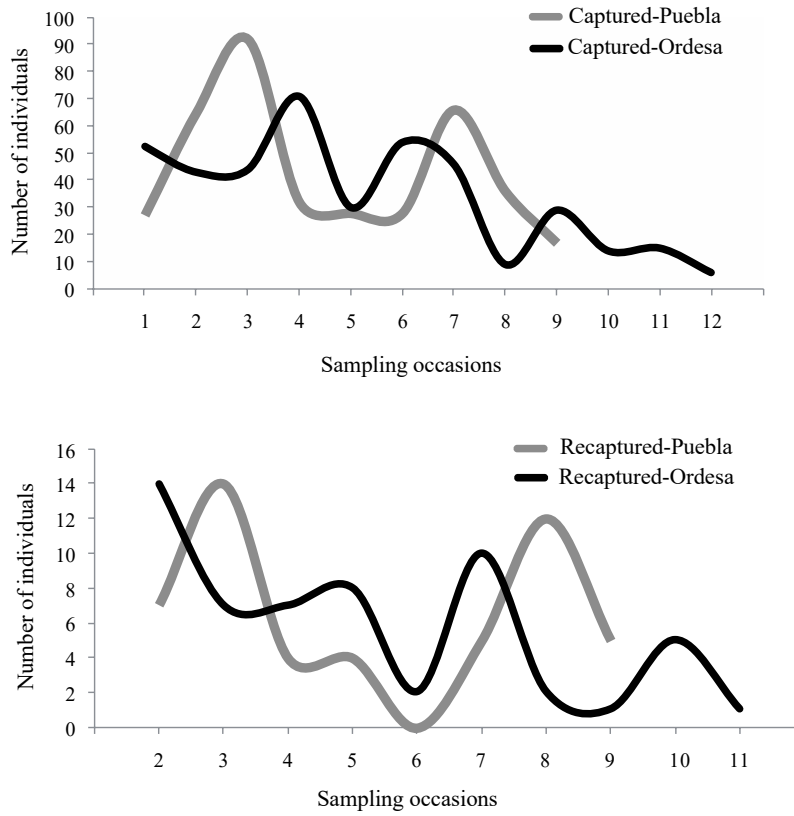


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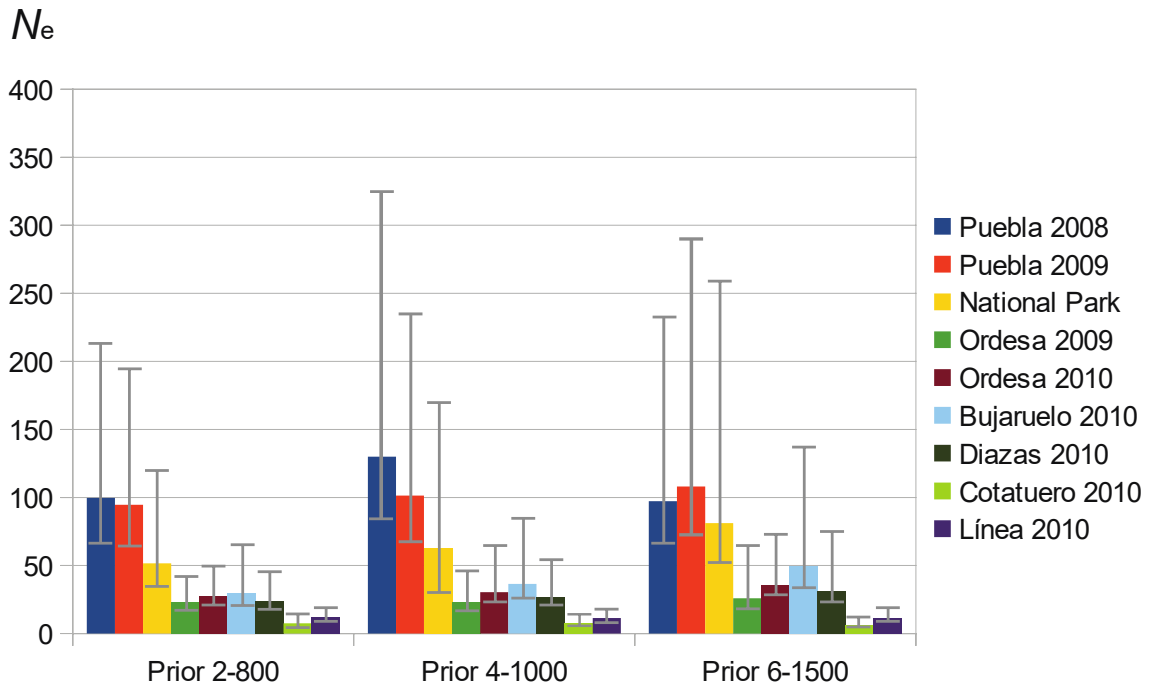
953

954 **Figure 3.** Emergence pattern of male *G. isabellae* in Puebla and Ordesa as from capture
955 and recapture data.



956

957 **Figure 4.** Mean effective population size and 95% confidence intervals obtained with
 958 the approximate Bayesian computation method implemented in ONeSAMP.



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