



Fragmentation, population features and intra-specific competition differently affect several life stages of a Mediterranean perennial herb

Journal:	<i>Plant Biology</i>
Manuscript ID	Draft
Manuscript Type:	Research Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Moncalvillo, Belén; Universidad Rey Juan Carlos, Biología y Geología, Física y Química Inorgánica Matesanz, Silvia; Universidad Rey Juan Carlos, Biología y Geología, Física y Química Inorgánica Sánchez, Ana; Universidad Rey Juan Carlos, Biología y Geología Escudero, Adrián; Universidad Rey Juan Carlos, Biología ay Geología
Keyword:	<i>Astragalus incanus</i> , fecundity, seed predation, offspring, gypsum

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5 Moncalvillo, B^{1,2*}., Matesanz, S^{1.}., Escudero, A^{1.}., Sánchez, A.M^{1.}.

6 ¹Biodiversity and Conservation Unit, Dep. Biology, Geology, Physics and Inorganic

7 Chemistry; ESCET, URJC; Móstoles, Madrid, Spain

8 ²Department of Ecology, Faculty of Biology, Philipps-University Marburg, D-35043

9 Marburg, Germany

10

11 *Corresponding author: Moncalvillo, B.

12 *Author e-mail: bmoncalvillourjc@gmail.com

13 *Author ORCID ID: <https://orcid.org/0000-0002-9958-5250>

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26 **Abstract**

27 Habitat fragmentation is one of the most pervasive environmental threats worldwide. Its
28 effects on plant population are complex as fragmentation may disrupt many ecological
29 processes, including plant-animal interactions. Studies often focus on a single life stage,
30 spatial scale and use fragment and/or population size as interchangeable indicators,
31 therefore frequently failing to assess the complexity of fragmentation effects. In order to
32 overcome these limitations, we conducted a study including several life stages of
33 *Astragalus incanus* subsp. *incanus*, a plant facultatively linked to gypsum soil islands in
34 Central Spain. We considered plant fecundity and pre-dispersive seed predation
35 obtained from field observations, and offspring performance measured in a common
36 garden. Fragmentation was assessed using landscape, community, population and
37 individual scale variables. Our results revealed different effects of fragmentation for
38 each life stage. Fragment size and connectivity had no effect on plant fecundity, e.g.
39 fruit set or seed set, but jointly determined fruit predation, while fragment size was
40 negatively related to offspring growth. Population density, rather than population size,
41 had a significant positive effect on predation but negatively affected plant fecundity and
42 offspring performance. Perennial cover, used as proxy of competition, reduced both
43 plant fecundity and predation incidence. Our results indicate that both landscape
44 (fragment size and connectivity), population features (population size and density) and
45 community (cover of perennials) affect plant reproductive performance in fragmented
46 habitats. Altogether, our study provides evidence that fragmentation effects at several
47 ecological scales operate in different ways concerning several life stages.

48

49 **Keywords:** *Astragalus incanus*, fecundity, seed predation, offspring, gypsum.

50

51 **Introduction**

52 Habitat fragmentation is a widely studied global change driver affecting terrestrial
53 ecosystems worldwide. However, there is increasing evidence showing that plant
54 studies based on individual aspects of fragmentation may not detect all the simultaneous
55 effects of this complex and long-term process (Ewers & Didham 2006; Haddad *et al.*
56 2015). Fragmentation has usually being quantified based solely on habitat loss and
57 isolation (Haddad *et al.* 2015); that is, fragment size and connectivity (Bennett &
58 Saunders 2011) and specific combinations of these factors, e.g. negative effects may
59 only be relevant in populations suffering simultaneously from highly reduced habitat
60 size and low connectivity (Haddad *et al.* 2015; Matesanz *et al.* 2015; Gómez-Fernández
61 *et al.* 2016). However, critical fragmentation thresholds may depend not only on these
62 factors but also on other properties such as population size, density or aggregation
63 (Reed 2005; Luzuriaga *et al.* 2006; Matesanz *et al.* 2009) which are not necessarily
64 correlated with habitat size but are frequently linked to habitat quality (Mortelliti *et al.*
65 2010; Lázaro-Nogal *et al.* 2012). These are, in turn, critical for animal interactions
66 (Brys *et al.* 2004; Rabasa *et al.* 2005; Luzuriaga *et al.* 2006). Moreover, fragmentation
67 may have long-term effects, acting throughout all plant life stages (Hobbs & Yates
68 2003; González-Varo *et al.* 2010).

69 Fragmentation have been often related to decreased female reproduction and
70 success (Aguilar *et al.* 2006). For instance, lower flower outputs and lower fruit size or
71 quantity in populations with lower habitat and/or population size may result from
72 reduced gene flow (Vergeer *et al.* 2003; Lienert 2004), which would provoke genetic
73 erosion or inbreeding (Young *et al.* 1996; Aguilar *et al.* 2008; Leimu *et al.* 2010).
74 Furthermore, plant fecundity, understood as seeds that successfully undergo maturation
75 and dispersion, may also reflect negative effects of fragmentation on plant-animal

76 interactions (Brudvig *et al.* 2015). Among the later, pollination has been extensively
77 assessed (Cunningham 2000; Santamaría *et al.* 2018), but other interactions have
78 received far less attention. For instance, seed predator abundance may be altered when
79 fragmentation occurs (Valladares *et al.* 2006). Given that seed predation is a key
80 determinant of the final number of viable seeds (Crawley 2000), especially in species
81 with synchronous flowering and fructification (Honek & Martinkova 2005), changes in
82 predators abundance usually have direct demographic consequences both in recruitment
83 (Louda *et al.* 1990; Crawley 2000) and in the dynamics of seeds in the soil (Azcárate &
84 Peco 2003).

85 Although fecundity has been traditionally assessed as the unique indicator of
86 plant fitness (Leimu *et al.* 2010), offspring performance is a more accurate surrogate
87 (Brudvig *et al.* 2015) and may also be affected by fragmentation (Lienert 2004). Indeed,
88 reduced offspring performance, estimated as germination (Pías *et al.* 2010), seedling
89 survival (Kolb 2005; Matesanz *et al.* 2017) or offspring growth (Michaels *et al.* 2008),
90 has been documented in fragmented populations. These outcomes can be a combination
91 of genetic (e.g. genetic erosion; Honnay and Jacquemyn 2007, González-Varo *et al.*
92 2010) and/or non-genetic causes, including habitat quality (Vergeer *et al.* 2003) or
93 transgenerational effects of the maternal environment, i.e. environmental circumstances
94 affecting mother plants that can pass on seed material (Galloway 2005; Pías *et al.*
95 2010). Therefore, several steps of plant reproduction can be expected to be affected by
96 fragmentation.

97 Gypsum outcrops in Central Spain constitute an ideal landscape model to
98 evaluate habitat fragmentation, as gypsum appears naturally immersed within other
99 types of soils. This edaphic island-like structure has been historically exacerbated by
100 agriculture (Escudero *et al.* 2015), which for decades has been one of the major

101 fragmentation drivers worldwide (Saunders *et al.* 1991). In this study we focused on
102 *Astragalus incanus* subsp. *incanus*, a perennial creeping herb facultatively linked to
103 gypsum soils that presents a high incidence of seed pre-dispersal predation, with a large
104 production of big, easily-accessible fruits. We aimed to determine how fragmentation
105 affects *A. incanus incanus* reproduction (in terms of plant fecundity, pre-dispersal seed
106 predation and offspring performance) through: (i) fragment size and connectivity, (ii)
107 presence of other perennials in the community, (iii) population size and density and (iv)
108 mother plant traits. We combined field data on plant fruit and seed production, as well
109 as seed predation, from 20 habitat fragments with a common garden experiment
110 exploring offspring from plants from these fragments. We expected plants from smaller
111 and more isolated fragments (Aguilar *et al.* 2006) to have lower fitness. Furthermore,
112 we also expected that population size and density (Leimu *et al.* 2010), together with
113 plant size (Brys *et al.* 2004), would modulate the effects of fragment-level factors, either
114 exacerbating or diminishing them.

115

116 **Materials and methods**

117 *Study species and sites*

118 *Astragalus incanus* L. subsp. *incanus* (Fabaceae), *A. incanus* hereafter, is a perennial
119 herb mostly found in the Iberian Peninsula but also present in southern France and
120 northwestern Morocco and Algeria (Podlech 1993). This plant usually appears in
121 gypsum soils, but not exclusively, i.e. it is a gypsovag. Flowering occurs in late spring,
122 usually in May or June. No study has assessed *A. incanus* pollination in depth, but
123 according to Santamaría *et al.*, (2018), main pollinators for *Fabaceae* with big flowers
124 in our study site are Hymenoptera, specially bees such as *Apis mellifera* and *Anthophora*
125 *atroalba*, and several species from the genera *Colletes*, *Osmia*, *Amegilla* and *Bombus*

126 (Santamaría *et al.* 2018). In any case, a certain rate of autogamy is common found in
127 similar legumes (Galloni *et al.* 2007; Sánchez *et al.* 2017) and cannot be discarded for
128 our species. The species produces an abundant crop of dry and indehiscent legumes with
129 no specific dispersion syndrome, and is thus considered autochorous.

130 Our study was performed in a gypsum landscape in the Tajo River Basin, near
131 Belinchón (754 m above sea level, 40°03' N, 3°03'O), central Spain. This area has a
132 semiarid Mediterranean climate with a mean annual rainfall of 429 mm and average
133 annual temperature of 12.6 °C (AEMET 2011). In these habitats natural vegetation has
134 been historically fragmented, due to the combined effect of natural and human-driven
135 processes, resulting in isolated gypsum outcrops (Luzuriaga *et al.* 2018; Matesanz *et al.*
136 2018). Plant communities are dominated by gypsum specialized chamaephytes such as
137 *Helianthemum squamatum* L. (*Cistaceae*) or *Lepidium subulatum* L. (*Brassicaceae*)
138 (Luzuriaga *et al.* 2006), as well as species that are facultatively linked to gypsum, i.e.
139 gypsovags, such as the study species. In addition, in these habitats the cover of
140 perennials is less than 30 % and exposed areas are occupied by a conspicuous biological
141 soil crust of lichens, mosses and cyanobacteria (Martínez *et al.* 2006) and a very rich
142 community of annual plants (Luzuriaga *et al.* 2018). Gypsophile species, i.e. specialized
143 gypsum species, very likely present specific adaptations to natural fragmentation
144 (Escudero *et al.* 2014), but that is not the case for gypsovags such as *A. incanus*.
145 Therefore, gypsovags constitute a good model to test the effects of human-related
146 habitat fragmentation.

147

148 ***Data collection***

149 *Fragment selection*

150 We selected 20 natural vegetation fragments in which *A. incanus* was present,
 151 representing a wide range of size and connectivity (Fig. 1, Appendix A). Using aerial
 152 photographs taken in 2011, we calculated fragment size and used the following
 153 connectivity index to assess fragment connectivity (Tremlová & Münzbergová 2007):

$$154 \quad C_j = \log_{10} \sum_{z=1}^n \frac{S_z}{d_{jz}^2}, j \neq z$$

155 in which C_j is the connectivity for fragment j , z is the total number of fragments found
 156 within 500 m of j , S_z is z fragment size and d_{jz} is the minimum distance between j and z
 157 borders. We used a value of 500 m of radius because movement of the most frequent
 158 pollinators (medium size bees and bumblebees) among fragments located at larger
 159 distances is unlikely (Aizen *et al.* 2002; Fontaine *et al.* 2008).

160

161 *Community-level competition and population-level variables*

162 We assessed the percentage of perennial plant cover using five 2.4 x 2.4 m quadrats per
 163 fragment located within areas in the fragment where the perennial vegetation was better
 164 preserved. This variable was regarded as a proxy of competition with other species in
 165 the community. In each fragment, the total number of *A. incanus* plants was counted
 166 prior to flowering to determine population size. To estimate population structure, we
 167 used 10-12 plants per fragment to calculate the mean distance of a focal *A. incanus* plant
 168 to its three nearest conspecific neighbors. Then we calculated a mean of all these
 169 Nearest Neighbour Distances (NND) to get a proxy of local plant density in each
 170 population. Population size was not correlated to NND ($R^2 = -0.03$; $p = 0.5189$), nor to
 171 fragment size ($R^2 < -0.01$; $p = 0.3349$).

172

173 *Plant fecundity and seed predation in natural conditions*

174 In May 2015, during *A. incanus* fructification, we randomly selected 10-12 plants per
175 fragment (the same used for the NND calculation) and collected fruits from 10
176 inflorescences per plant, or from all available inflorescences when we found less than
177 10. Total number of inflorescences, number of inflorescences containing fruits and
178 number of fruits per inflorescence were counted in each plant. Total number of fruits
179 per plant was calculated from these data. In addition, we measured height (a) and the
180 two largest diameters (b and c) of each plant in order to calculate its volume,
181 approaching it to an ellipsoid ($V = (4/3) \times \pi abc$), as a measure of plant size. Collected
182 fruits were maintained at -18°C until they were opened to prevent biological activity
183 such as degradation or parasite development and inspected under a magnifying glass.
184 Once opened, the presence of predation was assessed in each fruit as a binomial (1-0)
185 variable. Main seed predators are Hymenoptera and Lepidoptera larvae (personal
186 observation), that feed on the developing seed prior to its dispersion. They are easily
187 observed by the presence of the larvae inside the legume in some cases, while in other
188 cases they are perceived as the partial or total consumption of the legume content or the
189 presence of an exit hole in the legume. In case the fruit was not predated, the number of
190 healthy seeds and the total ovule number were recorded. Fruit set was calculated as the
191 ratio between seeds and ovules. Total number of seeds per plant and overall predation
192 incidence per plant were calculated from these data.

193

194 *Common garden experiment*

195 In June 2015, we selected a subsample of nine fragments from the previous 20,
196 maintaining the same area and connectivity ranges. In each of these nine fragments we
197 randomly selected 10-22 plants, estimated their volume and collected all their mature
198 fruits. Fruits were opened and seeds drawn. We discarded immature seeds and fruits

199 affected by predation. For three months, seeds were exposed to summer-like conditions
200 inside containers with silica gel to simulate natural field conditions and stimulate their
201 germination afterwards. Between 5-20 randomly selected seeds per plant were then
202 weighted in a microbalance (0.001 mg precision, Mettler Toledo MX5, Madrid Spain).
203 In addition, we randomly selected up to 40 seeds from 10 plants per fragment to carry
204 out a germination test (N = 2847). In January 2016, these 40 seeds per plant were
205 scarified with sandpaper to favor its rapid germination. Four seeds from the same plant
206 were sown in 5cm plastic alveoli that were watered twice a day. Between 2-4 weeks
207 after sowing up to 10 seedlings per mother plant (family hereafter) were transplanted to
208 $8 \times 10 \times 10$ cm plastic pots (final N = 674). During cultivation time, germination and
209 survival was monitored two times per week. In June 2016, four months after sowing we
210 plucked the aerial part of these seedlings, dried them during 3 days at 60°C and then
211 weighted their dry aerial biomass (0.1 mg precision) as a measure of their growth.
212 Therefore, offspring performance was estimated through germination and aboveground
213 biomass.

214

215 *Statistical analyses*

216 We built ten generalized linear mixed models (GLMM) using the “lme4” package
217 (Bates *et al.* 2015) from software R 3.3.2 (R Core Team 2016), each one for one
218 response variable related to *A. incanus* reproduction (see schematic design in Fig. 2).
219 Concretely, we made two models for predation (predation at plant and fruit level), five
220 for fecundity (fruits per plant, seeds per plant, ovules per fruit, seeds per fruit and total
221 fruit seed-set) and two for offspring performance (seed germination and final biomass).
222 Seed mass was used as an intermediate trait between plant fecundity and offspring. Link
223 functions varied depending on each variable distribution. An “identity” link function

224 was used for Gaussian distributions, a “log” link function for Poisson distributions and a
225 “logit” link function for binomial ones (Table 1, Table 2). All models were built with
226 predictors indicative of habitat fragmentation (fragment size, connectivity and their
227 interaction), community competition (cover of perennials), population characteristics
228 (size and density) and mother plant traits (plant size). The logarithm of fragment size
229 was used. Connectivity and cover of perennials were excluded from the models on
230 offspring traits as they were highly correlated with fragment size and population size,
231 respectively. To test the significance of fixed factors, we performed an ANOVA-like
232 analysis through the “stats” package (R Core Team 2016). Furthermore, we used
233 fragment identity and family identity (nested in fragment) as random factors when
234 appropriate. Total variance explained by the model, as well as by the fixed and random
235 factors, was estimated using the marginal and conditional coefficients of determination
236 (Nakagawa & Schielzeth 2013) with the “MuMIn” package (Barton 2018) (see
237 Appendix B).

238

239 **Results**

240 Habitat quality, population size and density and plant size were more relevant *A.*
241 *incanus* reproduction than fragmentation variables (fragment size and connectivity).
242 Furthermore, population density, estimated through Nearest Neighbor Distances (NND),
243 showed more significant effects than population size.

244 Specifically, fragment size and connectivity showed a marginally positive effect
245 on seeds per fruit but no significant effect on any other fecundity variables (Table 1).
246 There was no effect of fragment size or connectivity on predation incidence at the plant
247 level but we found an interaction between both in determining fruit predation. This
248 variable was higher in fragments with low size and connectivity (Fig. 3), while for large

249 or well-connected fragments, it depended on the value of the other factor (connectivity
250 or size, respectively). In addition, a positive effect of fragment size was observed for
251 seedling biomass (Table 2). In fragments with higher cover of perennials, the number of
252 fruits and seeds per plant were lower (Table 1, Fig. 4 A, B), but plants, and especially
253 fruits, presented a lower incidence of predation (Table 1).

254 Population density, estimated through NND, had a significant effect on more
255 variables than population size. Population density had a significantly positive effect on
256 the number of fruits and seeds per plant and a negative effect on predation incidence,
257 both at plant and fruit level (Table 1). Plants from populations where individuals were
258 farther from each other had bigger outputs and lower predation rates. In addition, these
259 populations presented higher seed mass, and seedling biomass (Table 2, Fig. 5 A, B).
260 Population size was only negatively related to predation at the fruit level. Larger plants
261 produced larger outputs in terms of fruits and seeds, had larger seeds and suffered more
262 predation (Tables 1 and 2). However, plant size had no significant influence on output
263 at the fruit level.

264 The variables included in our models accounted for 28-58% of the observed
265 variance (Fig. 6). Fixed factors (landscape, community, population and plant size
266 variables) had a remarkable influence on early reproductive stages, including pre-
267 dispersal predation and number of fruits and seeds produced per plant (Fig. 6 A).
268 Fragment identity had a similar influence on these early stages. However, mother plant
269 identity was the largest source of variance for several fecundity variables (Fig. 6 A),
270 seed mass (Fig. 6 B) and particularly for offspring variables (Fig. 6 C).

271

272 **Discussion**

273 Our study provided evidence that, for a plant living in a fragmented landscape,
274 fragmentation effects can depend on multiple simultaneous mechanisms operating at
275 different spatial scales. Consequently, the net effect of fragmentation may be difficult to
276 assess using only one or a reduced number of predictors -namely fragment size-, as
277 some authors have previously suggested (Ewers & Didham 2006). In fact, although
278 there is substantial evidence on the negative effects of fragmentation at the landscape
279 level on plant fecundity (Haddad *et al.* 2015), fragment size and connectivity had little
280 impact on our study. Their more relevant effect concerned predation, with more
281 predated fruits in plants from small and poorly connected fragments. This pattern of
282 high predation in low connected fragments was also found for some species of the same
283 habitat by Matesanz *et al.* 2015. This is probably because isolation might increase
284 herbivore and/or predation impact (Orrock and Damschen, 2005; Brudvig *et al.* 2015),
285 especially for predators with low mobility, that may tend to stay longer in isolated
286 fragments if they are unable to find plant patches nearby (Wilby & Shachak 2000). This
287 is consistent with the fact that *A. incanus* main predators are likely generalist insects
288 (Santamaría *et al.*, 2018) with limited landscape mobility, as is the case for other
289 *Astragalus* species (Platt *et al.* 1974; Green & Palmbald 1975). Taking into account that
290 these relatives also suffer from high rates of predation, and that a similar pattern has
291 also been found for other legumes present in the region as *Ononis tridentata* (Sánchez
292 *et al.* 2012) or *Colutea hispanica* (Rabasa *et al.* 2009), we suggest that habitat loss and
293 isolation can have a major impact in the predation dynamics of *A. incanus*. This finding
294 shows the importance of simultaneously considering fragment size and connectivity
295 when assessing fragmentation effects, in agreement with previous studies showing
296 similar results (e.g. Gómez-Fernández *et al.*, 2016).

297 Fragment size and connectivity also had a positive effect on seedling growth,
298 which agrees with several previous works (e.g. González-Varo et al., 2010; Matesanz et
299 al., 2017; Pías et al., 2010). Although there are almost no studies linking offspring
300 performance with its underlying mechanisms at a landscape level, González-Varo *et al.*
301 2010 related this to low outcrossing rates in small and low-connected fragments.
302 Accordingly, we suggest that this might also be the case for *A. incanus*. However, the
303 low explanatory power of fragment size and connectivity for *A. incanus* offspring
304 performance may indicate that other factors, namely mother plant effects, are more
305 relevant for plant fitness in our species.

306 Alongside fragment size and connectivity loss, the negative effects of reduced
307 population size on plant fecundity are well-documented (Aguilar *et al.* 2006) but were
308 not significant in our study. This is likely a consequence of the relatively high
309 population size of our sampled populations, which exceeds the thresholds necessary to
310 trigger the pernicious limitations of small populations. Population density, however,
311 affected all *A. incanus* life stages, supporting the idea that aggregation patterns can be
312 more relevant for plant fitness than population size *per se* (Luzuriaga *et al.* 2006). In
313 fact, we detected higher fecundity and offspring performance in plants occurring in
314 populations with large distances between neighbors. This, in turn, could be due to
315 reduced competition among individuals, even more considering the limited availability
316 of nutrients in gypsum habitats (Lázaro-Nogal *et al.* 2012). We cannot discard,
317 however, the influence of pollination effects, which were not assessed in our study, as
318 pollinators may invest more time and visit more flowers per plant in sparse populations
319 (Mustärvi *et al.* 2001).

320 Plants far from its neighbors also suffered less predation, suggesting that sparse
321 population structures reduced detectability by insects (Kolb *et al.* 2007) and agreeing

322 with our hypothesis that *A. incanus* main predators are insects with limited mobility. In
323 contrast, Platt, Hill and Clark (1974) detected the opposite pattern in *Astragalus*
324 *canadiensis*, while Sánchez et al. (2017) found no effect of population variables on
325 *Ononis tridentata* predation, another legume occurring in our study gypsum-soil system.
326 We therefore propose that population structure is relevant for pre-dispersal predation
327 but highly depends on the species and its associated predators. Reduced competition and
328 lower detection by predators in plants far from its neighbors could also explain the
329 heavier seeds produced by those plants. Seed mass, which integrates plant final
330 fecundity and initial resource storage for seedlings, was also larger in larger plants,
331 which likely take up more nutrients and invest more net resources in reproduction (Kéry
332 et al. 2000; Sletvold 2002). Although this larger individual size may also involve a
333 higher predator attraction, final fitness was not necessarily compromised by that, which
334 is a well-documented phenomenon in seed predation dynamics (Janzen 1971). Plant
335 competition for resources, as has been repeatedly detected in gypsum habitats
336 (Luzuriaga et al. 2012; Escudero et al. 2015), appears to be important also at an inter-
337 specific level, as we found a negative influence of perennial cover on plant fecundity.
338 High perennial cover was also related to lower predation, which can be due to a diluted
339 predation pattern in habitats with abundant and easy-to-find food (Wilby & Shachak
340 2000; Kolb et al. 2007).

341 Although perennial cover and population features had an impact on *A. incanus*
342 reproduction, and particularly predation, their explanatory power decreased for later
343 reproductive stages. Specifically, fragment identity and especially mother plant identity
344 were the most relevant factors for seedling germination and growth. A number of
345 characteristics differing between fragments could account for that, e.g. fragment shape
346 and topography (Brudvig et al. 2015; Escudero et al. 2015) or spatial and temporal

347 distribution of predators (Rabasa et al. 2009; Wilby & Shachak 2000). On the other
348 hand, it has been previously reported that offspring from a single individual may present
349 similar phenotypic traits because of their similar genotypic load and/or the growing
350 conditions of the mother plant (Mousseau & Fox 1998). In addition, this great relevance
351 of mother identity may also be indicative of large genetic differences among families,
352 which would imply that *A. incanus* populations have relevant genetic variability even if
353 they live in a fragmented landscape. These results show that plant-level factors besides
354 fragmentation may affect plant reproduction. For example, the environmental conditions
355 experienced throughout maturation can influence germination (Gutterman 2000) and in
356 fact accounted for a large proportion of variance in our model for *A. incanus*, where we
357 found no influence of fragment size and connectivity. We suggest that photoperiod,
358 temperature (Donohue 2005) and particularly moisture (Pías *et al.* 2010) experienced by
359 the mother plant during seed maturation, could be key factors for seed germination and
360 seedling emergence, as water is a limiting factor in drylands and specifically in gypsum
361 habitats. We consider this an important finding as germination and seedling
362 establishment have been identified as the main demographic filters in the life cycle of
363 other *Astragalus* species (Kaye 1999) and in other chamaephytes living on gypsum soils
364 (Soliveres *et al.* 2010; Tye *et al.* 2017), although its causes have not been frequently
365 studied.

366

367 **Conclusion**

368 Population density and mother plant traits were far more relevant than traditionally-
369 assessed surrogates of fragmentation (fragment or population size) for *A. incanus*. In
370 fact, inter- and intraspecific competition at a fine scale seem to be the main drivers
371 affecting all plant stages of our species reproduction. Our study provides a

372 comprehensive analysis of fragmentation-associated processes and highlights the
373 importance of assessing several scales and plant life stages to disentangle the complex
374 interacting effects of this phenomenon.

375

376 **Acknowledgements**

377 We thank Iván León de la Asunción, Javier López Jiménez, Roberto López Rubio and
378 Jesús Prieto for field assistance. We also thank the Spanish Metereological Agency
379 (AEMET) for providing climatic data. This study was supported by the Spanish Science
380 and Technology Comission (Roots CGL2015-66809-P), by the Regional Government of
381 Madrid (Remedinal 3-CM: S2013/MAE-2719) and by the European Union (Gypworld
382 H2020-MSCA-RISE-2007-777803).

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591

592 **Figure captions**

593 Fig. 1. Fragments of natural gypsum vegetation in the study area (grey patches).
594 Sampled fragments are highlighted in black (N = 20) (see Appendix A for fragment size
595 and connectivity values). All fragments were used for reproductive output and predation
596 incidence models. A subsample of 9 of them (fragments numbered 1, 10, 12, 13, 16, 33,
597 34, 35 and 42) was used for seed collection for seed mass model and offspring models.
598 White areas represent land subject to anthropogenic influence, mainly croplands. The
599 star symbol in the inset corresponds to the location of the study area in the Iberian
600 Peninsula.

601

602 Fig. 2. Diagram of our experimental design. The upper part shows fragmentation-related
603 factors considered as independent variables in all our models. The scale at which they
604 operate, from landscape to individual level, is also indicated. The lower part shows
605 fecundity variables, predation variables and offspring traits of *A. incanus* considered as
606 dependent variables in each of our models, specifying which plant life stage are they
607 indicative of. Number of samples used to assess each factor is also shown.

608

609 Fig. 3. Predation probability per fruit (0 to 1 scale) depending on fragment size (log of
610 size in m²) and connectivity (measured with the connectivity index used by Tremlová
611 and Münzbergová, 2007).

612

613 Fig. 4. Cover of perennials (considered as total percentage cover) influence on
614 reproductive output variables, concretely (A) number of fruits per plant, (B) number of
615 seeds per plant and (C) number of ovules per fruit. As cover of perennials was estimated
616 at fragment level, error bars show standard error of families for each fragment.

617

618 Fig. 5. Nearest Neighbor Distance (NND) (medium distance to the three nearer
619 neighbors, in m) influence on offspring traits, concretely (A) seed mass (in mg) and (B)
620 seedling biomass (in mg). As for these variables NND was estimated at fragment level,
621 error bars show standard deviation of families for each fragment.

622

623 Fig. 6. Percentage of variance explained by our GLMMs for (A) field variables
624 concerning reproductive output and predation incidence, (B) seed mass and (C)
625 common garden variables concerning offspring fitness. Factors responsible for detected
626 explained variance were included in our models as fixed variables (including fragment
627 size and connectivity, population size and density, perennial cover and mother plant
628 size) or random variables (fragment and family identity). Unexplained variance is
629 indicated as residual. Percentage of explained variance is indicated inside each bar.

630

631

Table. 1. Generalized linear mixed models (GLMM) showing the effect of variables related to fragment, population and plant on female reproductive output at plant and fruit level and on predation incidence. Signs (+ or -) refer to estimation coefficients of the model and represents the positive or negative influence of the corresponding fixed variable in the response variable. n.s., no significant effects. Letters between brackets note the distribution assumed for each model: B stands for binomial, P for Poisson and G for Gaussian. Level of signification, according to χ^2 statistic from an ANOVA test, is represented by the symbols: p<0,1, *p<0,05, **p<0,01, ***p<0,001.

	Fragment			Population			Plant	
	Frag. Size	Connectivity	Size * Connectivity	Cover of perennials	Vicinity index	Population Size	Plant Size	n
Fruits per plant (P)	n.s.	n.s.	n.s.	-.**	+***	n.s.	+***	193
Seeds per plant (P)	n.s.	n.s.	n.s.	-.*	+***	n.s.	+***	193
Ovules per fruit (G)	n.s.	n.s.	n.s.	+	n.s.	n.s.	n.s.	3152
Seeds per fruit (P)	+	+	n.s.	n.s.	n.s.	+	n.s.	3152
Fruit seed-set (G)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	3152
Predation per plant (B)	n.s.	n.s.	n.s.	-.	-.*	n.s.	+*	193
Predation per fruit (B)	-.***	+***	+***	-.***	-.***	-.***	+***	4332

Table. 2. Generalized linear mixed models (GLMM) showing the effect of variables related to fragment, population and plant on offspring fitness. Signs (+ or -) refer to estimation coefficients of the model and represents the positive or negative influence of the corresponding fixed variable in the response variable. The acronym 'ns' was used when no significant effects were found. Letters between brackets note the distribution assumed for each model: B stands for binomial, P for Poisson and G for Gaussian. Level of signification, according to χ^2 statistic from an ANOVA test, is represented by the symbols: $p < 0,1$, $*p < 0,05$, $**p < 0,01$, $***p < 0,001$.

	Fragment	Population		Plant	
	Frag. size	Vicinity index	Pop. size	Size	n
Seed mass (G)	n.s.	+***	n.s.	+***	2568
Seed germination (B)	n.s.	n.s.	n.s.	n.s.	2847
Seedlings biomass (P)	+	+	n.s.	n.s.	588

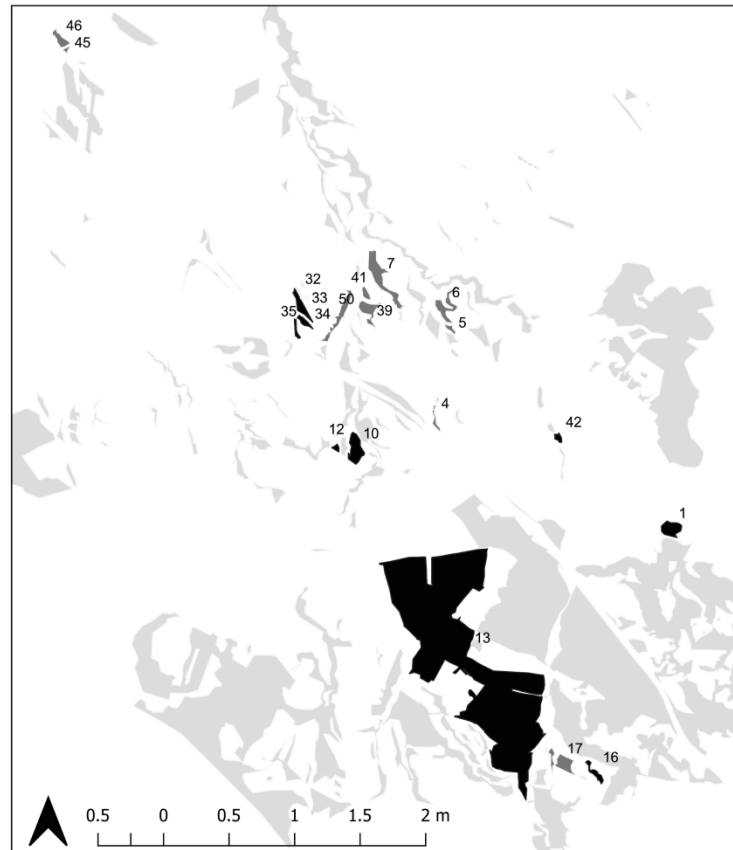


Fig. 1. Fragments of natural gypsum vegetation in the study area (grey patches). Sampled fragments are highlighted in black ($N = 20$) (see Appendix A for fragment size and connectivity values). All fragments were used for reproductive output and predation incidence models. A subsample of 9 of them (fragments numbered 1, 10, 12, 13, 16, 33, 34, 35 and 42) was used for seed collection for seed mass model and offspring models. White areas represent land subject to anthropogenic influence, mainly croplands. The star symbol in the inset corresponds to the location of the study area in the Iberian Peninsula.

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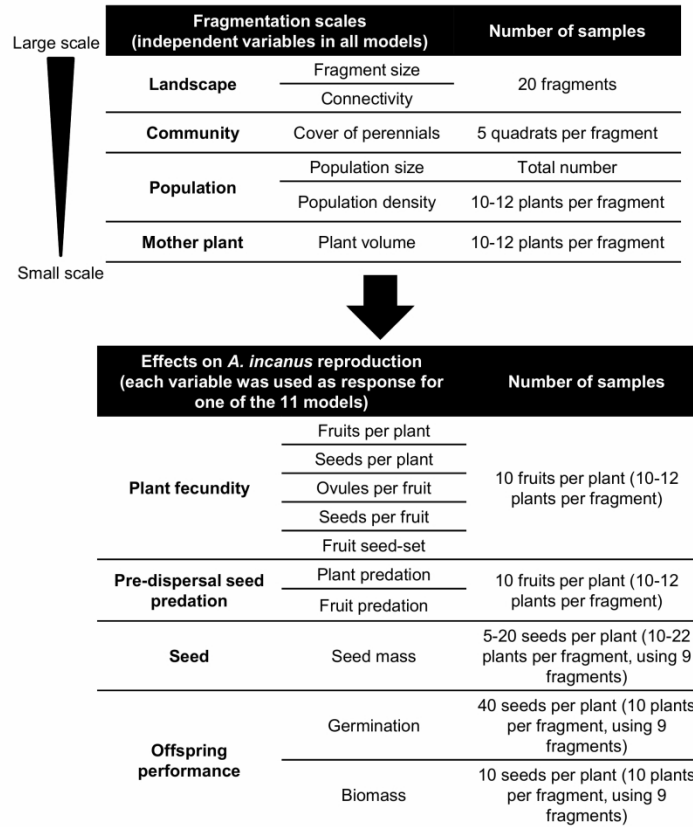


Fig. 2. Diagram of our experimental design. The upper part shows fragmentation-related factors considered as independent variables in all our models. The scale at which they operate, from landscape to individual level, is also indicated. The lower part shows fecundity variables, predation variables and offspring traits of *A. incanus* considered as dependent variables in each of our models, specifying which plant life stage are they indicative of. Number of samples used to assess each factor is also shown.

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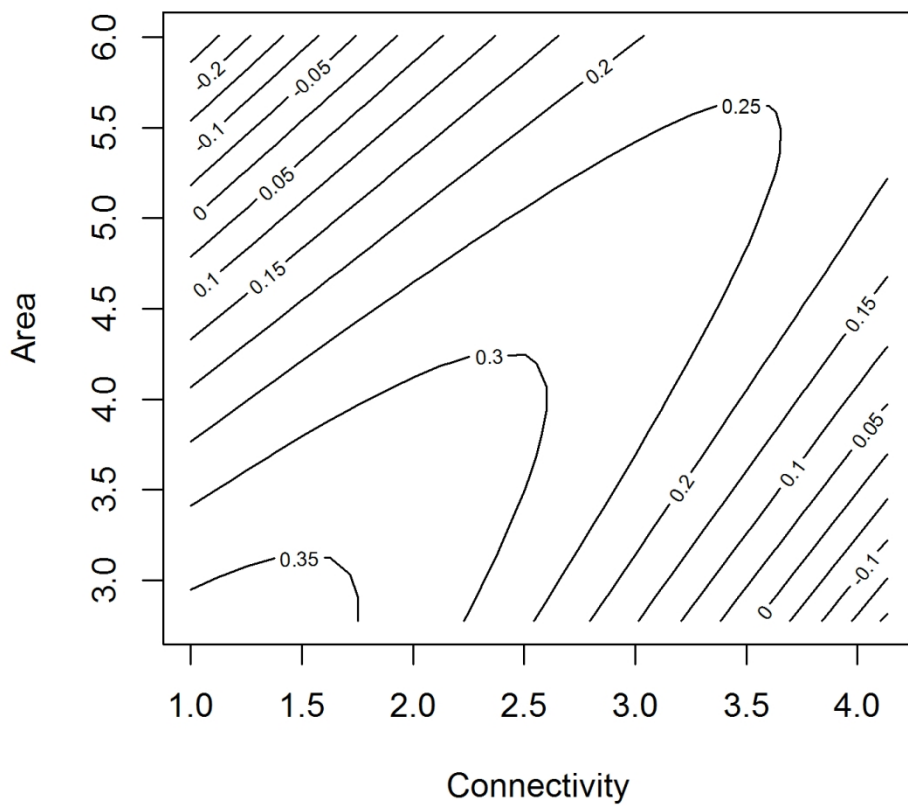


Fig. 3. Predation probability per fruit (0 to 1 scale) depending on fragment size (log of size in m²) and connectivity (measured with the connectivity index used by Tremlová and Münzbergová, 2007).

127x127mm (300 x 300 DPI)

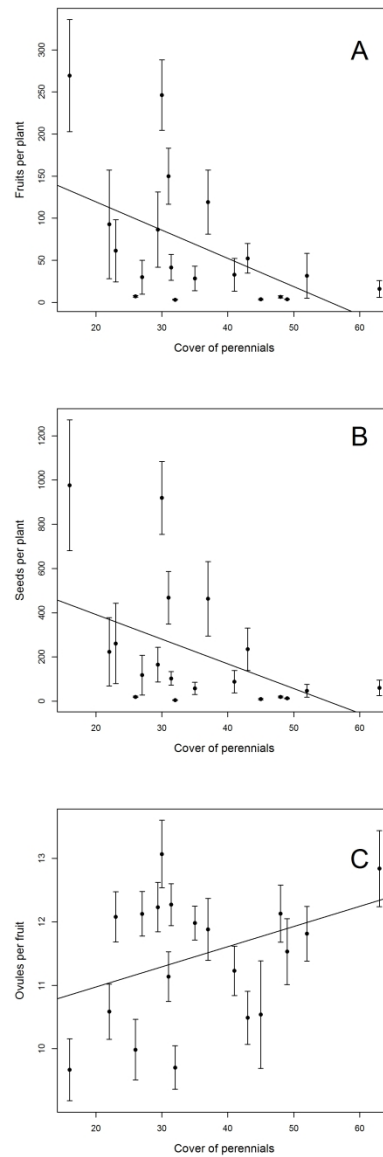


Fig. 4. Cover of perennials (considered as total percentage cover) influence on reproductive output variables, concretely (A) number of fruits per plant, (B) number of seeds per plant and (C) number of ovules per fruit. As cover of perennials was estimated at fragment level, error bars show standard error of families for each fragment.

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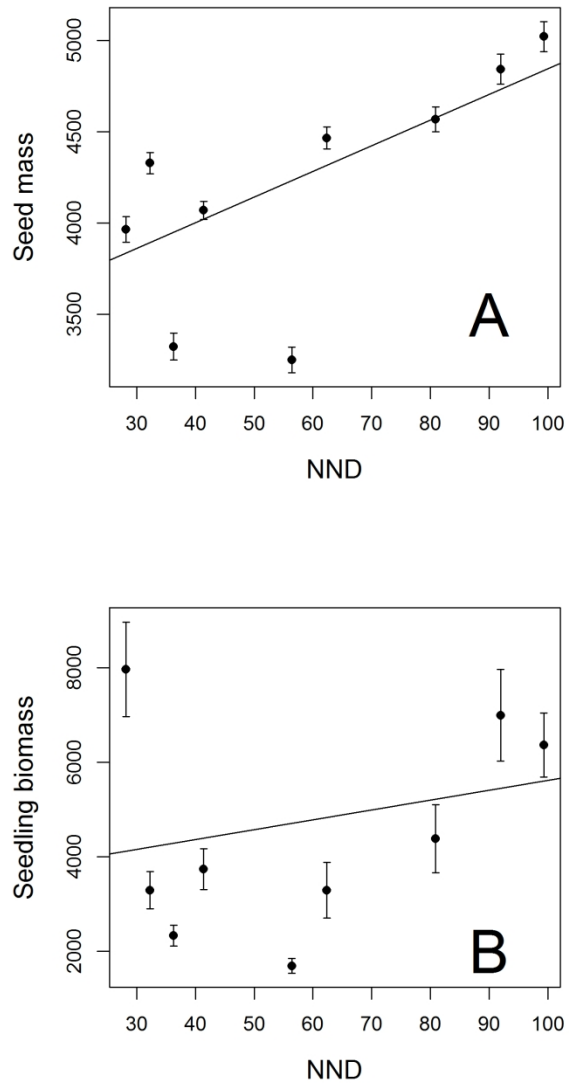


Fig. 5. Nearest Neighbor Distance (NND) (medium distance to the three nearer neighbors, in m) influence on offspring traits, concretely (A) seed mass (in mg) and (B) seedling biomass (in mg). As for these variables NND was estimated at fragment level, error bars show standard deviation of families for each fragment.

127x254mm (300 x 300 DPI)

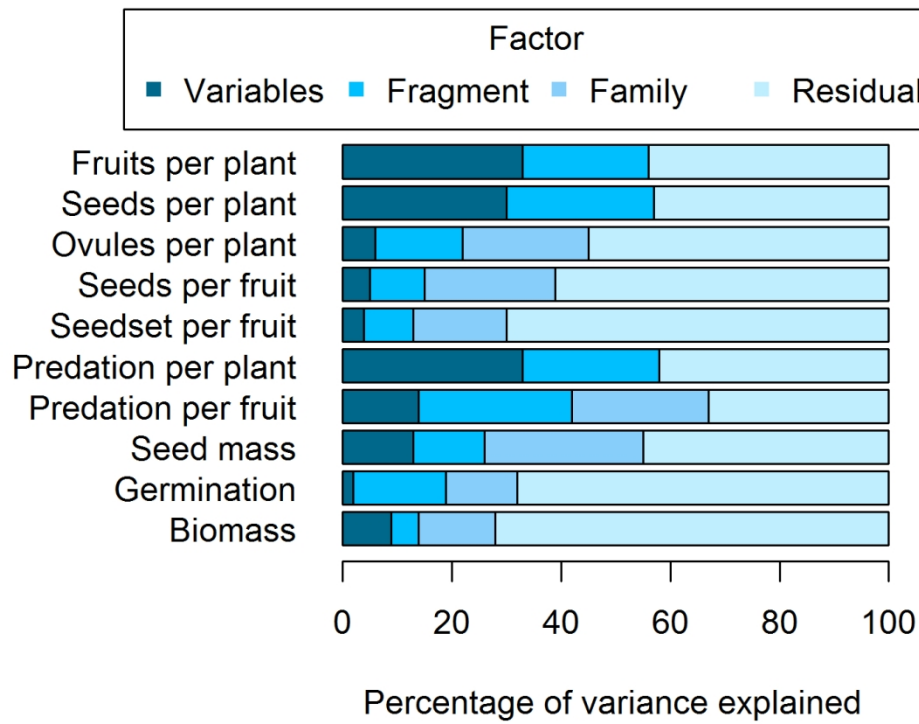


Fig. 6. Percentage of variance explained by our GLMMs for (A) field variables concerning reproductive output and predation incidence, (B) seed mass and (C) common garden variables concerning offspring fitness. Factors responsible for detected explained variance were included in our models as fixed variables (including fragment size and connectivity, population size and density, perennial cover and mother plant size) or random variables (fragment and family identity). Unexplained variance is indicated as residual. Percentage of explained variance is indicated inside each bar.

127x101mm (300 x 300 DPI)

1 Appendix A. Fragment size and connectivity values of the selected fragments.

Fragment identity	Size (m²)	Connectivity index
1	12700	3.43844859
4	4429	0.9994077
5	3171	2.72688679
6	22588	2.14428637
7	30680	1.27051316
10	20210	2.74026305
12	7702	2.53373112
13	1019191	4.13384261
16	4855	3.19195939
17	17793	2.82590595
32	595	2.23238735
33	7639	1.35922892
34	3372	1.37241288
35	1901	1.1239569
39	13204	1.4442337
41	3724	1.38633581
42	2224	1.34102384
45	950	1.93663642
46	8737	1.15152961
50	14773	1.18708324

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15 Appendix B. Calculation of the percentage of explained variance.

16 We calculated marginal (R^2c) and conditional (R^2m) coefficients of determination for our
 17 saturated models, that is, including all our variables. R^2m indicates the percentage of
 18 variance explained due to fixed factors while R^2c refers to total variance explained by the
 19 model. Therefore, subtracting them ($R^2c - R^2m$) we obtained the percentage of variance
 20 explained by random factors, that is, fragment and family. We then built models without
 21 family factor and calculate their coefficients. In this case, the subtract ($R^2c - R^2m$)
 22 resulted on the percentage of variance explained by fragment identity. Assuming that this
 23 percentage attributed to fragment was the same in the saturated model, and knowing the
 24 influence of fixed factors and total model explanation, we could calculate the remaining
 25 percentage of variance that corresponded to family in the saturated model. In the models
 26 built at plant level, the variance explained by random factors in the saturated model was
 27 all due to fragment identity.

28 Appendix B. Table 1. Percentage of variance explained by each variable or factor in our
 29 models.

	Saturated model			Model without family identity		$R^2c - R^2m$ (variance explained by fragment identity)	Variance of random factors minus variance of fragment identity (variance explained by family identity)
	R^2m (variance explained by fixed factors)	R^2c (variance explained by total model)	$R^2c - R^2m$ (variance explained by random factors)	R^2m	R^2c		
Fruits per plant	33	56	23			23	
Seeds per plant	30	57	27			27	
Ovules per fruit	6	45	39	6	22	16	23
Seeds per fruit	5	34	29	6	16	10	24
Fruit seed-set	4	30	26	5	14	9	17
Predation per plant	33	58	25			25	
Predation per fruit	14	53	39	9	37	28	25
Seed mass	13	55	42	13	26	13	29
Seed germination	2	30	28	3	20	17	13
Seedlings leaflets	15	29	14	17	18	1	13

Seedlings biomass	9	28	19	10	15	5	14
30							
31							

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