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## **Sex and the cost of reproduction through the life course of an extremely long-lived herb**

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Author Contributions: MBG and JE designed the experiments. MBG carried out the field work. All authors conceived the specific study questions. DAS and JPD conducted the statistical analyses. DAS wrote the first draft of the manuscript and all other authors contributed substantially with revisions.

1 **Abstract**

2 Despite being central concepts for life history theory, little is known about how reproductive  
3 effort and costs vary with individual age once plants have started to reproduce. We  
4 conducted a five-year field study and estimated age dependent reproductive effort for both  
5 sexes in the extraordinarily long-lived dioecious plant *Borderea pyrenaica*. We also  
6 evaluated costs of reproduction on vital rates for male and female plants, both by examining  
7 effects of differences in individual reproductive effort under natural conditions, and by  
8 conducting a flower removal experiment, aimed at decreasing reproductive effort.  
9 Reproductive effort was fairly constant and independent of age for males, which may reflect  
10 a strategy of adjusting overall reproductive output by spreading reproduction over the life  
11 course. Females had a higher total effort, which first increased and then decreased with age.  
12 The latter may be a response to an increasing reproductive value – an inverse of a terminal  
13 investment – or a sign of reproductive senescence due to an age-related physiological  
14 decline. Seed production was lower in plants with higher previous reproductive effort, and  
15 this effect increased with age. We found no evidence for costs of reproduction on other vital  
16 rates for either sex. Experimental flower removal only resulted in progressively more  
17 negative effects on flower production in older male plants, whereas female vital rates were  
18 unaffected. Overall, this study demonstrates that not only sex, but also age influences  
19 resource allocation trade-offs, and thus plant life history evolution.

20 **Key words:** Ageing, Demographic senescence, Life history, Reproductive effort,  
21 Reproductive value

22

23

24 **Introduction**

25 Reproduction is necessary for persistence of all species. Investing resources into producing  
26 offspring has short- and long-term consequences, and the optimal investment strategies  
27 depend on the abiotic and biotic environment. As a result, there is a substantial variation of  
28 reproductive strategies in terms of when to start, how often to reproduce, and how much to  
29 invest. Reproductive effort, the proportion of an organism's resources allocated to  
30 reproduction (Samson and Werk 1986), may be expected to change over the life span of  
31 individuals as an evolutionary response to changes in reproductive value (the expected  
32 average contribution of individuals of a given age to future population growth rate (Caswell  
33 2001)). However, although some studies have documented increases in reproductive effort  
34 with age for young plants (Lacey 1986; Ehlers and Olesen 2004), little is known about how  
35 reproductive effort changes with age in polycarpic plants that have started to reproduce. In  
36 species where survival decreases with advancing age (actuarial senescence), such as birds  
37 and mammals, future reproduction events become progressively less certain, and a  
38 progressive increase in reproductive effort could be expected to maximize fitness (Williams  
39 1966; Pianka and Parker 1975). In particular a large investment into reproduction very late in  
40 life has been called 'terminal investment' (Williams 1966), and seems to be common in  
41 animals (Galimberti et al. 2007; Descamps et al. 2007; but see Clutton-Brock 1984; Velando  
42 et al. 2006). Alternatively, reproductive senescence may accompany actuarial senescence,  
43 that is, reproductive effort may decline throughout an organism's life span due to  
44 physiological decline (Thomas 2013).

45

46 Plants show a wide variation in life span (Ehrlén and Lehtilä 2002), but few studies have  
47 investigated late-life changes in reproductive effort. Koenig and colleagues (2017) found no  
48 evidence for terminal investment, in terms of that trees of eight *Quercus* species in

49 California that died during the study had invested more into reproduction compared to  
50 surviving trees. In most investigated species, reproductive investment has been found to be  
51 relatively constant over age for established plants of similar size (Dahlgren and Roach 2017).  
52 However, we are not aware of studies explicitly investigating how reproductive effort and  
53 reproductive value change with age. For some plants, such as the extremely long-lived  
54 species *Pinus longaeva* and *Borderea pyrenaica*, increases in reproductive value due to  
55 increases in survival may occur (Hiebert and Hamrick 1983; García et al. 2011). In these  
56 species, where future reproduction becomes more certain with age, we may expect a  
57 decrease in reproductive effort as plants become older.

58

59 Likewise, reproductive “costs” (lowered future reproduction, growth and survival; Stearns  
60 1992; Roff 1992), may change over life spans. The concept of resource allocation trade-offs  
61 is central to life-history theory and reproductive costs have been shown in many taxa,  
62 including long-lived plants (Obeso 2002; Sletvold and Ågren 2015). Changes in the cost of  
63 reproduction over age could occur if individuals either senesce or become physiologically  
64 more robust as they age. However, little is known about how effects of reproduction on  
65 future performance change with age.

66

67 In dioecious organisms, reproductive effort and costs usually differ between sexes, and  
68 female plants often appear to invest more into reproduction than males (Meagher and  
69 Antonovics 1982a). A lower reproductive effort in males than in females would imply that  
70 male plants have more resources to invest in growth and survival (Stearns 1992). However,  
71 female plants may compensate for the higher reproductive investment by reproducing less  
72 frequently, delaying the onset of reproduction, and increasing their reproductive investment  
73 from low levels in young plants. If male reproduction is less costly, we may thus expect

74 earlier reproduction and overall flatter age trajectories of reproductive effort than in  
75 females.

76

77 In this study we used observational demographic data recorded over five years and field  
78 experiments, to examine age- and sex-dependent reproductive effort and costs of  
79 reproduction in the long-lived dioecious herb *Borderea pyrenaica*. Age determination in our  
80 study species is possible because of a morphological characteristic of the tubers, offering an  
81 unusual opportunity to explore how reproductive parameters change over the life course of  
82 individuals. Our data set included almost seven hundred individuals of ages up to 260 years  
83 in an ecologically stressful but stable alpine scree environment in the central Pyrenees.

84 Previous studies did not find evidence of demographic or physiological senescence in this  
85 plant, but instead showed that reproductive value increased with age for both sexes (García  
86 et al. 2011; Morales et al. 2013), and that males tend to flower more frequently than  
87 females (García and Antor, 1995b). Here we tested the following three hypotheses: (a)  
88 reproductive effort is similar in males and females over their life courses, because the higher  
89 investment per reproductive event in females is balanced by a lower frequency of  
90 reproductive events; (b) reproductive effort decreases with age in both sexes because the  
91 reproductive value increases, but the decrease in males is less pronounced because they  
92 have a lower overall effort; (c) reproductive costs are age dependent and decrease with age  
93 after that individuals have attained their maximum size and have more resources to invest  
94 into reproduction, again the decrease being less pronounced in male plants.

95

## 96 **Materials and Methods**

### 97 *Study System*

98 *Borderea pyrenaica* (Dioscoreaceae) is a small relict species endemic to limestone screes in  
99 the Central Pyrenees, usually at altitudes higher than 1800m a.s.l. Pollinators include ants,  
100 flies and lady beetles, fruit set is high and there is no evidence for pollen limitation (García et  
101 al. 1995). By the latter half of May each plant has grown one short aboveground stem,  
102 flowering begins in late June and fruit dispersal in early September. At the end of the  
103 growing season this stem dies back, leaving behind a scar on the tuber (García and Antor,  
104 1995a). Counting the number of scars gives an age estimate. Sex of reproductive individuals  
105 is usually easily identifiable throughout the growing season as flowers remain on the plant.  
106 Males start reproducing at younger ages (10-20 years) than females (15-35 years), have a  
107 larger number of flowers, and flower more frequently (almost every year) than females  
108 (García and Antor 1995b).

109

#### 110 *Data collection and preparation*

111 Data were collected yearly from 1995 to 1999 in the Pineta valley (42°41'N, 0°06'E; 2000 m  
112 a.s.l.), located in the central Pyrenees. The density of *B. pyrenaica* in this population varies  
113 considerably, reaching up to a few hundred plants per square metre (García et al. 2011).  
114 Individuals were carefully mapped to be relocated in successive years (aboveground parts  
115 wilt in fall), and for each plant and year we recorded state (dead or alive), sex (vegetative,  
116 male or female), number of leaves, length of the longest leaf, number of flowers in males,  
117 and the number of fruits and seeds within each fruit in females. Aerial vegetative biomass  
118 ("size" hereafter) was estimated for all plants as  $\log(\text{number of leaves} \times \text{leaf length}^2)$ . Tubers  
119 were collected the final year of the study and were weighed and aged. Due to a severe  
120 drought that made male flowers die back early in the season and counts unreliable, the  
121 number of flowers in males was not recorded in 1999. Age estimates for individuals of

122 unknown age but known size were imputed for both sexes based on generalised additive  
123 models (GAM) of age on size (García et al. 2011).

124

125 The analyses were based on two demographic data sets: observational data on 518  
126 individuals during the period 1995-1999 which was analysed previously to investigate  
127 changes in flowering probability, flower number, seed number and reproductive value with  
128 age (García et al. 2011), and experimental data on 181 reproductive individuals of both  
129 sexes, whose reproductive effort was manipulated by flower removal both in 1996 and  
130 1997. Experimental plants were assigned to one of two treatments: (1) control (no  
131 treatment) and (2) flower removal (in both years for individuals that flowered the second  
132 year, otherwise only in the first year). We conducted the experiment because we expected  
133 costs of reproduction to potentially be obscured by positive correlations between  
134 reproduction and plant condition.

135

136 In order to convert our measures of size and reproductive output into dry-weights (g), we  
137 also collected aerial parts of 32 female and 32 male reproductive plants, counted the  
138 number of fruits and male flowers, estimated size as defined above, and dried and weighed  
139 reproductive and vegetative parts separately. The dry weights were then regressed on  
140 corresponding counts of fruits or male flowers, or size, and the regression coefficients were  
141 used to translate field recordings of number of fruits, male flowers and size into  
142 corresponding dry-weights (Online Resource 1).

143

144 *Age trajectories of reproductive investment and effort*



145 Reproductive investment (weight of reproductive parts) and reproductive effort (proportion  
146 of aerial biomass allocated to flowers and fruits for males and females) were regressed on  
147 age using generalised additive models, which allow flexible nonlinear patterns reflecting  
148 potential non-monotonic changes in reproductive effort and investment with age. Each  
149 individual plant was assigned one value of reproductive investment or effort, being the sum  
150 of values for each year. We did not include tuber biomass in these calculations, as this was  
151 only measured once, at the end of the study. However, aerial and tuber biomass were tightly  
152 correlated (Online Resource 2), and our estimates of aerial reproductive investment and  
153 effort should therefore be proportional to values based on plant total biomass. We applied  
154 the default thin-plate smoothing splines in the “gam” function of the package “mgcv” for the  
155 statistical software R. Initially, models including all individuals were fitted, but since sex and  
156 the interaction term of sex and age had statistically significant effects on trajectories of  
157 flower number, seed number and size ( $p < 0.05$ ), separate models were fitted for the two  
158 sexes.

159

#### 160 *Effects of flower removal*

161 Generalised linear models (GLMs) were used to examine age-specific effects of flower  
162 removal for males and females separately. Treatment, individual age, and the interaction  
163 between treatment and age were used as predictor variables. Size in the previous year was  
164 also included as a covariate. We tested effects on five response variables: tuber biomass in  
165 the final year of the study, and size and fecundity in 1998 and 1999. Size was set to zero for  
166 plants that were dormant in one year and for 14 plants that did not emerge in the last year.  
167 Death is a very rare event in this species. Thus, survival was not modelled, and the potential  
168 effects of treatment on dormancy and survival are included in the analyses of size. Models of  
169 tuber biomass and size were fitted as ordinary linear regression models, in GLMs with

170 “identity” link functions and Gaussian error distributions. Fecundity was first analysed as  
171 total seed number and male flower number in all individuals (reproducing or not), in models  
172 with log link functions and quasipoisson error distributions (accounting for overdispersion  
173 relative to the Poisson distribution). Female fruit number and seed number were strongly  
174 correlated (Online Resource 3). Effects on fecundity were also examined for separate  
175 components: flowering probability, flower and fruit number in flowering individuals, and  
176 seed number in female plants that produced fruits. Both methods yielded similar results,  
177 and only the models of seed number and male flower number for all individuals are  
178 presented. Wald tests were used to calculate p-values.

179

#### 180 *Observational study of costs of reproduction*

181 Data from the observational study were used also to examine the cost of reproduction, in  
182 terms of relationships between past reproduction and current vital rates. The response  
183 variables were the same as in the analyses of the experimental flower-removal study and  
184 were analysed using the same types of regression models. All individuals that flowered at  
185 least once during the study were included in analyses. The total numbers of seeds produced  
186 per female (Online Resource 4) and flowers produced per male (Online Resource 5) in 1995,  
187 1996 and 1997 were regarded as measures of reproductive investment and used as  
188 predictor variables, together with age and size the previous year.

189

#### 190 **Results**

191 Generalised linear models indicated that for all ages, females invested more biomass into  
192 reproduction per year than males, also when accounting for the lower frequency of

193 reproductive events in females (Fig. 1 a & b). The pattern was similar for reproductive effort  
194 (Fig. 1 c & d).

195

196 There were also sex differences in the age-trajectories of reproductive investment and  
197 effort. In male plants, there was a continuous increase in reproductive investment with age.  
198 In contrast, in females there was an early increase in reproductive investment, followed by a  
199 decline starting at ages of about 70 years, when individuals attained their maximum size.  
200 The divergence of age-trajectories between sexes was slightly more pronounced for  
201 reproductive effort (Fig. 1 c & d). In males, there was no apparent increase with age in  
202 reproductive effort, whereas reproductive effort decreased in females at ages above 70  
203 years. The decreases in female reproductive investment and effort above 70 years were  
204 statistically significant (Online Resource 6).

205

206 Experimental flower removal did not affect size or seed production in female plants in the  
207 short term (Table 1). In males, flower removal reduced size in 1998, but not in 1999. There  
208 was also an interactive effect of age and treatment on male flower number; flower removal  
209 resulting in lower flower number in plants older than 40 years (median and mean ages were  
210 65 and 80.5, respectively), and effects of treatment being larger in older plants (note that  
211 effects in Table 1 are the effects of not receiving the flower removal treatment, i.e. of an  
212 increased reproductive investment, and thus corresponding to effects of increased  
213 reproductive investment in Table 2).

214

215 The results of the observational study suggest an age-dependent cost of reproduction for  
216 female plants: fecundity (in 1999 but not 1998) was lower in plants with higher previous

217 reproductive effort, and this negative effect of previous reproduction increased with age  
218 (Table 2; note that model predictions based on both main effects and the interaction term  
219 are that, except for plants with combinations of low previous reproduction and low age,  
220 effects of increasing previous reproduction are negative). For other response variables, our  
221 analyses suggested overall positive effects of having a higher previous reproduction in both  
222 sexes (Table 2). Female plants with higher previous reproductive investment had higher  
223 aerial biomass in 1998 and tuber biomass in 1999. However, older females with higher  
224 previous reproductive effort also tended to have a lower aerial biomass in 1999 and this  
225 effect became stronger with increasing age ( $p = 0.095$ ). Male plants with higher previous  
226 reproductive investment had a higher tuber biomass and subsequent flower number, with a  
227 tendency ( $p = 0.07$ ) of increasingly positive relationship between previous reproductive  
228 effort and flower number with age.

229

## 230 **Discussion**

231 Our results show that reproductive effort and costs of reproduction are age-dependent in  
232 the long-lived *Borderea pyrenaica*, and that age-trajectories differ between male and female  
233 plants. Males had an overall lower reproductive effort than females, and were hardly  
234 affected by plant age, while female reproductive effort first slightly increased and then  
235 decreased with age. Also costs of reproduction were age-dependent in females. Late-life age  
236 dependence of reproductive effort has been shown previously for animals (e.g. Ericsson et  
237 al. 2001), but not in plants, and we are not aware of any previous study with plants  
238 suggesting age-dependent reproductive costs.

239

240 In *Borderea pyrenaica*, the greater reproductive effort per reproductive event in females  
241 than in males is not fully compensated for by a lower frequency of female reproductive  
242 events. Sex differences in reproductive effort may reflect differing strategies of resource  
243 allocation. In polygynous animals, males appear to invest more resources into reproduction  
244 than females through high costs of traits favouring competition for mates (Promislow et al.  
245 1992; Clutton-Brock and Isvaran 2007). Male plants do not compete in other ways than by  
246 increasing pollen (flower) production, and females often invest more resources than males  
247 into reproductive tissue (Meagher and Antonovics 1982b). The higher female reproductive  
248 effort in *B. pyrenaica* is somewhat surprising given the lack of differences in growth or  
249 survival between sexes (García et al. 2011). It is possible that our measure of reproductive  
250 effort is not fully adequate, since it is only based on biomass and does not account for  
251 nutrient concentrations. Moreover, quantifying reproductive effort is difficult in our study  
252 species because both male inflorescences and female fruits are photosynthetically active.  
253 However, we would not expect higher nutrient concentrations in male tissues than in  
254 females, and despite that fruits might contribute to energy acquisition during ripening,  
255 resources invested in fruits are eventually lost at dispersal whereas nutrients may be  
256 resorbed from male inflorescences after the growing season. As a result, we expect the  
257 identified difference in reproductive effort to be conservative.

258

259 Also the shape of age trajectories of reproductive effort differed between sexes. One  
260 explanation of the observed differences in both overall effort and the shape of trajectories  
261 between sexes may be that a lower effort, per flower and also over their life course, allows  
262 males to spread their reproduction more evenly over their life spans than females. That  
263 females show lower reproductive effort early in life may be because the high minimum cost  
264 of reproduction, in terms of producing one fruit, makes it optimal for females to stay non

265 reproductive for a longer period than males. Our observation of a lower effort with  
266 advancing age in older female plants may be explained by the fact that their reproductive  
267 value seems to increase with age due to increasing survival (García et al. 2011). Under these  
268 circumstances it should not be beneficial for individuals to invest more resources into  
269 reproduction as they age if such investments affect survival negatively, because future  
270 reproduction becomes gradually more certain (Williams 1966). The observed tendency of  
271 decreasing reproductive effort with age could also simply be a consequence of physiological  
272 decline (Thomas 2013), even though no evidence of such declines has been detected  
273 previously in this species (Morales et al. 2013).

274

275 Our results regarding reproductive costs, in terms of effects of previous reproduction on  
276 vital rates, may at first glance seem contradictory. In our experimental study, we found no  
277 effect of flower removal on females, and a negative effect on male plants. Small effects of a  
278 two-year treatment for plants that can live longer than two centuries and have a prominent  
279 storage organ (tuber) is not surprising (Ehrlén and van Groenendael 2001; Obeso 2002; and  
280 see also Aragon et al. 2009 for effects on a short-lived plant). However, we still expect to  
281 detect age-dependent costs if the effect of age is strong. For male plants, it appears that  
282 negative effects of removing photosynthetic tissue associated with inflorescences  
283 outweighed any positive effects of reduced reproductive investment on future vital rates. It  
284 is possible that the treatment had negative effects also in females, which cancelled out  
285 positive effects of reduced reproductive effort. However, in several other studies flower  
286 removal has led to increases in future reproduction or in other vital rates (Hartemink et al.  
287 2004). Analysing the observational data, we found some positive relationships between high  
288 reproductive effort and future vital rates, which is likely a result of reproductive effort being  
289 positively being positively correlated with plant condition (Obeso 2002). However, we also

290 found that older female plants produced more seeds after having a lower previous  
291 reproductive effort, indicating a cost of reproduction, and that this effect increased with  
292 plant age. The fact that we did find evidence for an age-dependent cost of reproduction,  
293 despite the short study period in relation to the maximum life span of this plant, suggests to  
294 us that costs may be substantial. Taken together, and also considering the observed  
295 differences in reproductive investment and effort over the plants' life course, these results  
296 may illustrate causes of differences in life history strategies among sexes. Males maintain a  
297 fairly constant reproductive effort as they age, but at the cost of becoming frailer at higher  
298 ages, as indicated by the larger negative effect of the treatment in older males. Females, on  
299 the other hand, having higher costs per reproductive event than males, and a larger  
300 reproductive effort that first increases (young females) but then decreases (old females)  
301 seem to adjust their fecundity to their resource state.

302

303 In conclusion, the age-dependence of reproductive effort and reproductive costs, and the  
304 differences between sexes shown in this study, suggest that age and sex shape plant life  
305 history strategies. *Borderea pyrenaica* shows an increasing survival and reproductive value  
306 with advancing age, and no evidence for an increase of investment into reproduction at high  
307 ages, but instead a slight decline in female reproductive effort after reproductive maturity.  
308 This pattern may either be a response to an increasing reproductive value, or the  
309 consequence of physiological decline. More demographic studies considering plant ageing  
310 are needed in order to understand plant life history diversification, and how this is affected  
311 by resource allocation trade-offs.

312

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320

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322

323



324 **References**

- 325 Aragón CF, Méndez M, Escudero A (2009) Survival costs of reproduction in a short-lived  
326 perennial plant: Live hard, die young. *Am J Bot*, 96(5), 904-911. doi:  
327 10.3732/ajb.0800223
- 328 Caswell H (2001) *Matrix Population Models*. 2<sup>nd</sup> edn. Sinauer Associates, Sunderland
- 329 Clutton-Brock TH (1984) Reproductive effort and terminal investment in iteroparous  
330 animals. *Am Nat*, 123(2), 212–229. doi: 10.1086/284198
- 331 Clutton-Brock TH, Isvaran K (2007) Sex differences in ageing in natural populations of  
332 vertebrates. *Proc R Soc B Biol*, 274(1629), 3097–104. doi: 10.1098/rspb.2007.1138
- 333 Dahlgren JP, Roach DA (2017) Demographic senescence in herbaceous plants. In: Shefferson  
334 R, Jones OR, Salguero-Gómez R (eds) *The evolution of senescence in the tree of life*.  
335 Cambridge University Press, pp 303-319. doi: 10.1017/9781139939867.015
- 336 Descamps S, Boutin S, Berteaux D, Gaillard JM (2007) Female red squirrels fit Williams’  
337 hypothesis of increasing reproductive effort with increasing age. *J Anim Ecol*, 76(6),  
338 1192–1201. doi: 10.1111/j.1365-2656.2007.01301.x
- 339 Ehlers BK, Olesen JM (2004) Flower production in relation to individual plant age and leaf  
340 production among different patches of *Corydalis intermedia*. *Plant Ecol*, 174, 71-78.  
341 doi: 10.1023/B:VEGE.0000046060.77491.b9
- 342 Ehrlén J, van Groenendael J (2001) Storage and the delayed costs of reproduction in the  
343 understory perennial *Lathyrus vernus*. *J Ecol*, 89(2), 237-246. doi: 10.1046/j.1365-  
344 2745.2001.00546.x
- 345 Ehrlén J, Lehtilä K (2002) How perennial are perennial plants? *Oikos*, 98, 308–322. doi:  
346 10.1034/j.1600-0706.2002.980212.x

347 Ericsson G, Wallin K, Ball JP, Broberg M (2001) Age-related reproductive effort and  
348 senescence in free-ranging moose, *Alces alces*. *Ecology*, 82(6), 1613–1620. doi:  
349 10.1890/0012-9658

350 Galimberti F, Sanvito S, Braschi C, Boitani L (2007) The cost of success: reproductive effort in  
351 male southern elephant seals (*Mirounga leonina*). *Behav Ecol Sociobiol*, 62(2), 159-  
352 171. doi: 10.1007/s00265-007-0450-y

353 García MB, Antor RJ (1995a) Age and size structure in populations of a long-lived dioecious  
354 geophyte: *Borderia pyrenaica* (Dioscoreaceae). *Int J Plant Sci*, 156(2), 236–243. doi:  
355 10.1086/297246

356 García MB, Antor RJ (1995b) Sex ratio and sexual dimorphism in the dioecious *Borderia*  
357 *pyrenaica* (Dioscoreaceae). *Oecologia*, 101(1), 59–67. doi: 10.1007/BF00328901

358 García MB, Antor RJ, Espadaler X (1995) Ant pollination of the palaeoendemic dioecious  
359 *Borderia pyrenaica* (Dioscoreaceae). *Plant Syst Evol*, 198(1–2), 17–27. doi:  
360 10.1007/BF00985105

361 García MB, Dahlgren JP, Ehrlén J (2011) No evidence of senescence in a 300-year-old  
362 mountain herb. *J Ecol*, 99(6), 1424–1430. doi: 10.1111/j.1365-2745.2011.01871.x

363 Hartemink N, Jongejans E, de Kroon H (2004) Flexible life history responses to flower and  
364 rosette bud removal in three perennial herbs. *Oikos*, 105, 159–167. doi:  
365 10.1111/j.0030-1299.2004.12784.x

366 Hiebert RD, Hamrick JL (1983) Patterns and Levels of Genetic Variation in Great Basin  
367 Bristlecone Pine, *Pinus longaeva*. *Evolution*, 37(2), 302–310. doi: 10.1111/j.1558-  
368 5646.1983.tb05540.x

369 Lacey EP (1986) Onset of reproduction in plants: size versus age-dependency. *Trends Ecol*

370 Evol, 1(3), 72-75. doi: 10.1016/0169-5347(86)90021-2

371 Meagher TR, Antonovics JJ (1982a) Life history variation in dioecious plant populations: a  
372 case study of *Chamaelirium luteum*. In: Dingle H, Hegmann JP (eds) Evolution and  
373 Genetics of Life Histories. Springer, Berlin, pp 139-154. doi: 10.1007/978-1-4684-6270-  
374 8\_9

375 Meagher TR, Antonovics JJ (1982b) The populaiton biology of *Chamaelirium luteum*, a  
376 dioecious member of the lily family. Ecology, 63(6), 1690–1700. doi: 10.2307/1940111

377 Koenig WD, Knops JMH, Carmen WJ, Pesendorfer MB (2017) Testing the terminal  
378 investment hypothesis in California oaks. Am Nat 189 (5), 564-569. doi:  
379 10.1086/691161

380 Morales M, Oñate M, García MB, Munné-Bosch S (2013) Photo-oxidative stress markers  
381 reveal absence of physiological deterioration with ageing in *Borderea pyrenaica*, an  
382 extraordinarily long-lived herb. J Ecol, 101(3), 555–565. doi: 10.1111/1365-  
383 2745.12080

384 Obeso JR (2002) The costs of reproduction in plants. New Phytol, 155(139), 321–348. doi:  
385 10.1046/j.1469-8137.2002.00477.x

386 Pianka ER, Parker WS (1975) Age-specific reproductive tactics. Am Nat, 109(968), 453–464.  
387 doi: 10.1086/283013

388 Promislow DEL, Montgomerie R, Martin TE (1992) Mortality cost of sexual dimorphism in  
389 birds. Proc R Soc B Biol, 250(1328), 143–150. doi: 10.1098/rspb.1992.0142

390 Roff DA (1992) The evolution of life histories. Chapman and Hall, New York

391 Samson DA, Werk KS (1986) Size-dependent effects in the analysis of reproductive effort in  
392 plants. Am Nat, 127(5), 667–680. doi: 10.1086/284512

- 393 Sletvold N, Ågren J (2015) Nonlinear costs of reproduction in a long-lived plant. *J Ecol*,  
394 103(5), 1205–1213. doi: 10.1111/1365-2745.12430
- 395 Stearns SC (1992) *The Evolution of Life Histories*. Oxford University Press
- 396 Thomas H (2013) Senescence, ageing and death of the whole plant. *New Phytol*, 197, 696–  
397 711. doi: 10.1111/nph.12047
- 398 Velando A, Drummond H, Torres R (2006) Senescent birds redouble reproductive effort  
399 when ill: confirmation of the terminal investment hypothesis. *Proc R Soc B Biol*,  
400 273(1593), 1443–1448. doi: 10.1098/rspb.2006.3480
- 401 Williams GC (1966) Natural selection, the costs of reproduction, and a refinement of Lack's  
402 principle. *Am Nat*, 100(916), 687–690. doi: 10.1086/282461

403 **Table 1.** Effects of experimental flower removal, age (years) and their interactions on seed number, size  
 404 (aerial biomass) and tuber biomass for male and female plants of *Borderea pyreniaca*. Statistically  
 405 significant ( $P < 0.05$ ) regression coefficients for generalised linear models of the effects of no flower  
 406 removal (vs. flower removal; corresponding to a high reproductive investment (RI) in order for signs to be  
 407 comparable with Table 2) are presented without parentheses. Terms borderline significant at  $0.05 < P <$   
 408  $0.10$  are presented within parentheses. Size in the previous year was accounted for by its inclusion as a  
 409 covariate in all models.

	High RI (no flower removal)	Age	High RI × Age
<i>Female plants</i>			
Seed number 1998			
Seed number 1999			
Size 1998		(0.002)	
Size 1999		0.004	
Tuber biomass 1999		0.010	
<i>Male plants</i>			
Flower number 1998	-0.322	-0.004	0.0079
Size 1998	0.260		
Size 1999			
Tuber biomass 1999	(-0.507)	0.024	(0.0065)

410

411

412 **Table 2.** Relationships of observed reproductive investment (RI), age (years) and their interactions with  
 413 seed number, size (aerial biomass) and tuber biomass for male and female plants of *Borderea pyrenaica*.  
 414 Statistically significant ( $P < 0.05$ ) regression coefficients for generalised linear models are presented  
 415 without parentheses. Terms borderline significant at  $0.05 < P < 0.10$  are presented within parentheses.  
 416 Reproductive investment for observational data was quantified as the biomass of the number of fruits in  
 417 females, and flowers in males in the preceding years (see Methods). Average size over the same years was  
 418 accounted for by its inclusion as a covariate in all models.

	RI	Age	RI × Age
<i>Female plants</i>			
Seed number 1998		0.010	
Seed number 1999	0.018	0.005	-0.00038
Size 1998	0.005	0.003	
Size 1999	(0.005)	(0.003)	(-0.00010)
Tuber biomass 1999	0.006 (0.001)	0.011 (0.010)	(0.00007)
<i>Male plants</i>			
Flower number 1998	0.002 (0.001)	0.005 (-0.002)	(0.00002)
Size 1998			
Size 1999	(0.001)		
Tuber biomass 1999	0.005	0.015	

419

420 **Figure legends**

421 **Figure 1.** Reproductive investment measured as (ln) weight of reproductive structures (g) and reproductive  
422 effort (proportion of plant biomass invested in reproductive elements) over individual age for a, c) female  
423 and b, d) male individuals of *Borderea pyrenaica*, respectively. Each point represents an individual plant's  
424 summed reproductive investment or effort over the entire observation period. Continuous lines represent  
425 spline fits of GAM models, and dashed lines the 95% confidence intervals.