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Temporal cycles and spatial asynchrony in the reproduction and growth of a rare nectarless orchid

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22 ABSTRACT

23 The timing and intensity of plant reproduction vary due to both internal and external factors.

24 Although this variation has been widely studied in species exhibiting masting (intermittent

25 synchronous reproduction), it has attracted less attention in non-masting species. Here, we studied

26 intra-individual variation in the flowering intensity and plant size of a non-masting, rare terrestrial

27 orchid, *Cypripedium calceolus* using long-term monitoring data from three populations in Finland

28 and two populations in Estonia. Flowering intensity and plant size showed two-year cycles,

29 indicating that reproduction and growth were regulated by past costs of reproduction and extensive

30 clonal growth. In addition, flowering intensity and plant size were positively correlated with size

31 from the previous year, and were also affected by the weather conditions of spring and of the

32 previous growing season. However, there was little synchrony among plants, suggesting that the

33 climatic control of reproduction and growth is sufficiently low as to be masked by high annual

34 variation in these two vital rates. Together, these results indicate that the reproduction and growth

35 of *C. calceolus* depend on both individual demographic history as well as past weather conditions,

36 and that intrinsic factors can lead to cyclic fluctuation in reproduction also in non-masting species.

37 ADDITIONAL KEYWORDS: climate – cost of reproduction – orchids – pollinator limitation –

38 reproductive effort

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INTRODUCTION

45   INTRODUCTION

46   Among individual plants, the intensity of reproduction varies in time and space, and is regulated by

47   the interplay of external and internal factors, such as weather conditions and the quantity of

48   resources stored by individuals (Kelly & Sork, 2002; Pfeifer, Heinrich & Jetschke, 2006; Zywiec,

49   Holeksa & Ledwoń, 2012; Miyazaki, 2013; Miyazaki *et al.* 2014). Exceptionally intensive and

50   synchronous but intermittent reproduction is known as masting (Kelly & Sork, 2002), and occurs in

51   both woody (e.g., Kon *et al.*, 2005; Burns, 2012; Pearse, Koenig & Knops, 2014; Pearse, Koenig &

52   Knops, 2014) and herbaceous species (e.g., Rees, Kelly & Bjørnstad, 2002; Crone & Lesica, 2006).

53   Masting is widely considered an adaptive strategy through which plants escape from seed predation

54   by alternately starving seed predators and satiating them with excess food, which leads to increased

55   seed survival in masting years (the predation satiation hypothesis, Harper, 1987; Visser *et al.*,

56   2011). So far, however, temporal and spatial variability in reproduction in non-masting species has

57   attracted little interest and is often assumed to be mainly driven by external environmental

58   conditions such as weather. This assumption is true for lush habitats in particular, where

59   reproduction is not constrained by the amount of resources (Reznick, 1985). If vital rates (e.g.,

60   reproduction and growth) are directly proportional to environmental factors, temporal trends in

61   environmental factors should translate into temporal trends in vital rates depending on them.

62   However, there is evidence that reproduction and growth in non-masting species are also affected

63   by internal drivers such as reproductive costs (Primack & Stacy, 1998; Obeso, 2002), especially in

64   resource-poor habitats (Biere, 1995), which can lead to cyclic fluctuations in the intensity of

65   reproduction also in non-masting species. The relative importance of internal and external factors

66   for reproduction in non-masting species, has yet to be determined.

67   If plants actively use environmental signals as cues for the optimal timing of reproduction, or if the

68   environment has direct mechanistic effects on reproduction, synchrony in reproduction can arise

69   (Koenig *et al.*, 2015). For example, climatic factors such as precipitation (Burns, 2012), temperature

70   sum (Rees *et al.*, 2002) and temperature minimum (Kon *et al.*, 2005) appear to be reliable

71 environmental cues, and temperature has direct effects on plant reproduction as well (Cecich &

72 Sullivan, 1999; Pearse *et al.*, 2014). Using climatic factors as cues to synchronise flowering can be

73 evolutionarily adaptive, as plants that do not flower synchronously with conspecifics are often

74 pollen-limited (pollen coupling hypothesis, Crone, 2013; Moreira *et al.*, 2014; Pearse *et al.*, 2015).

75 Although reproductive synchrony has been extensively studied in relation to masting, few studies

76 have examined this phenomenon in other species.

77   Similar to reproductive costs causing cycles in flowering, the costs of producing a multitude of

78 shoots can lead to cycles in the number of ramets in clonal species (Kaitala & Kull 2002). Further,

79 Kaitala & Kull (2002) discussed that cycles in ramet numbers could also possibly arise due to self-

80 shading. Moreover, if growth in a given year mostly depends on the environment, we can expect

81 size to vary in synchrony among plants that experience similar environments.

82 In this study, we examined temporal and spatial variation in the reproduction and size of a non-

83 masting, rare deceptive orchid (the lady's slipper orchid, *Cypripedium calceolus* L). Deceptive

84 nectarless orchids do not provide any reward for pollinators and because pollinators learn to avoid

85 common decievers, these orchids may even benefit from asynchronous flowering (Parra-Tabla &

86 Vargas, 2007; Sun *et al.*, 2009; Tuomi *et al.*, 2015). Therefore deceptive orchids such as *C.*

87 *calceolus* represent an excellent contrast to masting species in regards of investigating variation in

88 vital rates. Using long-term monitoring data from three populations in Finland and two in Estonia,

89 we tested for the existence of temporal trends, cycles, and synchrony in reproduction (measured as

90 flowering intensity) and size (measured as the number of ramets) among individual clumps. We

91 specifically addressed four questions. First, is the variation in flowering intensity and clump size

92 cyclic, that is, are the temporal autocorrelations of these variables negative? We predicted that both

93 variables would exhibit cyclicity because of intrinsic costs of reproduction and extensive production

94 of biomass. Second, can the annual variation in flowering intensity and clump size be explained by

95 external climate variables such as temperature or precipitation? We hypothesised that, as *C.*

96 *calceolus* in Estonia and especially in Finland is near the northern edge of its distribution (Hultén &

97 Fries, 1986), it would benefit from warm summers and high precipitation in these two countries.

98 Third, is there a temporal trend in flowering intensity or clump size? We predicted that plant size

99 would increase with time, as older clones are usually larger due to rhizome branching, and that

100 flowering intensity would also show a positive trend due to the greater resource storage capacity of

101 larger plants. Finally, is the annual variation in flowering intensity and clump size synchronous

102 among plants within or between populations in each country? We hypothesised that if reproductive

103 effort and plant size were mainly under environmental control, they would vary in synchrony within

104 and between populations. However, we also predicted that, in the absence of strong environmental

105 control, as a deceptive species *C. calceolus* would have asynchronous reproduction.

106

107 MATERIALS AND METHODS

108 STUDY SPECIES

109 The lady's slipper orchid, *Cypripedium calceolus* L. (Orchidaceae), is a clonal, perennial herb that

110 forms horizontal, creeping rhizomes (Tutin *et al*., 1980). Above-ground parts of the plant wilt in the

111 autumn and the plant overwinters as a rhizome, meaning that it must produce a new set of

112 aboveground shoots every spring. Each rhizome tip annually produces two apical buds, of which

113 the larger one forms the next year's shoot, while the smaller one usually stays dormant (Kull &

114 Kull, 1991; Blinova, 2004). *C. calceolus* can grow as a single ramet, but often spreads vegetatively

115 and forms clumps of several ramets which share resources via rhizomal connections (Mossberg &

116 Nilsson, 1977; Kull, 1999). These clumps can consist of one or several clones (Kull & Kull, 1991)

117 which are long-lived and slow growing (Kull, 1988; Nicolè, Brzosko & Till-Bottraud, 2005).

118 Flowering occurs in May to early June in Estonia (Kuusk, 1984) and in late June to early July in

119 Finland (Mossberg & Nilsson, 1977). A flowering stalk usually supports one or two, rarely three,

120 flowers. Pollination takes place by food deception (Nilsson, 1979), and vegetative dormancy (i.e. a

121 state in which the plant does not sprout at all for one or more growing seasons) is common

5

122 (Shefferson *et al.*, 2001; Brzosko, 2002). In our populations the annual proportion of dormant adult

123 clumps was relatively low and stable during the study period (mean ± sd of 7 ± 3% and 1 ± 2% in

124 Finland and in Estonia, respectively). *C. calceolus* is a circumpolar, mainly boreal species (Hultén

125 & Fries, 1986) which inhabits mesic, semi-shaded sites and prefers calcium-rich substrate (Kull,

126 1999). The species is classified as near-threatened in both Finland (Rassi *et al.*, 2010) and Estonia

127 ('Red Data Book of Estonia', 2008).

128

129 DEMOGRAPHIC DATA

130 We monitored three populations in Oulanka National Park, Kuusamo, Finland from 2002-2014 and

131 two populations in Estonia from 1987-2012 (Table 1, Fig. S1). In Finland, two populations

132 (Korvasvaara I and II) were located close to each other, with about 150 m of unsuitable habitat (a

133 dense spruce forest) between them. Despite the short distance between these two populations, they

134 grew in different habitat types (Table 1) and were therefore considered separately. The third

135 population (Ampumavaara) was approximately 12 km away from Korvasvaara. The distance

136 between the populations in Estonia was approximately 160 km, and the distances between the

137 Estonian and Finnish populations were over 800 km (Fig. S1). At each study site, we established a

138 10 × 10 m permanent square. In these squares, we marked each single ramet that was clearly

139 separate from others and each group of ramets growing densely together as a distinct demographic

140 unit. Marking was done with individually numbered plastic tags secured to the ground with metal

141 sticks. Ramets in a clump grew so densely together that we could not reliably differentiate between

142 individual ramets, and differentiation between clones was impossible without genetic analysis or

143 digging up the plants. While in the Estonian populations each clump consisted of a single clone

144 (verified by isoenzyme analysis; Kull & Kull, 1991), in the Finnish populations the clumps may

145 have consisted of several different intermingling genets, and large clones may have also appeared in

146 two or more clumps (A. Jäkäläniemi, unpublished data). All populations were censused once a year

147 in June (Estonia) or in July (Finland) by counting the number of ramets and the number of flowers

148 in each clump, except in 1998 when one Estonian population (Muhu) was not visited. Only plants

149 that flowered at least once during the observation period were included in the final data set, which

150 consisted of 171 clumps from Finland and 37 clumps from Estonia (Table 1). Herbivory by reindeer

151 and voles in the study populations was rare.

152

153 STATISTICAL METHODS

154 The response variables in all statistical models were flowering intensity and clump size that we used

155 as proxies for reproduction and growth, respectively. Note that due to the low fruiting success of the

156 species (only 13% of sprouting ramets in the populations studied here produced a capsule), we were

157 unable to include fruiting probability in the models. We defined flowering intensity of a clump as

158 the proportion of flowering ramets, i.e. the number of flowering ramets divided by the total number

159 of ramets in a clump. The proportion of flowering ramets reflects a plant's reproductive intensity

160 better than the number of flowering ramets or the number of flowers does, as the latter two

161 measurements depend heavily on the total number of ramets in a clump. Clump size was defined as

162 the number of ramets in a clump, and was used to estimate annual vegetative growth (i.e. the

163 amount of vegetative biomass produced). All analyses were conducted using the statistical package

164 R 3.2.2 (R Developement Core Team, 2015).

165

166 *Temporal patterns: cyclicity and trends*

167 To assess temporal cyclicity in flowering intensity and clump size, we calculated autocorrelations

168 based on de-trended data, i.e. data in which a temporal trend was removed by differencing

169 (calculating differences between two consecutive years). Kaitala & Kull (2002) previously found

170 two-year cycles in flowering intensity in Estonian populations of *C. calceolus*. Therefore, we

171 calculated autocorrelations in flowering intensity and clump size with a lag of one year for each

172 clump (n = 208). Visual assessment of the correlograms showed that autocorrelations with longer

173 lags were negligible. The statistical significance ($\alpha = 0.05$) of the autocorrelations was determined

174 based on bootstrapped data sets with 1000 replicates. The average strength of temporal

175 autocorrelations in flowering intensity and clump size in each country was then examined with

176 general linear mixed-effects models (function "lmer" in the lme4 package in R; Bates et al. 2014),

177 and the proportions of significant temporal autocorrelations were analysed with generalized linear

178 mixed-effects models (binomial distribution with the logit link function, function "glmer" in the

179 lme4 package in R; Bates et al. 2014). To account for the possible effect of location and for the

180 spatial relatedness of the populations within countries, all models included country as a fixed

181 explanatory variable and population nested within country as a random factor. The models included

182 no intercept as we were interested in exploring the average temporal autocorrelations within each

183 country rather than testing differences between countries.

184 We examined temporal trends in flowering intensity and clump size of adult plants using linear

185 regression. Possible explanations for the temporal changes in these two variables were explored

186 using the climate models described below. Flowering intensity was logit-transformed as

187 recommended in Warton & Hui (2011) and was analysed with a general linear mixed-effects model

188 (function "lmer" in the lme4 package in R; Bates et al. 2014). Clump size was analysed using a

189 generalized linear mixed-effects model with the zero-inflated Poisson distribution and a log link

190 function (function 'glmmadmb' in the glmmADMB package in R, Fournier et al. 2012). Both trend

191 models included time as a fixed continuous explanatory variable and clump, nested within

192 population, as a grouping variable with a random intercept and slope. The inclusion of this random

193 term in the model took into account the fact that repeated measures were conducted from the same

194 populations and clumps over time. The model fit was visually verified from residuals and by

195 examining possible over-dispersion (no over-dispersion was detected, dispersion factor < 1 in all

196 the models). Due to the different lengths and years of the study periods in Finland and Estonia

197 (Table 1), we examined temporal trends in flowering intensity and clump size separately for each

198 country.

199

200 *Climate models*

201 To investigate how climate variables were associated with the flowering intensity and size of *C.*

202 *calceolus* clumps, we used linear mixed-effects models fitted separately for each country. The most

203 parsimonious model for each response variable was selected based on AIC (Akaike's Information

204 Criterion) values. Model specifics were as in the temporal trend models above, except that random

205 effects (clump nested within population) included a random intercept only. As explanatory factors,

206 we considered climate variables that were readily available or that have been reported to correlate

207 with orchid reproduction or growth (Shefferson *et al*., 2001; Blinova, 2008). We had the following

208 climate measurements for each spring (March-May): the number of freezing days (days when the

209 minimum temperature dropped below 0˚C), the minimum temperature, the cumulative temperature

210 sum (sum of the amount by which mean daily temperatures exceeded 5˚C), the precipitation sum,

211 and the mean temperature. Because buds for the year are formed during the previous autumn, in

212 selecting models we also considered the same climate variables from the previous year's growing

213 season, as well as the length of the previous growing season. The growing season was defined as

214 the snow-free period when the 24-hour average temperature was at least 5˚C for ten consecutive

215 days. The tested climate variables included also the winter precipitation sum and the maximum and

216 mean spring snow depth. In addition to these climate variables, the clump sizes of the previous and

217 current growing season (with logarithmic transformation) were considered as explanatory variables.

218 The Finnish climate data were provided by the Finnish Meteorological Institute from the

219 Kiutaköngäs weather station, which was located within 13 km of the Finnish study populations. The

220 Estonian climate data for the Ussisoo and Muhu populations were provided by the Türi and Virtsu

221   weather stations of the Estonian Weather Service, which were about 25 km and 13 km away from

222   the populations, respectively.

223   The demographic data from the first year in the Muhu and Finnish populations (1987 and 2002,

224   respectively) and from the year 1999 in the Muhu population (the year following the missing year)

225   were excluded from the analyses because they lacked estimates of the prior year's clump size.

226   Moreover, the year 2010 in Finland had some missing values for climate variables, and so the years

227   2010 and 2011 were removed from the analysis. As a result, the climate models for the Finnish

228   populations of *C. calceolus* included 10 years, while the models for the Estonian populations

229   included 25 and 26 years (Muhu and Ussisoo, respectively). The model fit was assessed visually

230   from residuals as with the trend models. We also examined the variance inflation factors (VIF) for

231   possible multicollinearity of the explanatory variables (no multicollinearity was detected, all VIFs <

232   3).

233

234   *Spatial synchrony among clumps*

235   We used cross-correlations to estimate the synchrony of flowering intensity and size among clumps

236   both within and between populations. We calculated all pairwise correlations in flowering intensity

237   and clump size between the clumps in each population (within-population synchrony) and between

238   the clumps of different populations (between-population synchrony) using the previously de-

239   trended time series. The average strength of correlations and the proportions of significant (both

240   negative and positive) correlations were calculated as in the temporal autocorrelation analyses

241   above.

242

243   RESULTS

244   TEMPORAL PATTERNS: CYCLICITY AND TRENDS

245 The autocorrelation analysis points towards two-year cycles in both flowering intensity and clump

246 size over time, as the majority of the clumps (94% and 97% for flowering intensity and clump size,

247 respectively) showed negative, although only moderate, autocorrelation coefficients at a lag of one

248 year (Table 2). This indicates that years of intensive flowering were followed by years of sparse

249 flowering and vice versa. Similarly, years of extensive vegetative growth were usually followed by

250 years of meager vegetative growth. In addition, clump size increased over time in both countries

251 (Fig. 1), while the flowering intensity of the clumps showed no significant temporal trend (Table 3).

252

253 CLIMATE MODELS

254 In general, variation in flowering intensity was best explained by the temperature of the previous

255 summer and clump size during the previous growing season, although the best variables for summer

256 temperatures differed between countries, as did the effects of temperature (Table 4). In Finland, the

257 most parsimonious model for flowering intensity also included the spring cumulative temperature

258 sum, which was negatively correlated with subsequent flowering (Table 4).

259 For clump size, the best single predictor was clump size in the previous year, which correlated

260 positively with current size (Table 4). Current clump size was also positively associated with spring

261 snow depth in both countries (Table 4). In Estonia, clump size increased with the increasing

262 cumulative temperature sum of the previous growing season. The strongest effect of this was seen

263 in the smallest clumps (Table 4, Fig. 2), indicating that the smallest plants were the most sensitive

264 to temperature. Moreover, clump size in Estonia was associated with spring weather conditions,

265 with the precipitation sum having a positive effect and the cumulative temperature sum having a

266 negative effect on size (Table 4).

267

268 SPATIAL SYNCHRONY

11

269   Overall, there was little synchrony in reproduction and vegetative growth among clumps both

270   within and between populations, as the cross-correlations among clumps were moderate and mostly

271   non-significant in both countries as well as between countries (Table 5). Although some pairs of

272   clumps showed perfect positive synchrony, while others showed perfect negative synchrony, the

273   variation in correlation coefficients was high (Fig S2), with over half of the pairs showing no

274   correlation in either of the variables (Table S1). Furthermore, the pairs of clumps flowering in

275   synchrony were usually not the same ones that were synchronous in size (Table S1).

276

DISCUSSION

277

278   TEMPORAL CYCLICITY AND THE IMPORTANCE OF DEMOGRAPHIC HISTORY

279   Our findings suggest that internal factors can lead to cyclic reproduction also in non-masting

280   species, as the autocorrelation analysis revealed two-year cycles in flowering intensity and clump

281   size in the study populations (Table 2). In other words, consecutive years tended to be dissimilar to

282   each other, with years of intensive flowering (or growth) being followed by years of less intensive

283   flowering (or growth) High variability in reproduction is one of the defining aspects of the masting

284   phenomenon (Kelly & Sork, 2002) and two-year cycles similar to the ones observed here have been

285   reported also in masting legumes (Crone, Miller & Sala, 2009) and trees (Kon *et al.*, 2005; Pearse

286   *et al.*, 2014). These cycles are assumed to be due to the depletion of resource by the costs of

287   reproduction (Rees *et al.*, 2002; Crone *et al.*, 2009; Miyazaki, 2013; Pesendorfer *et al.*, 2016). The

288   cyclicity observed here indicates that the internal demographic history of individuals matters also in

289   non-masting species. In finding two-year cycles, our study agrees with that of Kaitala & Kull

290   (2002), although the autocorrelations observed in both studies were only moderate and mostly

291   non-significant.

292   In the present study, both flowering intensity and clump size increased with the increasing clump

293   size of the previous year (Table 4). Such a positive relationship is expected, as the largest clones

have the most resources available to allocate to both flowering and growth. Moreover, these results indicate that flowering depends on resources collected during the past growing season (as opposed to relying on the photosynthesis of the current growing season), which enables cycles in reproduction. In addition to high levels of stored resources, large clumps with extensively branched rhizomes also have a large number of meristems. Because of this, large clumps can be expected to produce a multitude of ramets year after year, while small clumps with meagerly branched rhizomes will always have only a few ramets due to their small numbers of meristems (Geber, 1990).

TEMPORAL TRENDS

We observed that the clump size of *C. calceolus* increased over time in both countries, which was in line with our predictions and with previous studies of this species (Kaitala & Kull 2002, Laitinen, 2006). An increase in ramet numbers with time probably occurred via rhizome branching as the clones aged. It is also possible that the branching structure itself did not change over time, but instead, that the sprouting rate increased due to a positive change in the environment (either directly through reduced bud mortality or indirectly via an increase in resource levels), which enabled the plant to support a larger number of ramets sprouting from the existing meristems.

In contrast to our predictions, flowering intensity showed no increase over time. This finding differs from the results of Kaitala & Kull (2002), who observed a positive trend in flowering based on the same Estonian populations studied here. This discrepancy between the results can partially be explained by the different methods used to measure flowering intensity. While we used the proportion of flowering ramets, the previous study used the total number of flowers per clone, which is dependent also on the number of ramets in the clone (and is thus affected by changes in clone size). Kaitala & Kull (2002) also had data from shorter time periods and partly different years compared to the present study. Overall, the different results between these studies highlight the

318   importance of considering the time-period, its length, and the response variable used when

319   examining temporal trends in vital rates.

320

321   CLIMATE, REPRODUCTION, AND GROWTH

322   In addition to clump size, weather conditions of the previous year and current spring affected the

323   performance of *C. calceolus* clumps, with the temperature of the previous growing season and

324   spring snow depth being the most influential weather aspects for flowering intensity and clump size,

325   respectively. However, the effects of weather conditions varied between countries (Table 4). The

326   negative effect of high summer temperatures on flowering in Estonia may be due to the costs of

327   increased respiration. Instead, warm summers had a positive effect on flowering intensity in

328   Finland, possibly because, in general, the average summer temperatures there are lower than in

329   Estonia and the clumps are, on average, smaller, and therefore do not suffer from excess respiration.

330   In this respect, the differences observed here between countries are not particularly surprising, as

331   other studies have also reported that the effects of summer temperatures on orchids may vary by

332   species, study location, or the measure used to describe plant performance (Hutchings 2010;

333   Sletvold et al. 2013; Shefferson, Warren & Pulliam, 2014).

334   The negative effect of high cumulative spring temperature on flowering in the Finnish populations

335   and on clump size in Estonia may have appeared because warm springs induce plants to start

336   growing earlier, which exposes them more frequently to frosts. Unsurprisingly, snow cover seemed

337   to have a protective effect on plant growth. In addition, melting snow also provides moisture. In

338   Estonia, where the climate is not as humid as in Finland and the plants are more likely to experience

339   drought, clump size was further increased by high spring precipitation (Table 4) which ensured

340   favorable water conditions for growth. This finding suggests that the growth of *C. calceolus* will

341   benefit from the predicted increase in precipitation at high latitudes (IPCC, 2014). However, spring

342   snow cover has continued to decrease in the Northern Hemisphere during the past two decades

343 (IPCC, 2014), and this can lead to more frequent bud damage from freezing in the spring, as has

344 already been observed in montane wildflowers (Inouye 2008).

345 The results from our climate models should, however, be viewed with some caution, as the model

346 outcomes were sensitive to the dataset used, and this may have consequences for the generality of

347 our results. While individual estimates for the explanatory variables were rather robust, the rank

348 order of the models varied depending on the exact dataset used. We also note that the correlations

349 observed here between plant performance and weather variables do not prove causality, and

350 manipulative experiments are needed to more explicitly assess the impact of weather conditions on

351 reproduction and growth (see e.g. Crone & Lesica, 2006). Nevertheless, as the same climate

352 variables (spring and summer temperatures, spring snow depth) were the best predictors in both

353 countries despite differences in habitats and partial differences in the years under study, our

354 findings provide strong support for the importance of these climate variables in regulating the

355 reproduction and growth of *C. calceolus*.

356

357 SPATIAL ASYNCHRONY IN REPRODUCTION AND GROWTH AMONG CLUMPS

358 Despite the significant associations between climate variables, flowering intensity, and clump size,

359 the overall synchrony in the examined variables between clumps was low both within and between

360 populations (Table 5). This suggests that while environmental factors do affect reproduction and

361 growth in the study populations, within-population variability in microhabitats and in clump

362 demographic history overwhelm any synchrony caused by local weather. This view is supported by

363 the fact that significant positive correlations within populations (8-33%) were more common than

364 significant positive correlations between populations within a country (5-14%), which in turn were

365 more common than positive correlations between populations of different countries (5-6%). In other

366 words, the closer to each other the clumps grow, the more likely they were to behave similarly,

367 which is to be expected considering that environmental factors are often spatially autocorrelated.

368   *C. calceolus* is a nectarless species with low overall pollination success and seed production (Kull,

369   1999). Thus, it seems likely that neither predation satiation nor pollen coupling play any important

370   role in the reproductive success of this species, which could further explain why we did not observe

371   synchrony among clumps. In fact, as a deceptive species, *C. calceolus* might even benefit from

372   asynchrony because of the negative frequency-dependent selection. Selective advantages could

373   arise due to either decreased competition for pollinators (Parra-Tabla & Vargas, 2007) or because

374   high frequencies of deceptive flowers enable pollinators to more quickly learn to discriminate and

375   avoid them (Smithson & MacNair, 1996). Previous studies in deceptive orchids have demonstrated

376   that frequency-dependent selection operates at least on flowering phenology (Parra-Tabla & Vargas,

377   2004; Sun et al., 2009) and the frequency of floral color morphs (Gigord, Macnair & Smithson,

378   2001; but see Pellegrino et al 2005).

379

380   CONCLUSIONS

381   In this long-term study of Finnish and Estonian populations of the rare deceptive orchid *C.*

382   *calceolus*, we observed that reproduction and vegetative growth were regulated by both internal and

383   external factors. Although this species is non-masting, our data suggest two-year cyclicity in

384   reproduction and growth which arises due to variation in a plant's internal resource levels which, in

385   turn, depend on its demographic history. Despite the fact that flowering intensity and plant size

386   were associated with external weather conditions, we observed no synchrony in these two traits

387   among clumps either within or between populations. We thus obtained no support for the

388   hypothesis that individuals use weather as a cue for flowering, and our results indicate that the

389   direct effects of external factors are overridden by the internal ones. This is not very surprising

390   considering that *C. calceolus* is a non-masting species and therefore is not expected to actively

391   synchronize its reproduction with conspecifics. We suggest that, as a deceptive species, *C.*

16

calceolus may even benefit from asynchronous flowering because of negative frequency-dependent selection.

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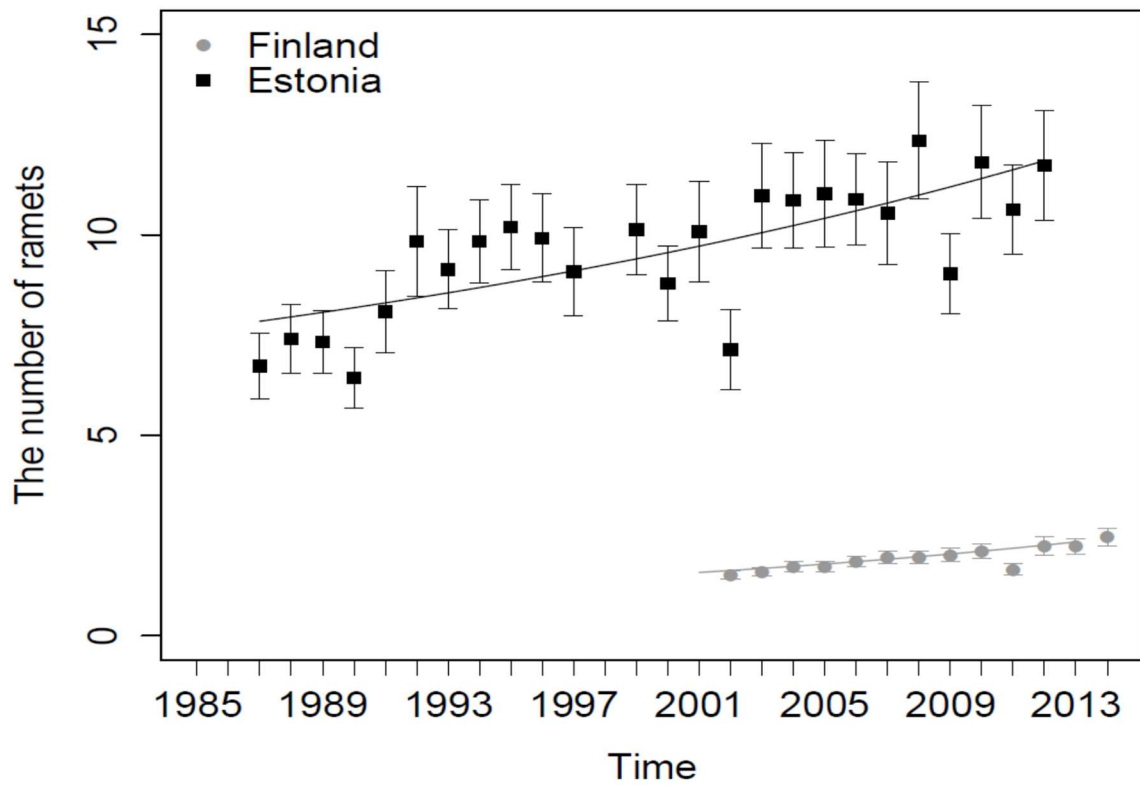
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Figure 1. Temporal trends in clump size (number of ramets) of *Cypripedium calceolus* in Finland

and Estonia. Vertical bars show the observed annual mean value (± SE). Note that year 1998 is

excluded due to missing data from one of the two Estonian populations.

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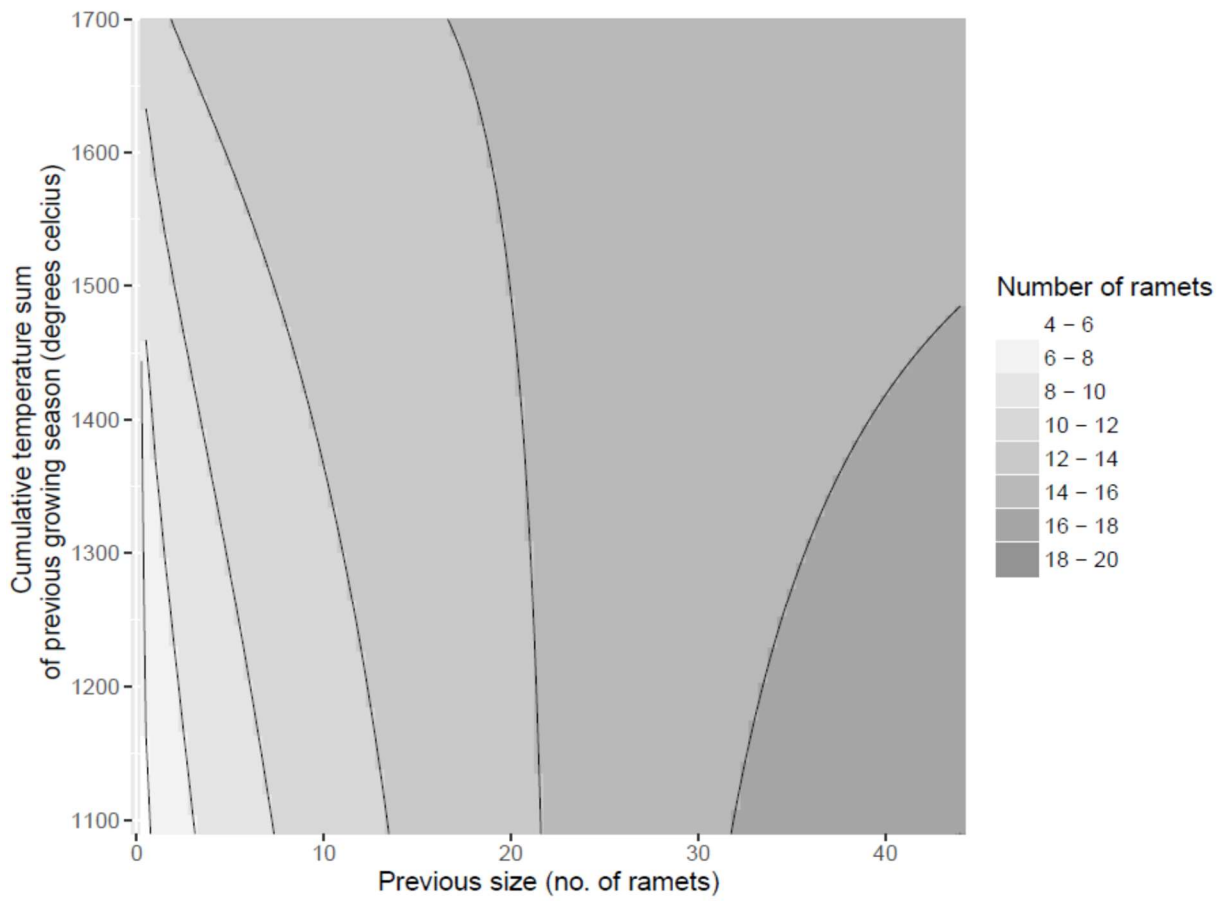
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Figure 2. The effects of the cumulative temperature sum and the previous year's clump size on

clump size of *Cypripedium calceolus* in Estonia.

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Table 1. Details of the studied populations of *Cypripedium calceolus.*

Population	Country	Location	Habitat	No. clumps	Years monitored
1. Ampumavaara	Finland	Oulanka	moist herb-rich forest	73	2002-2014
2. Korvasvaara I	Finland	Oulanka	moist herb-rich forest	29	2002-2014
3. Korvasvaara II	Finland	Oulanka	rich spruce-birch fen	69	2002-2014
4. Ussisoo	Estonia	Ussisoo	boreo-nemoral spruce forest	14	1987-2012
5. Muhu	Estonia	Muhu	alvar pine forest	23	1987-2012*

*year 1998 excluded because of missing data

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Table 2. Autocorrelations in flowering intensity (measured as the proportion of flowering ramets) and clump size (measured as the number of ramets) in Finnish and Estonian populations of *Cypripedium calceolus*.

| Dependent variable | Average autocorrelation | | % negative autocorrelations | |
	Country	Estimate ± SE	Country	Estimate-SE – Estimate+SE
Flowering intensity	Estonia	-0.358 ± 0.050***	Estonia	0.398 – 0.576
	Finland	-0.368 ± 0.031***	Finland	0.270 – 0.357***
Clump size	Estonia	-0.445 ± 0.037***	Estonia	0.567 – 0.723
	Finland	-0.319 ± 0.018***	Finland	0.150 – 0.212***

Average correlation is the mean autocorrelation coefficient across all clumps, and % negative autocorrelations is the proportion of significant negative autocorrelations out of all autocorrelations. Proportion is back-transformed from logit and therefore the p-values indicate whether the estimates significantly differ from 0.5.

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ based on Satterthwaite's approximation (average autocorrelations) or Wald tests (% negative autocorrelations)

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Table 3. Results from general and generalized linear mixed-effects models that were used to examine temporal trends in the flowering intensity (proportion of flowering ramets) and clump size (number of ramets) of *Cypripedium calceolus* populations in Finland and Estonia.

	Finland	Estonia
Flowering intensity at the start of the study	0.455 – 0.544	0.610 – 0.826
Clump size at the start of the study	1.084 – 1.403	5.285 – 7.278***
Annual increase in odds of flowering intensity	1.067 – 4.696%	-0.924 – -3.600%
Annual increase in clump size	1.248 – 2.939%*	1.207 – 1.845%***

Values given are the interval between the Estimate-SE and Estimate+SE.

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ based on Wald tests

Table 4. Results from linear mixed-effects models that were used to explore associations between selected climatic variables and the flowering intensity (proportion of flowering ramets) and clump size (number of ramets) of *Cypripedium calceolus* populations in Finland and Estonia.

Dependent variable	Finland		Estonia	
	Parameter	Estimate ± SE	Parameter	Estimate ± SE
Flowering intensity	Intercept	0.1806±0.1066*	Intercept	0.6276±0.5899
	Cumulative spring temperature	-0.0048±0.0001***	Cumulative temperature of the previous growing season	-0.0016±0.0003***
	Log(clump size of the previous growing season)	0.5106±0.1142***	Log(clump size of the previous growing season)	0.5606±0.0981***
	Min temperature of the previous growing season	0.2212± 0.0328***		
Clump size	Intercept	-0.1105±0.1046	Intercept	2.0529±0.1285***
	Log(clump size of the previous growing season)	0.8125±0.0813***	Log(clump size of the previous growing season)	0.2572±0.0029***
	Mean spring snow depth	0.0167±0.0043**	Max spring snow depth	0.0040±0.0009***
			Cumulative temperature of the previous growing season	0.0007±0.0001***
			Spring precipitation sum	0.0017±0.0003***
			Cumulative spring temperature	-0.0011±0.0002***
			Log(clump size of the previous growing season) × Cumulative temperature of the previous growing season	-0.0006±0.0001***

All predictors are centered, and the models include clump nested within population as a random factor.

Parameter estimates are on logit (flowering intensity) and log (clump size) scales.

*: p < 0.05, **: p < 0.01, ***: p < 0.001 based on Wald tests

Table 5. Pairwise correlations in flowering intensity (proportion of flowering ramets) and clump size (number of ramets) among clumps within Finnish and Estonian populations of *Cypripedium calceolus*.

Dependent variable	Average correlation		% positive correlations		% negative correlations	
	Country	Estimate ± SE	Country	Estimate-SE – Estimate+SE	Country	Estimate-SE – Estimate+SE
Flowering intensity within population	Estonia	0.1944 ± 0.0446*	Estonia	0.1761 – 0.2886***	Estonia	0.0049 – 0.0155***
	Finland	0.0567 ± 0.0325	Finland	0.0740 – 0.1152***	Finland	0.0467 – 0.0556***
Clump size within population	Estonia	0.2287 ± 0.0637*	Estonia	0.2748 – 0.3921**	Estonia	0.0065 – 0.0189***
	Finland	0.0222 ± 0.0500	Finland	0.0625 – 0.0915***	Finland	0.0373 – 0.0523***
Flowering intensity between populations	Estonia	0.1229 ± 0.050*	Estonia	0.1135 – 0.1788***	Estonia	0.0070 – 0.0235***
	Finland	0.0417 ± 0.0263	Finland	0.0839 – 0.1054***	Finland	0.0545 – 0.0807***
	Between countries	0.0202 ± 0.0192	Between countries	0.0516 – 0.0630***	Between countries	0.0514 – 0.0691***
Clump size between populations	Estonia	0.0031 ± 0.0355	Estonia	0.0343 – 0.0620***	Estonia	0.0472 – 0.0736***
	Finland	-0.0013 ± 0.0178	Finland	0.0495 – 0.0609***	Finland	0.0440 – 0.049 ***
	Between countries	0.0121 ± 0.0132	Between countries	0.0536 – 0.0637***	Between countries	0.0400 – 0.0451 ***

Average correlation is the mean of correlations over all pairwise comparisons between clumps, and % negative and positive correlations are the numbers of significant negative and positive correlations, respectively, divided by the total number of pairwise correlations. Note that the proportions are back-transformed from logit and therefore p-values indicate whether the estimates significantly differ from 0.5

***: $p < 0.001$, **: $p < 0.01$, **: $p < 0.05$ based on Satterthwaite's approximation (average correlations) or Wald tests (% correlations)