

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

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4 HURSKAINEN, SONJA^{1*}, JÄKÄLÄNIEMI, ANNE¹, KAITALA, VEIJO², KULL, TIJU³,
5 MÕTLEP, MARILIN³, RAMULA, SATU⁴, TUOMI, JUHA^{1,4}

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7 *¹Department of Ecology, University of Oulu, Pentti Kaiteran katu 1, 90014 Oulu, Finland*

8 *²University of Helsinki, Viikinkaari 1, P.O.Box 65, 00014 University of Helsinki, Finland*

9 *³Estonian University of Life Sciences, Friedrich Reinhold Kreutzwaldi 1, 51014 Tartu, Estonia*

10 *⁴Section of Ecology, Department of Biology, University of Turku, 20014 Turku, Finland*

11 *Corresponding author.

12 Address: Department of Ecology, University of Oulu, Pentti Kaiteran katu 1, 90014 Oulu, Finland

13 Tel. +358 400 641 225

14 E-mail: sonja.hurskainen@oulu.fi

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21 ABSTRACT

22 The timing and intensity of plant reproduction vary due to both internal and external factors.
23 Although this variation has been widely studied in species exhibiting masting (intermittent
24 synchronous reproduction), it has attracted less attention in non-masting species. Here, we studied
25 intra-individual variation in the flowering intensity and plant size of a non-masting, rare terrestrial
26 orchid, *Cypripedium calceolus* using long-term monitoring data from three populations in Finland
27 and two populations in Estonia. Flowering intensity and plant size showed two-year cycles,
28 indicating that reproduction and growth were regulated by past costs of reproduction and extensive
29 clonal growth. In addition, flowering intensity and plant size were positively correlated with size
30 from the previous year, and were also affected by the weather conditions of spring and of the
31 previous growing season. However, there was little synchrony among plants, suggesting that the
32 climatic control of reproduction and growth is sufficiently low as to be masked by high annual
33 variation in these two vital rates. Together, these results indicate that the reproduction and growth
34 of *C. calceolus* depend on both individual demographic history as well as past weather conditions,
35 and that intrinsic factors can lead to cyclic fluctuation in reproduction also in non-masting species.

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

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44 INTRODUCTION

45 Among individual plants, the intensity of reproduction varies in time and space, and is regulated by
46 the interplay of external and internal factors, such as weather conditions and the quantity of
47 resources stored by individuals (Kelly & Sork, 2002; Pfeifer, Heinrich & Jetschke, 2006; Zywiec,
48 Holeksa & Ledwoń, 2012; Miyazaki, 2013; Miyazaki *et al.* 2014). Exceptionally intensive and
49 synchronous but intermittent reproduction is known as masting (Kelly & Sork, 2002), and occurs in
50 both woody (e.g., Kon *et al.*, 2005; Burns, 2012; Pearse, Koenig & Knops, 2014; Pearse, Koenig &
51 Knops, 2014) and herbaceous species (e.g., Rees, Kelly & Bjørnstad, 2002; Crone & Lesica, 2006).
52 Masting is widely considered an adaptive strategy through which plants escape from seed predation
53 by alternately starving seed predators and satiating them with excess food, which leads to increased
54 seed survival in masting years (the predation satiation hypothesis, Harper, 1987; Visser *et al.*,
55 2011). So far, however, temporal and spatial variability in reproduction in non-masting species has
56 attracted little interest and is often assumed to be mainly driven by external environmental
57 conditions such as weather. This assumption is true for lush habitats in particular, where
58 reproduction is not constrained by the amount of resources (Reznick, 1985). If vital rates (e.g.,
59 reproduction and growth) are directly proportional to environmental factors, temporal trends in
60 environmental factors should translate into temporal trends in vital rates depending on them.
61 However, there is evidence that reproduction and growth in non-masting species are also affected
62 by internal drivers such as reproductive costs (Primack & Stacy, 1998; Obeso, 2002), especially in
63 resource-poor habitats (Biere, 1995), which can lead to cyclic fluctuations in the intensity of
64 reproduction also in non-masting species. The relative importance of internal and external factors
65 for reproduction in non-masting species has yet to be determined.

66 If plants actively use environmental signals as cues for the optimal timing of reproduction, or if the
67 environment has direct mechanistic effects on reproduction, synchrony in reproduction can arise
68 (Koenig *et al.*, 2015). For example, climatic factors such as precipitation (Burns, 2012), temperature
69 sum (Rees *et al.*, 2002) and temperature minimum (Kon *et al.*, 2005) appear to be reliable

70 environmental cues, and temperature has direct effects on plant reproduction as well (Cecich &
71 Sullivan, 1999; Pearse *et al.*, 2014). Using climatic factors as cues to synchronise flowering can be
72 evolutionarily adaptive, as plants that do not flower synchronously with conspecifics are often
73 pollen-limited (pollen coupling hypothesis, Crone, 2013; Moreira *et al.*, 2014; Pearse *et al.*, 2015).
74 Although reproductive synchrony has been extensively studied in relation to masting, few studies
75 have examined this phenomenon in other species.

76 Similar to reproductive costs causing cycles in flowering, the costs of producing a multitude of
77 shoots can lead to cycles in the number of ramets in clonal species (Kaitala & Kull 2002). Further,
78 Kaitala & Kull (2002) discussed that cycles in ramet numbers could also possibly arise due to self-
79 shading. Moreover, if growth in a given year mostly depends on the environment, we can expect
80 size to vary in synchrony among plants that experience similar environments.

81 In this study, we examined temporal and spatial variation in the reproduction and size of a non-
82 masting, rare deceptive orchid (the lady's slipper orchid, *Cypripedium calceolus* L). Deceptive
83 nectarless orchids do not provide any reward for pollinators and because pollinators learn to avoid
84 common deceivers, these orchids may even benefit from asynchronous flowering (Parra-Tabla &
85 Vargas, 2007; Sun *et al.*, 2009; Tuomi *et al.*, 2015). Therefore deceptive orchids such as *C.*
86 *calceolus* represent an excellent contrast to masting species in regards of investigating variation in
87 vital rates. Using long-term monitoring data from three populations in Finland and two in Estonia,
88 we tested for the existence of temporal trends, cycles, and synchrony in reproduction (measured as
89 flowering intensity) and size (measured as the number of ramets) among individual clumps. We
90 specifically addressed four questions. First, is the variation in flowering intensity and clump size
91 cyclic, that is, are the temporal autocorrelations of these variables negative? We predicted that both
92 variables would exhibit cyclicity because of intrinsic costs of reproduction and extensive production
93 of biomass. Second, can the annual variation in flowering intensity and clump size be explained by
94 external climate variables such as temperature or precipitation? We hypothesised that, as *C.*
95 *calceolus* in Estonia and especially in Finland is near the northern edge of its distribution (Hultén &

96 Fries, 1986), it would benefit from warm summers and high precipitation in these two countries.
97 Third, is there a temporal trend in flowering intensity or clump size? We predicted that plant size
98 would increase with time, as older clones are usually larger due to rhizome branching, and that
99 flowering intensity would also show a positive trend due to the greater resource storage capacity of
100 larger plants. Finally, is the annual variation in flowering intensity and clump size synchronous
101 among plants within or between populations in each country? We hypothesised that if reproductive
102 effort and plant size were mainly under environmental control, they would vary in synchrony within
103 and between populations. However, we also predicted that, in the absence of strong environmental
104 control, as a deceptive species *C. calceolus* would have asynchronous reproduction.

105

106 MATERIALS AND METHODS

107 STUDY SPECIES

108 The lady's slipper orchid, *Cypripedium calceolus* L. (Orchidaceae), is a clonal, perennial herb that
109 forms horizontal, creeping rhizomes (Tutin *et al.*, 1980). Above-ground parts of the plant wilt in the
110 autumn and the plant overwinters as a rhizome, meaning that it must produce a new set of
111 aboveground shoots every spring. Each rhizome tip annually produces two apical buds, of which
112 the larger one forms the next year's shoot, while the smaller one usually stays dormant (Kull &
113 Kull, 1991; Blinova, 2004). *C. calceolus* can grow as a single ramet, but often spreads vegetatively
114 and forms clumps of several ramets which share resources via rhizomal connections (Mossberg &
115 Nilsson, 1977; Kull, 1999). These clumps can consist of one or several clones (Kull & Kull, 1991)
116 which are long-lived and slow growing (Kull, 1988; Nicolè, Brzosko & Till-Bottraud, 2005).
117 Flowering occurs in May to early June in Estonia (Kuusk, 1984) and in late June to early July in
118 Finland (Mossberg & Nilsson, 1977). A flowering stalk usually supports one or two, rarely three,
119 flowers. Pollination takes place by food deception (Nilsson, 1979), and vegetative dormancy (i.e. a
120 state in which the plant does not sprout at all for one or more growing seasons) is common

121 (Shefferson *et al.*, 2001; Brzosko, 2002). In our populations the annual proportion of dormant adult
122 clumps was relatively low and stable during the study period (mean \pm SD of $7 \pm 3\%$ and $1 \pm 2\%$ in
123 Finland and in Estonia, respectively). *C. calceolus* is a circumpolar, mainly boreal species (Hultén
124 & Fries, 1986) which inhabits mesic, semi-shaded sites and prefers calcium-rich substrate (Kull,
125 1999). The species is classified as near-threatened in both Finland (Rassi *et al.*, 2010) and Estonia
126 ('Red Data Book of Estonia', 2008).

127

128 DEMOGRAPHIC DATA

129 We monitored three populations in Oulanka National Park, Kuusamo, Finland from 2002-2014 and
130 two populations in Estonia from 1987-2012 (Table 1, Fig. S1). In Finland, two populations
131 (Korvasvaara I and II) were located close to each other, with about 150 m of unsuitable habitat (a
132 dense spruce forest) between them. Despite the short distance between these two populations, they
133 grew in different habitat types (Table 1) and were therefore considered separately. The third
134 population (Ampumavaara) was approximately 12 km away from Korvasvaara. The distance
135 between the populations in Estonia was approximately 160 km, and the distances between the
136 Estonian and Finnish populations were over 800 km (Fig. S1). At each study site, we established a
137 10×10 m permanent square. In these squares, we marked each single ramet that was clearly
138 separate from others and each group of ramets growing densely together as a distinct demographic
139 unit. Marking was done with individually numbered plastic tags secured to the ground with metal
140 sticks. Ramets in a clump grew so densely together that we could not reliably differentiate between
141 individual ramets, and differentiation between clones was impossible without genetic analysis or
142 digging up the plants. While in the Estonian populations each clump consisted of a single clone
143 (verified by isoenzyme analysis; Kull & Kull, 1991), in the Finnish populations the clumps may
144 have consisted of several different intermingling genets, and large clones may have also appeared in
145 two or more clumps (A. Jäkäläniemi, unpublished data). All populations were censused once a year

146 in June (Estonia) or in July (Finland) by counting the number of ramets and the number of flowers
147 in each clump, except in 1998 when one Estonian population (Muhu) was not visited. Only plants
148 that flowered at least once during the observation period were included in the final data set, which
149 consisted of 171 clumps from Finland and 37 clumps from Estonia (Table 1). Herbivory by reindeer
150 and voles in the study populations was rare.

151

152 STATISTICAL METHODS

153 The response variables in all statistical models were flowering intensity and clump size that we used
154 as proxies for reproduction and growth, respectively. Note that due to the low fruiting success of the
155 species (only 13% of sprouting ramets in the populations studied here produced a capsule), we were
156 unable to include fruiting probability in the models. We defined flowering intensity of a clump as
157 the proportion of flowering ramets, i.e. the number of flowering ramets divided by the total number
158 of ramets in a clump. The proportion of flowering ramets reflects a plant's reproductive intensity
159 better than the number of flowering ramets or the number of flowers does, as the latter two
160 measurements depend heavily on the total number of ramets in a clump. Clump size was defined as
161 the number of ramets in a clump, and was used to estimate annual vegetative growth (i.e. the
162 amount of vegetative biomass produced). All analyses were conducted using the statistical package
163 R 3.2.2 (R Development Core Team, 2015).

164

165 *Temporal patterns: cyclicity and trends*

166 To assess temporal cyclicity in flowering intensity and clump size, we calculated autocorrelations
167 based on de-trended data, i.e. data in which a temporal trend was removed by differencing
168 (calculating differences between two consecutive years). Kaitala & Kull (2002) previously found
169 two-year cycles in flowering intensity in Estonian populations of *C. calceolus*. Therefore, we

170 calculated autocorrelations in flowering intensity and clump size with a lag of one year for each
171 clump ($n = 208$). Visual assessment of the correlograms showed that autocorrelations with longer
172 lags were negligible. The statistical significance ($\alpha = 0.05$) of the autocorrelations was determined
173 based on bootstrapped data sets with 1000 replicates. The average strength of temporal
174 autocorrelations in flowering intensity and clump size in each country was then examined with
175 general linear mixed-effects models (function “lmer” in the lme4 package in R; Bates et al. 2014),
176 and the proportions of significant temporal autocorrelations were analysed with generalized linear
177 mixed-effects models (binomial distribution with the logit link function, function “glmer” in the
178 lme4 package in R; Bates et al. 2014). To account for the possible effect of location and for the
179 spatial relatedness of the populations within countries, all models included country as a fixed
180 explanatory variable and population nested within country as a random factor. The models included
181 no intercept as we were interested in exploring the average temporal autocorrelations within each
182 country rather than testing differences between countries.

183 We examined temporal trends in flowering intensity and clump size of adult plants using linear
184 regression. Possible explanations for the temporal changes in these two variables were explored
185 using the climate models described below. Flowering intensity was logit-transformed as
186 recommended in Warton & Hui (2011) and was analysed with a general linear mixed-effects model
187 (function “lmer” in the lme4 package in R; Bates et al. 2014). Clump size was analysed using a
188 generalized linear mixed-effects model with the zero-inflated Poisson distribution and a log link
189 function (function ‘glmmadmb’ in the glmmADMB package in R, Fournier et al. 2012). Both trend
190 models included time as a fixed continuous explanatory variable and clump, nested within
191 population, as a grouping variable with a random intercept and slope. The inclusion of this random
192 term in the model took into account the fact that repeated measures were conducted from the same
193 populations and clumps over time. The model fit was visually verified from residuals and by
194 examining possible over-dispersion (no over-dispersion was detected, dispersion factor < 1 in all
195 the models). Due to the different lengths and years of the study periods in Finland and Estonia

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

198

199 *Climate models*

200 To investigate how climate variables were associated with the flowering intensity and size of *C.*
201 *calceolus* clumps, we used linear mixed-effects models fitted separately for each country. The most
202 parsimonious model for each response variable was selected based on AIC (Akaike's Information
203 Criterion) values. Model specifics were as in the temporal trend models above, except that random
204 effects (clump nested within population) included a random intercept only. As explanatory factors,
205 we considered climate variables that were readily available or that have been reported to correlate
206 with orchid reproduction or growth (Shefferson *et al.*, 2001; Blinova, 2008). We had the following
207 climate measurements for each spring (March-May): the number of freezing days (days when the
208 minimum temperature dropped below 0°C), the minimum temperature, the cumulative temperature
209 sum (sum of the amount by which mean daily temperatures exceeded 5°C), the precipitation sum,
210 and the mean temperature. Because buds for the year are formed during the previous autumn, in
211 selecting models we also considered the same climate variables from the previous year's growing
212 season, as well as the length of the previous growing season. The growing season was defined as
213 the snow-free period when the 24-hour average temperature was at least 5°C for ten consecutive
214 days. The tested climate variables included also the winter precipitation sum and the maximum and
215 mean spring snow depth. In addition to these climate variables, the clump sizes of the previous and
216 current growing season (with logarithmic transformation) were considered as explanatory variables.
217 The Finnish climate data were provided by the Finnish Meteorological Institute from the
218 Kiutaköngäs weather station, which was located within 13 km of the Finnish study populations. The
219 Estonian climate data for the Ussisoo and Muhu populations were provided by the Türi and Virtsu

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

222 The demographic data from the first year in the Muhu and Finnish populations (1987 and 2002,
223 respectively) and from the year 1999 in the Muhu population (the year following the missing year)
224 were excluded from the analyses because they lacked estimates of the prior year's clump size.
225 Moreover, the year 2010 in Finland had some missing values for climate variables, and so the years
226 2010 and 2011 were removed from the analysis. As a result, the climate models for the Finnish
227 populations of *C. calceolus* included 10 years, while the models for the Estonian populations
228 included 25 and 26 years (Muhu and Ussisoo, respectively). The model fit was assessed visually
229 from residuals as with the trend models. We also examined the variance inflation factors (VIF) for
230 possible multicollinearity of the explanatory variables (no multicollinearity was detected, all VIFs <
231 3).

232

233 *Spatial synchrony among clumps*

234 We used cross-correlations to estimate the synchrony of flowering intensity and size among clumps
235 both within and between populations. We calculated all pairwise correlations in flowering intensity
236 and clump size between the clumps in each population (within-population synchrony) and between
237 the clumps of different populations (between-population synchrony) using the previously de-
238 trended time series. The average strength of correlations and the proportions of significant (both
239 negative and positive) correlations were calculated as in the temporal autocorrelation analyses
240 above.

241

242 RESULTS

243 TEMPORAL PATTERNS: CYCLICITY AND TRENDS

244 The autocorrelation analysis points towards two-year cycles in both flowering intensity and clump
245 size over time, as the majority of the clumps (94% and 97% for flowering intensity and clump size,
246 respectively) showed negative, although only moderate, autocorrelation coefficients at a lag of one
247 year (Table 2). This indicates that years of intensive flowering were followed by years of sparse
248 flowering and vice versa. Similarly, years of extensive vegetative growth were usually followed by
249 years of meager vegetative growth. In addition, clump size increased over time in both countries
250 (Fig. 1), while the flowering intensity of the clumps showed no significant temporal trend (Table 3).

251

252 CLIMATE MODELS

253 In general, variation in flowering intensity was best explained by the temperature of the previous
254 summer and clump size during the previous growing season, although the best variables for summer
255 temperatures differed between countries, as did the effects of temperature (Table 4). In Finland, the
256 most parsimonious model for flowering intensity also included the spring cumulative temperature
257 sum, which was negatively correlated with subsequent flowering (Table 4).

258 For clump size, the best single predictor was clump size in the previous year, which correlated
259 positively with current size (Table 4). Current clump size was also positively associated with spring
260 snow depth in both countries (Table 4). In Estonia, clump size increased with the increasing
261 cumulative temperature sum of the previous growing season. The strongest effect of this was seen
262 in the smallest clumps (Table 4, Fig. 2), indicating that the smallest plants were the most sensitive
263 to temperature. Moreover, clump size in Estonia was associated with spring weather conditions,
264 with the precipitation sum having a positive effect and the cumulative temperature sum having a
265 negative effect on size (Table 4).

266

267 SPATIAL SYNCHRONY

268 Overall, there was little synchrony in reproduction and vegetative growth among clumps both
269 within and between populations, as the cross-correlations among clumps were moderate and mostly
270 non-significant in both countries as well as between countries (Table 5). Although some pairs of
271 clumps showed perfect positive synchrony, while others showed perfect negative synchrony, the
272 variation in correlation coefficients was high (Fig S2), with over half of the pairs showing no
273 correlation in either of the variables (Table S1). Furthermore, the pairs of clumps flowering in
274 synchrony were usually not the same ones that were synchronous in size (Table S1).

275

276 DISCUSSION

277 TEMPORAL CYCLICITY AND THE IMPORTANCE OF DEMOGRAPHIC HISTORY

278 Our findings suggest that internal factors can lead to cyclic reproduction also in non-masting
279 species, as the autocorrelation analysis revealed two-year cycles in flowering intensity and clump
280 size in the study populations (Table 2). In other words, consecutive years tended to be dissimilar to
281 each other, with years of intensive flowering (or growth) being followed by years of less intensive
282 flowering (or growth) High variability in reproduction is one of the defining aspects of the masting
283 phenomenon (Kelly & Sork, 2002) and two-year cycles similar to the ones observed here have been
284 reported also in masting legumes (Crone, Miller & Sala, 2009) and trees (Kon *et al.*, 2005; Pearse
285 *et al.*, 2014). These cycles are assumed to be due to the depletion of resource by the costs of
286 reproduction (Rees *et al.*, 2002; Crone *et al.*, 2009; Miyazaki, 2013; Pesendorfer *et al.*, 2016). The
287 cyclicity observed here indicates that the internal demographic history of individuals matters also in
288 non-masting species. In finding two-year cycles, our study agrees with that of Kaitala & Kull
289 (2002), although the autocorrelations observed in both studies were only moderate and mostly
290 non-significant.

291 In the present study, both flowering intensity and clump size increased with the increasing clump
292 size of the previous year (Table 4). Such a positive relationship is expected, as the largest clones

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

300

301 TEMPORAL TRENDS

302 We observed that the clump size of *C. calceolus* increased over time in both countries, which was in
303 line with our predictions and with previous studies of this species (Kaitala & Kull 2002, Laitinen,
304 2006). An increase in ramet numbers with time probably occurred via rhizome branching as the
305 clones aged. It is also possible that the branching structure itself did not change over time, but
306 instead, that the sprouting rate increased due to a positive change in the environment (either directly
307 through reduced bud mortality or indirectly via an increase in resource levels), which enabled the
308 plant to support a larger number of ramets sprouting from the existing meristems.

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

317 importance of considering the time-period, its length, and the response variable used when
318 examining temporal trends in vital rates.

319

320 CLIMATE, REPRODUCTION, AND GROWTH

321 In addition to clump size, weather conditions of the previous year and current spring affected the
322 performance of *C. calceolus* clumps, with the temperature of the previous growing season and
323 spring snow depth being the most influential weather aspects for flowering intensity and clump size,
324 respectively. However, the effects of weather conditions varied between countries (Table 4). The
325 negative effect of high summer temperatures on flowering in Estonia may be due to the costs of
326 increased respiration. Instead, warm summers had a positive effect on flowering intensity in
327 Finland, possibly because, in general, the average summer temperatures there are lower than in
328 Estonia and the clumps are, on average, smaller, and therefore do not suffer from excess respiration.
329 In this respect, the differences observed here between countries are not particularly surprising, as
330 other studies have also reported that the effects of summer temperatures on orchids may vary by
331 species, study location, or the measure used to describe plant performance (Hutchings 2010;
332 Sletvold et al. 2013; Shefferson, Warren & Pulliam, 2014).

333 The negative effect of high cumulative spring temperature on flowering in the Finnish populations
334 and on clump size in Estonia may have appeared because warm springs induce plants to start
335 growing earlier, which exposes them more frequently to frosts. Unsurprisingly, snow cover seemed
336 to have a protective effect on plant growth. In addition, melting snow also provides moisture. In
337 Estonia, where the climate is not as humid as in Finland and the plants are more likely to experience
338 drought, clump size was further increased by high spring precipitation (Table 4) which ensured
339 favorable water conditions for growth. This finding suggests that the growth of *C. calceolus* will
340 benefit from the predicted increase in precipitation at high latitudes (IPCC, 2014). However, spring
341 snow cover has continued to decrease in the Northern Hemisphere during the past two decades

342 (IPCC, 2014), and this can lead to more frequent bud damage from freezing in the spring, as has
343 already been observed in montane wildflowers (Inouye 2008).

344 The results from our climate models should, however, be viewed with some caution, as the model
345 outcomes were sensitive to the dataset used, and this may have consequences for the generality of
346 our results. While individual estimates for the explanatory variables were rather robust, the rank
347 order of the models varied depending on the exact dataset used. We also note that the correlations
348 observed here between plant performance and weather variables do not prove causality, and
349 manipulative experiments are needed to more explicitly assess the impact of weather conditions on
350 reproduction and growth (see e.g. Crone & Lesica, 2006). Nevertheless, as the same climate
351 variables (spring and summer temperatures, spring snow depth) were the best predictors in both
352 countries despite differences in habitats and partial differences in the years under study, our
353 findings provide strong support for the importance of these climate variables in regulating the
354 reproduction and growth of *C. calceolus*.

355

356 SPATIAL ASYNCHRONY IN REPRODUCTION AND GROWTH AMONG CLUMPS

357 Despite the significant associations between climate variables, flowering intensity, and clump size,
358 the overall synchrony in the examined variables between clumps was low both within and between
359 populations (Table 5). This suggests that while environmental factors do affect reproduction and
360 growth in the study populations, within-population variability in microhabitats and in clump
361 demographic history overwhelm any synchrony caused by local weather. This view is supported by
362 the fact that significant positive correlations within populations (8-33%) were more common than
363 significant positive correlations between populations within a country (5-14%), which in turn were
364 more common than positive correlations between populations of different countries (5-6%). In other
365 words, the closer to each other the clumps grow, the more likely they were to behave similarly,
366 which is to be expected considering that environmental factors are often spatially autocorrelated.

367 *C. calceolus* is a nectarless species with low overall pollination success and seed production (Kull,
368 1999). Thus, it seems likely that neither predation satiation nor pollen coupling play any important
369 role in the reproductive success of this species, which could further explain why we did not observe
370 synchrony among clumps. In fact, as a deceptive species, *C. calceolus* might even benefit from
371 asynchrony because of the negative frequency-dependent selection. Selective advantages could
372 arise due to either decreased competition for pollinators (Parra-Tabla & Vargas, 2007) or because
373 high frequencies of deceptive flowers enable pollinators to more quickly learn to discriminate and
374 avoid them (Smithson & MacNair, 1996). Previous studies in deceptive orchids have demonstrated
375 that frequency-dependent selection operates at least on flowering phenology (Parra-Tabla & Vargas,
376 2004; Sun et al., 2009) and the frequency of floral color morphs (Gigord, Macnair & Smithson,
377 2001; but see Pellegrino et al 2005).

378

379 CONCLUSIONS

380 In this long-term study of Finnish and Estonian populations of the rare deceptive orchid *C.*
381 *calceolus*, we observed that reproduction and vegetative growth were regulated by both internal and
382 external factors. Although this species is non-masting, our data suggest two-year cyclicity in
383 reproduction and growth which arises due to variation in a plant's internal resource levels which, in
384 turn, depend on its demographic history. Despite the fact that flowering intensity and plant size
385 were associated with external weather conditions, we observed no synchrony in these two traits
386 among clumps either within or between populations. We thus obtained no support for the
387 hypothesis that individuals use weather as a cue for flowering, and our results indicate that the
388 direct effects of external factors are overridden by the internal ones. This is not very surprising
389 considering that *C. calceolus* is a non-masting species and therefore is not expected to actively
390 synchronize its reproduction with conspecifics. We suggest that, as a deceptive species, *C.*

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

393

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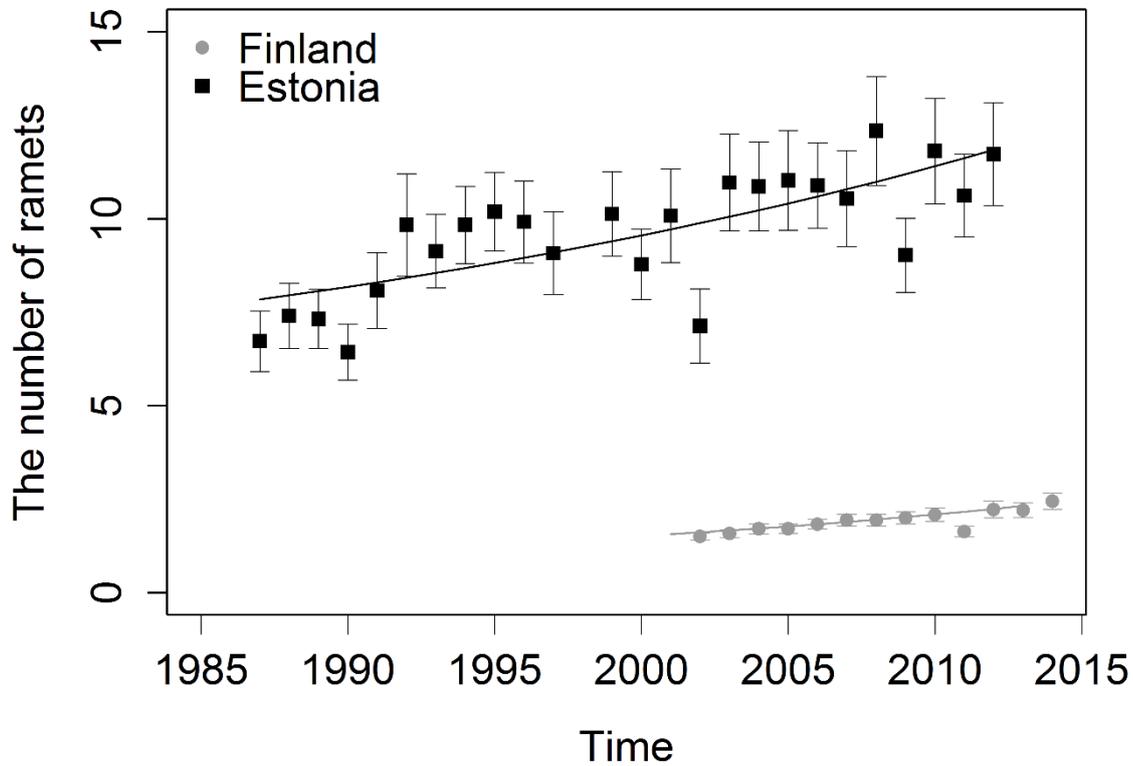
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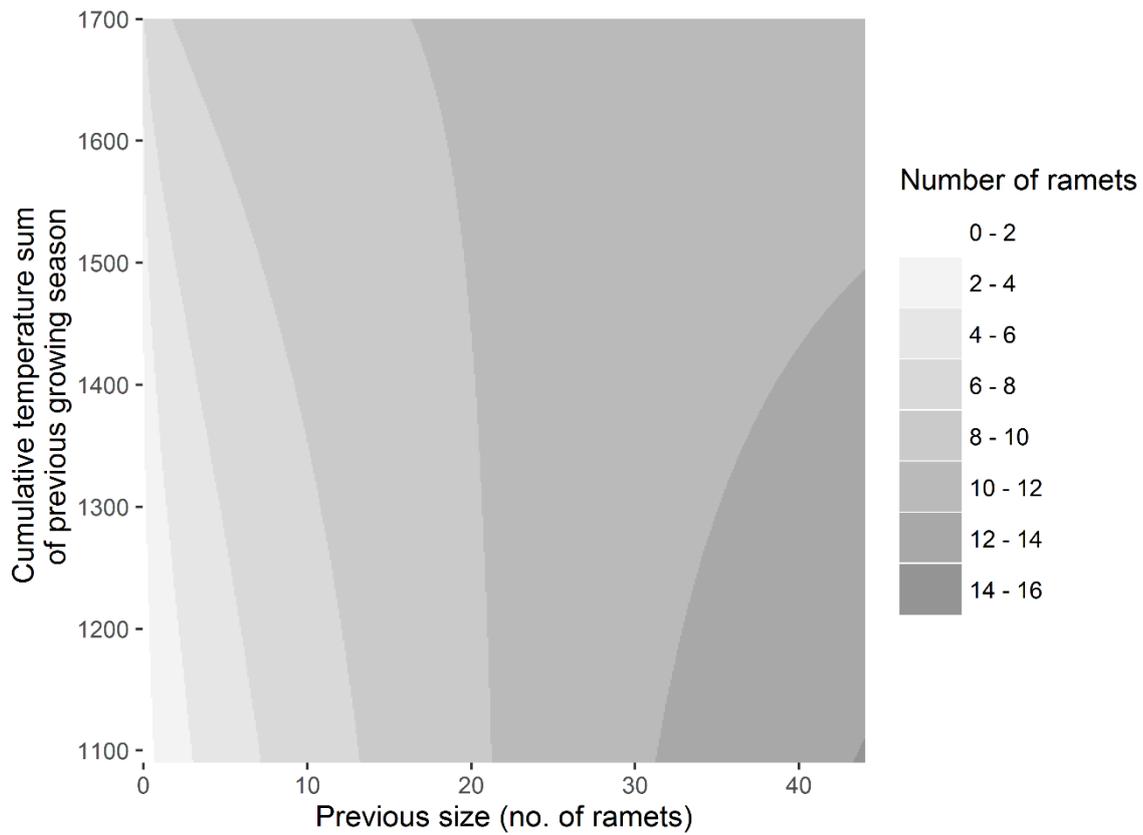
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529 **Figure 1.** Temporal trends in clump size (number of ramets) of *Cypripedium calceolus* in Finland
 530 and Estonia. Vertical bars show the observed annual mean value (\pm SE). Note that year 1998 is
 531 excluded due to missing data from one of the two Estonian populations.



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

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 \pm SE	Country	Estimate-SE – Estimate+SE
Flowering intensity	Estonia	-0.358 \pm 0.050***	Estonia	0.398 – 0.576
	Finland	-0.368 \pm 0.031***	Finland	0.270 – 0.357***
Clump size	Estonia	-0.445 \pm 0.037***	Estonia	0.567 – 0.723
	Finland	-0.319 \pm 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) ×	-0.0006±0.0001***
			Cumulative temperature of the previous growing season	

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)