Population viability analysis of *Cypripedium calceolus* in a protected area: longevity, stability and persistence

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Summary

1 *Cypripedium calceolus* L. is an emblematic long-lived clonal orchid which has suffered an alarming decline throughout Eurasia. We performed a population viability analysis on three island populations in a protected area to estimate population viability and thus to gain a better understanding of the species and its needs. We developed a 10-stage matrix model, comprising the seed bank, the first 4 years underground, juveniles, young and adult clump stages and three dormant stages, from an 11-year census.

2 All analyses indicate that the three populations have remarkably slow and stable dynamics, with stochastic growth rates close to 1 after 100 years and very slow convergence to equilibrium. The system is probably stabilized by the long life span of clumps (mean longevity from 110 to 350 years). Retrospective and perturbation analyses showed that adult dormancy had an important role in the dynamics, and adult survival and seed persistence were key factors in maintaining population stability and persistence.

3 A young, uniformly distributed population had fewer, shorter lived adult clumps than two older, aggregated populations. Although no perceptible change was predicted for the next 100 years, the younger population was more sensitive to environmental variations and may go extinct in the next 250–500 years, depending on longevity of the seed bank.

4 *C. calceolus* populations can persist in a protected area where there are only slow changes in habitat through secondary forest succession. The dramatic decrease in *C. calceolus* population size and area over 20 years in Eurasia suggests that many populations have experienced unforeseeable extrinsic disturbances or unfavourable habitat disturbances.

5 Our Population Viability Analysis indicated the importance of habitat vs. individual conservation for the protection of *C. calceolus* populations. As this species is usually found within rich orchid communities, it should be used as an umbrella species in management plans.

Key-words: adult dormancy, age-based parameters, *Cypripedium calceolus*, environmental and demographic stochasticities, longevity, persistence, perturbation analysis, population viability analysis, stability

Introduction

Population Viability Analysis (PVA) is both a process for identifying the threats faced by a species and an analytical modelling tool (Soulé 1986; Beissinger 2002). Contrasting methods of PVA provide different kinds of analyses giving insight into the viability of populations. One approach is to assess extinction risks quantitatively, and, as a result, to propose conservation solutions, by integrating stochastic phenomena. Assuming that models predict the fate of a population, their output should allow estimation of absolute risks of extinction. This approach has, however, been questioned recently by authors identifying poor-quality data, difficulties in achieving good estimates of certain demographic

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parameters, different types of uncertainties (structural uncertainty among models, sampling error and biases, and stochasticities) and low capacity to validate models (Beissinger & Westphal 1998; Burgman & Possingham 2000; Beissinger 2002; Reed *et al*. 2002). Thus, an emerging consensus is that PVA should be used to estimate relative rather than absolute risks of extinction. This allows an understanding to be reached about species or systems, and thus research needs and priorities, by ranking risks (Ralls *et al*. 2002). We used this kind of qualitative analysis to investigate the population biology of *Cypripedium calceolus* L., an emblematic orchid of temperate habitats, in a comparison of three island populations in a protected area in Poland. As observed in other European populations (Kull 1999), these populations display different types of spatial distribution ranging from aggregated (very dense groups of ramets separated from other dense groups of ramets) to uniformly distributed, with most clumps composed of only a few ramets.

Few studies have used matrix models and PVA techniques to investigate the global life cycle of temperate orchids, or even more generally those of long-lived clonal plants (Cochran & Ellner 1992; Eriksson 1993; Ehrlen 1995; Oostermeijer *et al*. 1996) because data are especially scarce (Menges 2000). The quality of modelling is limited by our ability to gather census data of adequate quality. Some events are intractable, or are so rarely observed that only enlarging sample sizes or number of years allows accurate estimation of the demographic parameters concerned. There is very little field information on the early life stages of *C. calceolus* (Terschuren 1999) or of other orchids (Eriksson 1993; Rasmussen & Whigham 1998). This is a recurrent problem in modelling plant dynamics (Doak *et al*. 2002) and we therefore allowed for such uncertainties by simulating different scenarios.

The approach used by Terschuren (1999) was to perform a quantitative PVA using approximate biological parameters based on anecdotal data from different locations and authors. In spite of its wide distribution throughout Eurasia (Cribb 1997), populations of *C. calceolus* throughout its range are frequently small, isolated and declining in size. The loss of distribution area at a regional scale is alarming: between 50% and 90% decline was estimated between 1975 and 1995 (Terschuren 1999). No PVA has previously been performed on *C. calceolus* using empirical data on the whole life cycle of a wild population.

Orchids from temperate regions may have different types of below-ground organs. For rhizomatous species, such as *Cypripedium* spp., vegetative growth can lead to large clumps of ramets having the same genotype. However, studies using allozymes have shown that several *Cypripedium* genets can be intermingled within a clump (Case 1993; Kull & Paaver 1997; Brzosko *et al*. 2002a). Below-ground clonal growth, higher recruitment in the vicinity of mother plants and possible fragmentation of the rhizome in these species mean that

genotyping of each ramet is needed to confirm the genetic identity of individuals within a clump. Because ramet growth and recruitment mean that this genotyping would have to be repeated over the course of any study, the demography of rhizomatous orchids has received much less attention than that of non-clonal species. Studies on *Cypripedium* have focused on different demographic units: ramet, genet or clump (usually assumed to be a genet or clone). Kéry & Gregg (2004) chose to study dormancy and survival of marked ramets of *Cypripedium reginae* using capture–recapture models. Brzosko (2002) studied the demography of 'clones' of *Cypripedium calceolus* that were later identified as mixtures of genets (Brzosko *et al*. 2002b). Also in *C. calceolus*, isoenzymes have been used to detect genets and to focus on clonality by modelling ramet vs. genet dynamics (Kull 1995). Kull (1988; see also Kull 1995; Kull & Kull 1991; Kull & Paaver 1997) used one well-resolved polyallelic locus and two or three other variable loci to identify clones, and found that three of seven Estonian populations were composed of multigenet clumps. When two ramets differ by even one allele at one locus they clearly belong to different genets, but, as underlined by Kull (1988, p. 195), 'In order to prove that two ramets belong to the *same* clone it is not enough to show that they are identical in some few loci. The more loci are examined and found to be identical, the greater is the probability that the ramets are really genetically identical.' Shefferson *et al*. (2001) studied 'genets' of *Cypripedium calceolus* ssp. *parviflorum*, assuming that genets were spatially segregated and non-overlapping due to the low number of shoots (ramets) found per clump and the low overall shoot density in the population. However, an allozyme study indicated that a Polish population, also with few ramets per clump and low density of ramets, had clumps with more than one genet (mean number of ramets per clump = 3.1, mean genets per clump = 2.1 ± 1.4; Brzosko *et al*. 2002b). The genotyping of clumps in the three populations studied here, using five polymorphic allozyme loci (Brzosko *et al*. 2002b), indicated that the mean number of genets per clump was 2.5 ± 1.7. The physical distances between distinct genets were very small (a few cm) and it was not possible to distinguish them within a given clump. Thus, in our populations of *C. calceolus*, it was almost impossible to work on individual genets. This led us to use the clump as the demographic unit. Furthermore, the clump is a more practical and reliable field unit than the genet or the ramet, as it is easier to ensure correct identification of each above-ground unit and therefore to compare results between populations.

Given the emblematic status of *C. calceolus* in Eurasia, our aims were to assess population viability and, more particularly, to compare and link spatial patterns, population dynamics and conservation consequences in different situations, by identifying qualitative differences in the population dynamics, the longevity of plants and persistence of different populations, as well

as the important demographic parameters for maintenance of each population.

We surveyed three populations with different spatial distributions and each consisting of nearly 130 clumps over a period of 11 years (1989–2000). We combined the census data with bibliographic data and our field experience of the species to construct a biologically meaningful matrix model of 10 stages. We then estimated the above-ground parameters (survival, growth, fruiting rates and recruitment) and the probabilities of becoming dormant and emerging from dormancy. Loglinear analyses of transition matrices (Caswell 2001) were used to identify population and/or time effects on the fate of clumps and were followed by a retrospective analysis and prospective analyses including a perturbation analysis, the estimation of the longevity of *C. calceolus* clumps and stochastic projections.

Materials and methods

STUDY SPECIES, STUDY SITE AND DEMOGRAPHIC CENSUS

Cypripedium calceolus L. is a long-lived orchid of temperate regions. It occurs in shady deciduous and mixed woodlands, occasionally in full sunlight at higher altitudes or, rarely, on slopes, predominantly on calcareous soils (reviewed in Cribb 1997; Kull 1999; Terschuren 1999). *C. calceolus* reproduces sexually by outcrossing and pollination by bees (*Andrena* sp.) but, owing to pollen limitation, low fruit set (0–57%, Kull 1998) has been observed in different countries. Reproduction also occurs asexually through growth and ramification of a horizontal rhizome. The aerial parts are absent in winter. Each bud of the branched rhizome may grow into a subunit (annual size increment of 0.5–1.1 cm, Kull & Kull 1991).

The populations studied are in a protected area of the Biebrza National Park in north-east Poland, characterized by marshes and peat-bogs. Of the few thousand mineral islands that have resulted from accumulation of sediment from the Biebrza River, only six have been colonized by *C. calceolus.* The three studied here differ in community composition (Brzosko 2002; Brzosko *et al.* 2002a), with populations on Zabudnik (Zab) and Pogorzaly (Pog) being aggregated, with very large and dense clumps of ramets (sometimes more than 70 stems), but clumps on Oparzelisko (Opa) being uniformly distributed. Each clump (a spatially delimited group) was mapped on a 1×1 m grid and followed from 1989 for Pog and Zab, and from 1991 for Opa, until 2000. Every year, the presence of each clump was recorded and the number of flowering and non-flowering ramets in each clump was counted. The number of flowers per clump was recorded throughout the study, and the number of fruits was recorded from 1994. We also recorded juveniles (individuals with one or two small and thin leaves) when visible (i.e. those found close to adult clumps).

CONSTRUCTION OF THE LIFE CYCLE DIAGRAM AND PARAMETER ESTIMATION

The stages used to construct the life cycle diagram for *C. calceolus* were defined so as to be biologically meaningful and to limit within-stage variability. The life cycle was composed of 10 life stages (Fig. 1).

Seeds, protocorms and juveniles

Although the presence of a seed bank in *C. calceolus* is strongly presumed (Curtis 1943), we are not aware of any data on *in situ* seed survival. The success of *in vitro* germination is highly variable and unpredictable (Light
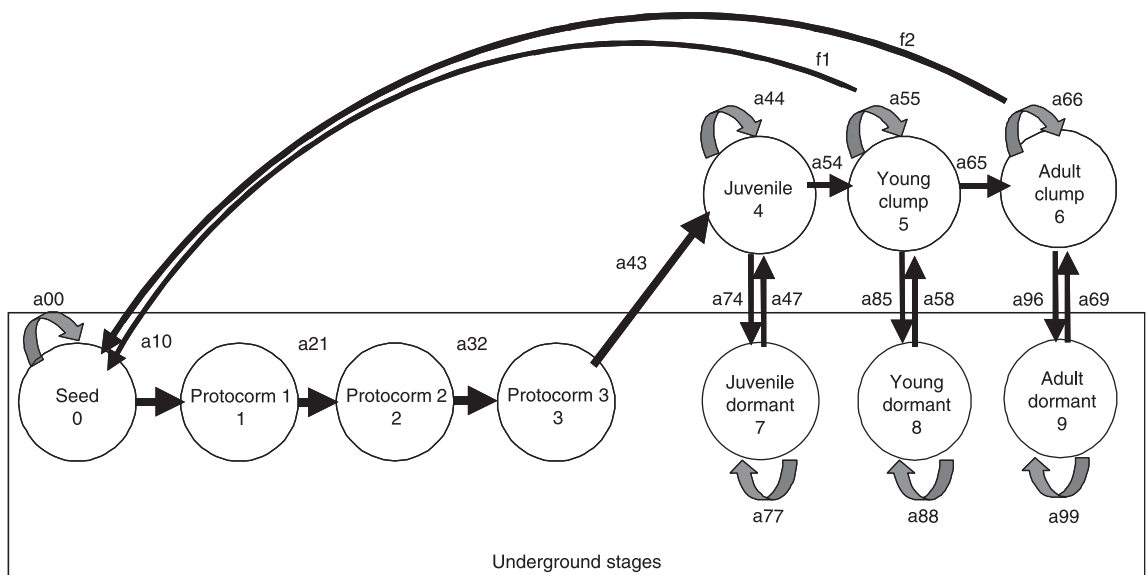
Fig. 1 Life cycle diagram of *Cypripedium calceolus*. Each circle represents a stage numbered from 0 to 9 and each arrow represents a possible transition from year *t* to year *t* + 1.

& MacConaill 1998; Terschuren 1999). In nature, seeds germinate if they develop a symbiotic relationship with a fungus (*Rhizoctonia*, Kull 1999). Seeds then become protocorms. Recruitment is microsite-limited and higher in the vicinity of clumps where the environment appears to be more favourable. The protocorms grow and store reserves underground for 3 years before the first leaves appear above ground (Curtis 1943). It was necessary to include these three stages in the model because above-ground organs only emerge 4 years after germination. We directly estimated the overall recruitment from seed to juvenile in each population by dividing the total number of newly observed juveniles at time $t + 4$ by the number of seeds produced at time t. To estimate the transitions between the different protocorm stages, we used our overall estimate of recruitment and values for the relative proportions of the different protocorm ages from Curtis (1943). A careful search for juveniles was performed, but once they became adult plants (young clumps), if they were within or close to a pre-existing clump, they could not be distinguished from the other stems. Therefore, we have no information on their density-dependent growth.

Clumps

All clumps comprising more than seven stems were assigned to the 'adult' category. We analysed the census data from the whole life history of each clump and, once assigned to adult status, surviving clumps remained in this stage even if the highly variable numbers of stems throughout the census period meant that ramet number sometimes fell below seven.

Young clumps, which exhibited gradual slow growth throughout the 11 years of survey, comprised those that had never been recorded as having more than seven stems. To encode clumps unambiguously, we considered that the growth increment of young clumps was less than three ramets per year over the whole census.

Dormancy

Juveniles, young and adult clumps can be dormant. Adult dormancy interfered with our ability to estimate the survival of dormant clumps and to distinguish dormancy and death at the end of the census period. It is theoretically possible to estimate apparent survival and dormancy rate, corrected for detection probability, using indirect Capture–Mark–Recapture (CMR) methods (Shefferson *et al.* 2001, 2003; Kéry & Gregg 2003, 2004). Unfortunately, at present, the multi-strata methods of CMR lack the power to estimate all the parameters for complex models with multiple states. For that reason, we used a classical method to estimate the dormancy rate.

Each year, we checked carefully for previously mapped clumps. Using spatially delimited groups as the demographic unit reduces the detection error and we also deleted from the data set any clumps that were not clearly spatially delimited or for which detection was ambiguous in at least one year. We thus assumed that detection probability was close to one, enabling good estimates of dormancy parameters to be obtained in the middle of the census period. A clump was considered dormant if it had one or several 'presence–absence–presence' sequences throughout the whole census period. At the end of the census period, we assumed that those clumps that had disappeared for fewer than four consecutive years (Shefferson *et al.* 2001) had been dormant, whereas those that had disappeared for longer were considered dead (and recorded as dead in their first year of absence). Without evidence and data about different survival rates for underground stages in our populations, we hypothesized the same survival rates for dormant and above-ground clumps for each stage.

Fecundity

Fecundity was defined for each stage as the mean number of seeds produced per clump (i.e. mean number of fruits per clump × mean number of seeds per fruit). The number of fruits produced by clumps of a given stage was variable because the number of ramets per clump varied. We observed a significant linear relationship between the size of the clump and the number of fruits ($F = 766.4$, d.f. = 1774, $P < 0.001$). We estimated the mean number of seeds per fruit to be approximately 13 000 ($n = 5$, mean = 13 000 ± 8000, range = 5540–23 470); this compares with Kull's (1998) data from Estonia ($n = 5$, range = 5940–16 700). We assumed that seeds are immediately dispersed, and need a minimum of 1 year to develop into first-year protocorms.

We used our census data to estimate growth, stasis and survival probabilities for juveniles, young and adult clumps. We then combined these estimates into transitions between stages. This allowed us to carry out perturbation analyses on growth rate at the level of the transitions and at the level of the parameters. Demographic parameters were estimated for each population × pair of consecutive year matrices.

PRELIMINARY ANALYSES: OBSERVED STAGE STRUCTURE AND LOGLINEAR ANALYSIS OF TRANSITION MATRICES

We compared the observed stage structure of the three populations and carried out a loglinear analysis of a four-way contingency table to test the effect of population and year on the demographic fate of clumps (Caswell 2001). We removed the seed and the different protocorm stages because they are invariant, and we added mortalities. We performed the analysis on 21 life-tables (the three populations surveyed from 1992 to 1999).

RETROSPECTIVE ANALYSIS

To identify the transitions that have most affected growth rate over the past 11 years, we performed a

retrospective analysis (λ_d variance decomposition analysis, Caswell 2000, 2001, using MATLAB 2003). We summed the absolute values of the contributions of each matrix entry to prevent cancelling out of positive and negative values, and calculated the relative contribution of each transition to population growth.

DETERMINISTIC MATRIX ANALYSES

Deterministic models assume equilibrium and constant vital rates. To include interannual variation, the deterministic population growth rate (λ_d), was computed as the mean value of the growth rates estimated from each pair of consecutive year matrices, using MATLAB (2003). The damping-ratio is the ratio of the first two dominant eigenvalues and represents the rate of convergence to equilibrium.

Perturbation analyses were performed using the ULM software (Legendre & Clobert 1995) to estimate sensitivities and elasticities of both parameters and transitions (Caswell 2001). Following Silvertown & Franco (1993), we summed the elasticities of transitions in three major categories: G, positive growth; L, stasis and retrogression (including dormancy); and F, seed production and recruitment.

ESTIMATION OF AGE-BASED LIFE HISTORY PARAMETERS

Age-based parameters were calculated from the deterministic matrix model using STAGECOACH (Cochran & Ellner 1992). We estimated the probability of a seed reaching maturity and the age at sexual maturity (eq. 15 in Cochran & Ellner 1992), the mean age of residence in each stage (eq. 29), the generation time (without assuming stable stage distribution, eq. 27) and the mean conditional life span of the oldest adult stage (i.e. the expected mean number of years until death of a clump that has survived to the adult clump stage, eq. 3).

STOCHASTIC ANALYSES: ASSESSMENT OF THE PROBABILITY OF FUTURE RISK

As dormant stages were variable and, in some years, included few individuals, we had to consider demographic stochasticity. Additionally, environmental stochasticity was incorporated in all transitions to take into account interannual variations and uncertainties of the estimates. These variations were incorporated as observed interannual standard deviations that include both sampling biases and natural stochasticities. Note that they may overestimate the theoretical standard deviation of the transitions, leading to a less conservative PVA. Stochastic models were studied with Monte-Carlo simulations.

Fecundity was divided into two normally distributed terms: the number of fruits per clump and the number of seeds per fruit. A beta distribution was assumed for parameters ranging between 0 and 1. As stochastic pro-

jections are sensitive to initial stage size, we fixed the initial stage size to the size during the last year of the census period. We used four initial values for seed bank size: 250 000, 1 million, 4.8 million and 24 million seeds, and modified the numbers in the protocorm stages accordingly. A seed bank size of 250 000 seeds is equivalent to only 1 year of underground survival, and the higher seed bank values represent scenarios in which seeds stay alive for up to 4 years, 20 years and 100 years, respectively.

For each population, we performed 3000 trajectories with Monte-Carlo simulations using the ULM software (Legendre & Clobert 1995). The probability of extinction ($P_{ext}(t)$) was calculated as the number of trajectories that were extinct after t years divided by the total number of trajectories. The mean stochastic growth rate (λ_t) was obtained by taking the average value for all trajectories including extinct trajectories.

Results

OBSERVED STAGE STRUCTURE AND LOGLINEAR ANALYSIS

The observed stage structure differed significantly between populations (Fig. 2). The major differences lay in the proportions of adult clumps (highest contribution to χ^2): Opa had a smaller proportion of adult clumps than Zab and Pog.

In the loglinear analysis, the Population–Time interaction was not significant, but all population and time effects were highly significant (Table 1). The variability is best explained by the model that includes both population and time effects. The time effect is essentially due to an increase in dormancy in 1997, especially in the population Opa. Both the stage structure and the transition parameters were significantly different between populations, and we therefore analysed the three populations separately. Moreover, the strongly significant time effect required the use of both
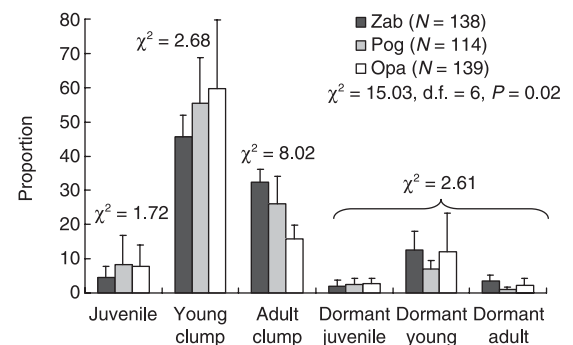
Fig. 2 Observed stage structure of surveyed stages for the three populations (mean percentage + standard deviations). N: mean number of observed clumps per population. The difference in stage structure between populations was tested using a χ^2 test of independence (SAS 1999). Because of small sample sizes, dormant stages were grouped in this analysis. The contribution of each stage to the χ^2 value is presented above the bars.

Table 1 Loglinear analysis to test population and time effects on life-tables. (a) Comparing pairs of models that differed by one explanatory factor tests for the effects of time, population and their interaction (significant effect given in bold type). The significance of an effect is measured by the difference between the goodness-of-fit of two models (ΔG^2). ΔG^2 follows a χ^2 distribution with Δd.f. degrees of freedom. (b) The terms of the hierarchical models are F: fate; S: state; T: time; P: population. The best model (in bold type) minimizes the Akaike Information Criterion (AIC) calculated as AIC = $G^2 - 2$d.f. This criterion takes into account the trade-off between the proportion of variation explained by the model and the number of parameters estimated. It shows which model fits the data best. If the difference in AIC (ΔAIC) between the best model and a second model is greater than 10, the second model has no support and is not considered (Caswell 2001)

(a) Tested effect	Contrast	ΔG^2	Δd.f.	P
Population	**M2 vs. M1**	**78.8**	**22**	**< 0.0001**
Population (including time effect)	**M3 vs. M4**	**79.2**	**22**	**< 0.0001**
Time	**M3 vs. M1**	**188.6**	**66**	**< 0.0001**
Time (including population effect)	**M2 vs. M4**	**188.9**	**66**	**< 0.0001**
Population × Time	M5 vs. M4	142.2	132	0.256

(b) Model	AIC	| ΔAIC |
M1 = FS STP	−30.1	92
M2 = FSP STP	−64.9	57
M3 = FST STP	−86.6	35
M4 = FST FSP STP	**−121.8**	
M5 = FSTP	0	122

Table 2 Deterministic parameters (deterministic growth rate (λ_d) and age-based parameters) and retrospective parameters (contribution to the variance of λ_d) for the three Polish populations of *C. calceolus* and mean ± standard deviation across populations. Contribution of dormant stages is the sum of the contributions of stasis transitions in dormant stages and all transitions from underground to above-ground stages. Contributions of the adult stage include contributions of stasis in the adult clump stage and the probability of becoming dormant

	Zab	Pog	Opa	Mean over populations ± SD
Deterministic analysis				
Mean λ_d ± SD	1.001 ± 0.001	0.997 ± 0.009	0.990 ± 0.021	0.996 ± 0.012
Probability for a seed to reach maturity	8.9E-07	6.4E-07	9.1E-07	8.1E-0.7 ± 1.5E-0.7
Age at sexual maturity (years)	8.1	9.7	8.4	8.8 ± 0.9
Generation time (mean years ± SD)	281 ± 270	334 ± 332	56 ± 53	223.6 ± 147.6
Mean conditional lifespan of the oldest adult stage (years)	270	332	66	222.7 ± 139.2
Retrospective analysis				
Contribution of dormant stages	0.39	0.47	0.34	0.40 ± 0.07
Contribution of the adult stage	0.55	0.48	0.62	0.55 ± 0.07
Contribution of juveniles and young stages	0.06	0.05	0.04	0.05 ± 0.01

demographic and environmental stochasticities in the non-deterministic models.

RETROSPECTIVE ANALYSIS

Throughout the survey in all three populations, the transitions that contributed most to the variance in λ_d ($V(\lambda_d)$) were those concerning adult clumps (Table 2). Dormant stage transitions also had an important impact on $V(\lambda_d)$ (40%), whereas the transitions concerning juveniles and young clumps made negligible contributions for the three populations (around 5%).

DETERMINISTIC MODEL

The mean deterministic growth rate calculated over the course of the study is close to 1 for each of the three

populations, indicating stability in population size, even at Opa where λ_d was slightly lower, but was still not statistically different from 1 (Table 2). The damping ratio was very similar in all populations, indicating a very slow convergence to equilibrium (1.03, 1.04 and 1.01, respectively, for Zab, Pog and Opa). In all three populations, most of the elasticity on λ_d was due to the matrix transitions estimated from field data (> 99% for all populations). On average, over all populations, most of the elasticity on λ_d was due to stasis (84.1%) and dormancy (14.7%) (thus global stasis, according to Silvertown & Franco 1993, equals 98.8%), whereas growth and fecundity had extremely low elasticities (1% and 0.2%, respectively). For individual parameters, we observed a similar pattern in all populations, with only those related to survival showing significant elasticity, and adult survival (s6, Fig. 3) having by far
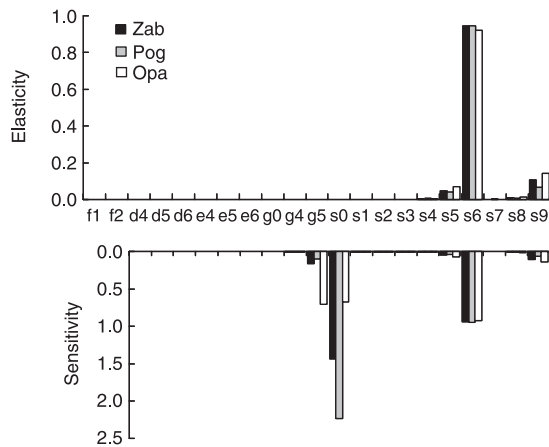
Fig. 3 Elasticity and sensitivity of the parameters for the three populations (f = fecundity parameters; d = probability of becoming dormant; e = probability of emerging from dormancy; g = probability of growth; s = probability of survival; the number refers to the stage, see Fig. 1).

Fig. 4 Mean residence time in each stage for the three Polish *C. calceolus* populations.

the highest value. Sensitivities, by contrast, exhibited different patterns in each population (Fig. 3). The highest sensitivities were on seed survival (s0), adult clump survival (s6) and young clump growth (g5). In the Opa population the most sensitive parameter was adult clump survival, because of its slightly lower value and higher range of variation compared with Zab and Pog (0.985 ± 0.03, 0.997 ± 0.008, 0.996 ± 0.011, respectively). Young clump growth, the sensitivity of which was negligible in the Zab and Pog populations, was the second most sensitive parameter in Opa, because very few young clumps were observed to grow into adult clumps over the census period. In the populations Zab and Pog seed survival had the highest sensitivity, especially for Pog. This result indicates a need to improve the estimation of this parameter.

AGE-BASED LIFE HISTORY PARAMETERS

The probability of a seed reaching maturity was extremely low (Table 2). The age at maturity (age at which a clump entered the young clump stage) ranged from 8.1 to 9.7 years. Mean residence time in each stage was very similar in all three populations except for adult clumps and dormant adults (Fig. 4), for which mean longevity was longer in the aggregated populations (Zab and Pog) than in Opa (on average, 310 years for Zab, 370 years for Pog and 110 years for Opa).

Not surprisingly, the generation time was high, and longer for Zab and Pog than Opa (280 ± 224 years, 334 ± 332 and 56 ± 53 years, respectively). The mean conditional life span of the adult stage was 270 years for Zab, 333 years for Pog and 66 years for Opa.

STOCHASTIC ANALYSES: ASSESSMENT OF THE PROBABILITY OF FUTURE RISK

The populations Pog and Zab had stochastic growth rates close to 1 and were considered likely to persist
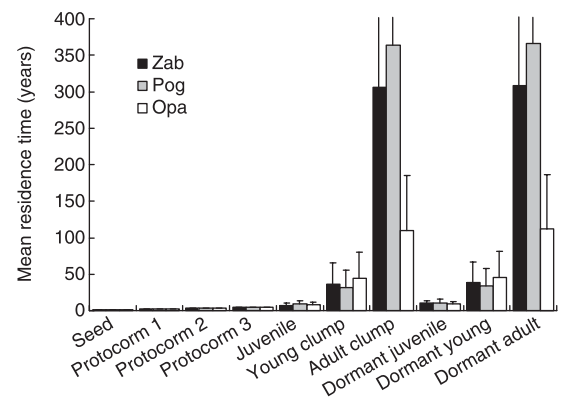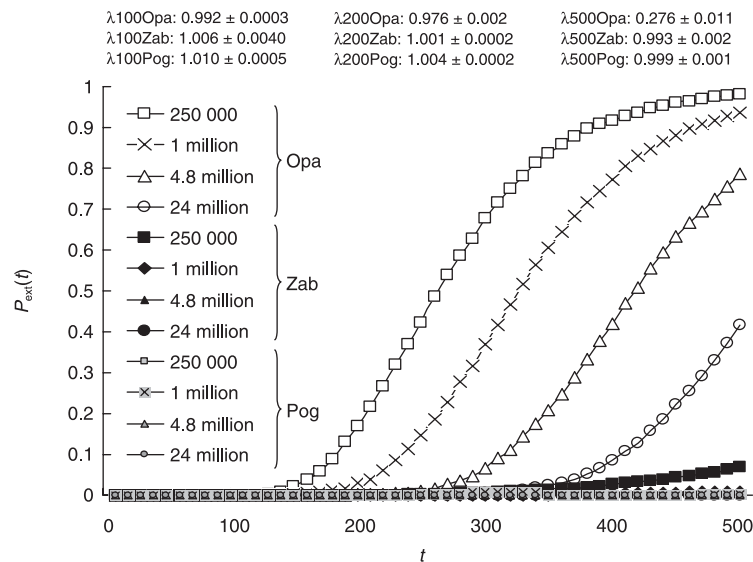
for the next 500 years for all initial seed bank values (Fig. 5). However, stochastic phenomena decreased the growth rate of the Opa population (to slightly lower than 1 after 100 years and falling to 0.976 after 200 years) and led the population to extinction. The initial number of seeds in the seed bank strongly influenced its probability of extinction at time *t*: increasing the size of the seed bank (and thus supposing longer seed survival) increased the probability of recruitment from seed and thus reduced the probability of extinction. We believe that seed survival in the seed bank is between 1 and 20 years, which means that the probability of extinction lies between the curves for the simulations assuming seed bank sizes of 250 000 and 4.8 million. If seeds live only 1 year underground, the Opa population has a 50% chance of becoming extinct after 260 years. If seeds live up to 20 years underground, the 50% probability of extinction is postponed until 420 years.

Discussion

Examination of the dynamics of the three *C. calceolus* populations using different types of analysis gave convergent results for the three populations and underlined three major biological characteristics of *C. calceolus*.

First, for the young stages, the mean residence time was similar in all three populations: *C. calceolus* is a very slow-growing plant and we estimated that 8–10 years are necessary for seeds to form a clump, and even longer for a young clump to flower. This estimate of time from seed to flowering is in agreement with existing data (from 10 to 16 years; reviewed by Curtis 1943; Terschuren 1999), partially validating our model.

Second, adult dormancy played a crucial role in the dynamics, possibly influenced by environmental factors. In our model, dormancy rate was similar to the estimation of Shefferson *et al.* (2001) (mean probability of dormancy = 0.21 ± 0.23 vs. 0.32 ± 0.024). This contrasts with Kull (1995), who concluded that dormancy was negligible in Estonian *C. calceolus* populations. Shefferson *et al.* (2001) observed a positive

λ100Opa: 0.992 ± 0.0003   λ200Opa: 0.976 ± 0.002   λ500Opa: 0.276 ± 0.011
λ100Zab: 1.006 ± 0.0040   λ200Zab: 1.001 ± 0.0002   λ500Zab: 0.993 ± 0.002
λ100Pog: 1.010 ± 0.0005   λ200Pog: 1.004 ± 0.0002   λ500Pog: 0.999 ± 0.001

Fig. 5 Probability of extinction $P_{ext}(t)$ calculated for different projected times t. We used four initial values of seed bank size for the three populations (see text). The stochastic growth rates estimated over 100, 200 and 500 years are reported above the curves.

relationship between the number of freezing days in spring and dormancy of *C. calceolus* ssp. *parviflorum*, and Kéry & Gregg (2004) found a climatic effect on ramet transitions in *C. reginae*. In our populations, dormancy was particularly common in one year (1997) and in one population (Opa), suggesting a local environmental effect.

Third, the global dynamics is similar to that of extremely long-lived perennials such as those studied by Silvertown & Franco (1993). To compare the dynamics of plants species, Silvertown & Franco summed the elasticities of transitions in three categories, positive growth (G), stasis and retrogression (L), and seed production and recruitment (F). *C. calceolus* populations fall close to the positions of Saguaro cactus (*Carnegiea gigantea*) and the common guava (*Psidium guajava*) in their 'GLF triangle'. Life span was estimated to be 125–175 years for the Saguaro cactus, with a potential life span of almost three centuries (Pierson & Turner 1998), and 176 years for the guava (Silvertown *et al.* 2001).

These characteristics might explain why the three Polish populations of *C. calceolus* we analysed showed remarkably stable dynamics, with deterministic growth rates and stochastic growth rates close to 1 after 100 years and a very slow convergence to equilibrium. Without major disturbances, all three populations should persist for at least 100 years. Therefore, there seems to be no intrinsic cause for the dramatic extinction rates observed in Eurasia.

However, the results of PVAs need to be interpreted with caution. Their principal purpose was to allow us to compare populations with different spatial distributions in order to understand the processes underlying the population dynamics of *C. calceolus* and, as expected, the three populations did show significantly different stage structures, dynamics (longevities and stability) and persistence.

The major differences lay between the uniformly distributed population (Opa) and the two aggregated populations (Pog and Zab) and mostly stem from differences in mean residence time in adult stages. Adult clumps were more common at Zab and Pog and lived longer (estimated at > 300 years vs. 110 years) than at Opa, where young clumps were dominant.

The life spans we obtained for the Opa population are in agreement with those in the literature: Shefferson *et al.* (2001) estimated that the longevity of *C. calceolus* ranges from 25 to 100 years, and Ehrlen & Lehtilä (2002) estimated the mean conditional life span of *C. acaule* at 26 years. Kull (1995) estimated the longevity of *C. calceolus* in Estonia by excavating the rhizomes, and obtained an average value of 30–35 years, with a maximum longevity greater than 100 years. The extreme longevity we found in Zab and Pog could be explained by the larger size of adult clumps in these populations, as survival is often size-dependent in clonal plants (Peterson & Jones 1997) or by the use of clumps as our demographic unit, as it is possible that several genets are present per clump, each clump being a composite of several physiologically integrated units (Silvertown *et al.* 2001). The higher adult clump longevity introduced more inertia to the dynamics of Pog and Zab. Indeed, Opa was the most sensitive population to environmental variations. Had we been able to use genet as the unit of study, we would have expected a more dynamic picture for Zab and Pog.

Unlike the two aggregated populations (Zab and Pog), which were remarkably persistent, the Opa population showed a long-term decrease in projected population size. Although extinction was not predicted during the next 100 years, the Opa population may become extinct in the next 250–500 years, depending on the seed bank size.

Extending the time scale of model predictions beyond 100 years is important for two reasons. First, 100 years

is not even one generation for the lady's slipper orchid (Lande 2002). Second, in addition to catastrophic events and human disturbances that occur over a much shorter time scale, the persistence of *Cypripedium calceolus* populations is linked to the slow changes in its habitat through successional processes over centuries.

The sequence of appearance of woody species in a secondary succession was studied in the oak (*Quercus robur*) – lime (*Tilia cordata*) – hornbeam (*Carpinus betulus*) forest where our Polish populations originated (Falinski 1988). The presence of *Quercus robur* at Pog indicates that it is an old community, and the small fragments of forest with *Fraxinus excelsior* at Zab indicate that this community is older than the young *Betula pendula* forest at Opa. The two aggregated *C. calceolus* populations are thus located within older secondary successional communities than the more vulnerable Opa population. However, forest successional processes are likely to cause the demographics on Opa to change in the next 200–300 years, probably becoming similar to those of the other two populations. This will be achieved though an increase in growth of young clumps, the maintenance of adult clumps and decreased variation in demographic parameters.

However, in extending the time frame to 500 years, estimates of extinction probability cannot be subject to absolute interpretation. There are good reasons to believe that the parameters will change over such a long time period (for example due to successional processes), and that the effects of both slow environmental changes, catastrophic events and uncertainties are complex and difficult to predict (Ludwig 1999; Burgman & Possingham 2000; Fieberg & Ellner 2000).

Spatial patterns can be good indicators of the ecology of a species or a population and may thus be useful as a diagnostic tool in management (Anon. 1995). Clumps are simple to detect and populations with more than 20% of adult clumps appear to be relatively stable and less sensitive to environmental variations than those with fewer adult clumps. Accordingly, we found that the maintenance of a high adult survival is a key factor in population persistence. All perturbations concerning adult survival (e.g. uprooting by gardeners or collectors, digging up of rhizomes by wild animals, damage to habitat quality) would greatly affect the persistence of both aggregated and uniformly distributed populations.

Although early demographic parameters do not strongly influence population dynamics, they may actually be very variable and thus contribute greatly to growth rate variations as shown by Pfister (1998) and Fréville *et al.* (2004). Unfortunately, the lack of year-to-year field data on seed/protocorm survival and growth prevented us from estimating their variance and consequently their role. We estimated that the mean probability of a seed reaching maturity was very low (8.1×10^{-7}), and that sensitivity of seed survival was not negligible. Consequently, seed survival had a strong impact on the persistence of the Opa population. Our results confirmed that it is necessary to focus on early stages to obtain better demographic estimates and thus better projections. Investigation of the soil surface and below-ground ecology, including study of the demography of seeds and of the mycorrhizal symbiont, will be of crucial importance in planning management strategies for *C. calceolus*.

The relative stability and persistence of the populations in the Biebrza National Park (a protected area), compared with the recent alarming decrease both in population numbers and in distribution area across Eurasia, strongly indicates that many populations of *C. calceolus* are at risk from catastrophic events or unfavourable successional processes (canopy closure). Modification of the forestry regime and canopy closure modifies light conditions, water availability and drainage, and soil eutrophication (Kull 1999; Terschuren 1999) from those required by the species and these factors directly affect recruitment, heterotrophic growth and attractiveness of plants to pollinators. Biebrza National Park was created in 1993 and, as a wetland site of global significance, it is under the protection of the RAMSAR Convention. Its management, focusing on habitat protection and thus limiting extrinsic events that affect adult survival and the seed bank, seems to be highly beneficial for the maintenance of *C. calceolus* populations.

Since the first description of PVA (Soulé 1986), both practice and research have largely focused on animals (especially vertebrates) rather than plants (Menges 2000; Doak *et al.* 2002). One reason is that some plant species have life histories that are especially difficult to study (long-lived with dormant or resting stages), requiring long censuses and study of multiple populations. Such data acquisition is, however, a necessary step for reliable predictions of future population behaviour (Reed *et al.* 2002). Our results underline the importance of successional processes in shaping the dynamics of populations of *C. calceolus* and the lack of an intrinsic threat to population viability indicates the importance of habitat vs. individual conservation. *C. calceolus* is usually found within rich orchid communities including both common and endangered species. Saving this species, which means managing its habitat, would automatically result in conservation of many other species. The emblematic status of the European lady's slipper should enhance conservation at the habitat level and make it an umbrella species for sympatric orchid communities (Simberloff 1998).

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