

# Costs and benefits of fruiting to future reproduction in two dormancy-prone orchids

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## Summary

**1** Reproduction is expected to occur at a cost to survival, growth or future reproduction. However, trade-offs in long-lived, clonal herbs have proven difficult to assess, particularly when they are prone to adult dormancy.

**2** We assessed the costs of fruiting in a study of two species of lady's slipper orchid, *Cypripedium candidum* and *C. parviflorum*, growing sympatrically in a wet meadow in north-eastern Illinois, USA, from 2000 to 2005.

**3** First, we characterized flowering and fruiting in both populations. We found some differences between species, with  $68.6 \pm 5.7\%$  (mean  $\pm$  SE) and  $43.5 \pm 1.4\%$  of sprouting plants flowering, while  $33.6 \pm 10.0\%$  and  $33.5 \pm 8.1\%$  of flowering plants fruited in *C. candidum* and *C. parviflorum*, respectively.

**4** Next, we tested the survival, sprouting and flowering response to current fruiting using multistate mark–recapture statistics. The best-fit model posited no cost of fruiting. However, according to a model parsimonious with the best-fit model, fruiting may have resulted in a small cost to survival visible primarily in small-sized individuals of *C. parviflorum* (decrease from 0.846 in non-fruiting but flowering plants to 0.824 in fruiting plants). In all cases, fruiting resulted in an increased probability of future flowering, suggesting that reproduction may have a higher priority in resource allocation than survival.

**5** Finally, we tested the effects of fruiting on future fruiting using logistic regression for two years in which fruiting was particularly high, but detected no change in the probability of fruiting after fruiting.

**6** Fruiting may increase in response to internal cues, perhaps related to nutrient uptake or storage, in addition to the obvious effects of pollination. The result may be that plants with greater access to nutrients or with greater stored reserves are more likely to flower each season. We suggest a need for further research exploring the internal mechanisms governing fruiting response in long-lived, clonal herbs.

*Key-words:* *Cypripedium*, demographic costs, lady's slipper orchids, life history, long-lived plants, multistate mark–recapture, physiological allocation, trade-offs

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## Introduction

Reproduction may incur costs to survival, growth or reproduction in the same or a future breeding season (Fisher 1930; Stearns 1992). Such costs are often referred

to as reproductive trade-offs, because resources allocated to one beneficial trait may result in detriment to another. Trade-offs are generally detected as either correlated demographic responses among different reproductive classes (Fox *et al.* 2003; Kraaijeveld & Godfray 2003), a negatively correlated evolutionary response in one trait to imposed selection on another (Rose & Charlesworth 1981; Navara & Hill 2003), or shifts in physiological allocation among life-history traits (Alonzo & Warner 2000; Sakai & Harada 2004).

All such studies must be conducted long enough to observe trade-offs, and so short-lived organisms such as annual plants are most frequently used because trade-offs are demonstrable within a short timeframe.

Long-lived organisms require longer time periods to observe results. In long-lived, iteroparous plants, observation of reproductive trade-offs is further confounded by indeterminate growth (Winkler & Fischer 2001; Hartemink *et al.* 2004). Costs of reproduction differ in plants of different size (Obeso 2002), making annual root expansion and clonal growth confounding factors. Furthermore, patterns of resource allocation may change over time depending on growth-related changes in resource acquisition (van Noordwijk & de Jong 1986). These issues can result in delayed costs (Ehrlén & Van Groenendael 2001), or even observation of positive relationships among life-history traits that theory predicts should be negatively related.

The phenomenon of adult whole-plant dormancy, also known as prolonged dormancy, is a relatively little studied aspect of the life history of many long-lived geophytes. It has been most commonly studied in the orchid genera *Cypripedium* and *Cleistes* (Shefferson *et al.* 2001; Brzosko 2002; Kéry & Gregg 2003, 2004; Shefferson *et al.* 2003; Gregg & Kery 2006; Shefferson 2006). Life-history questions related to dormancy have generally focused on its relationship to survival. Through these studies, it has become apparent that the probability of dormancy is negatively correlated to plant size, while the probability of survival is positively correlated to plant size (Primack & Stacy 1998; Gregg & Kery 2006; Shefferson 2006), and that the probabilities of dormancy and survival *in situ* are often negatively correlated (Hutchings 1987; Shefferson *et al.* 2003). However, dormancy buffers survival against environmental stochasticity at least in the short term, suggesting it may confer adaptive benefits (Shefferson *et al.* 2005a).

Life-history relationships between dormancy and reproduction have been less studied, perhaps because of the difficulties in estimating reproductive success in the most commonly studied dormancy-prone group of plants, the orchid family. A 7-year study of *Cypripedium* species growing sympatrically in north-eastern Illinois suggested the impact of reproduction on dormancy was minimal, because flowering was more likely in years following flowering, and dormancy was more likely in years following dormancy (Shefferson *et al.* 2003). Similar results were obtained in an 11-year study of two populations of *C. reginae* in West Virginia (Kéry & Gregg 2004). However, an 11-year hand-pollination experiment in three populations of *C. acaule* suggested that while flowering does not result in increased dormancy, fruiting may (Primack & Stacy 1998). Contradicting these results, an observational assessment of fruiting costs in *C. calceolus*, in which 3500 flowers in eight populations were tracked over 11 years, found no effect of fruiting on growth or further fruiting (Kull

1998), although pollen limitation may have acted to keep statistical power low. Thus, observations on the relationship between reproduction and dormancy have been mixed.

Here, we assessed reproductive costs in two long-lived, dormancy-prone orchid species, *Cypripedium candidum*, the white lady's slipper, and *C. parviflorum*, the small yellow lady's slipper, growing sympatrically at a site in north-eastern Illinois, USA. This is the same system in which flowering costs were tested in Shefferson *et al.* (2003). We specifically asked whether fruiting results in decreased survival, increased dormancy and/or decreased flowering, which would suggest costs of fruiting to survival, sprouting and/or sexual reproduction, respectively. We addressed this question with multistate mark-recapture statistics with individual covariates in program MARK (White & Burnham 1999). This method provides plant demographers and evolutionary ecologists with the tools necessary to estimate life-history parameters in populations of dormancy-prone plant species without bias (Shefferson *et al.* 2001; Shefferson 2002; Kéry *et al.* 2005). We then tested whether fruiting results in costs to future fruiting via logistic regression.

## Methods

### STUDY ORGANISMS AND FIELD SITE

Two terrestrial lady's slipper orchid taxa, the white lady's slipper, *Cypripedium candidum* Muhl. ex Willd., and the small yellow lady's slipper, *C. parviflorum* Salisb., were censused from 2000 to 2005 in a 3-ha wet meadow in Gavin Prairie Nature Preserve, Illinois, USA. These taxa are perennial geophytes occurring primarily in the Great Lakes region of the United States, but reaching into Canada and the American West as far as the Rocky Mountains (Case 1987; Swink & Wilhelm 1994). They typically occur in tamarack swamps, wet woodland boundaries, wet meadows and fens. In Lake County, Illinois, flowering occurs annually from mid-May through to mid-June (Swink & Wilhelm 1994). Pollination is by deceit of insect vectors, and the tiny seeds lack nutritional reserves, requiring colonization by the appropriate mycorrhizal fungi for germination and growth (Shefferson *et al.* 2005b, 2007). The first aerial leaf typically develops 3 years after germination, with the first mature flowering shoot appearing 7–13 years later (Curtis 1943). The lateral rhizome of a single genet can initiate multiple stems, which grow from adjacent nodes that can be as little as 0.5–1.1 cm apart (Kull & Kull 1991).

This study was conducted at Gavin Prairie Nature Preserve in Lake County, Illinois, USA (42°23'N, 88°8'W). The meadow had standing water in the western and southern sections, and was dominated by *Carex* species growing on tussocks. The meadow grades at its eastern end into a wet prairie dominated by tallgrass species. Four soil series have been identified in the wet meadow,

with a pH range from 5.6 to 7.8 (Nuzzo 1990). Annual precipitation, measured from the start of one monitoring period to the start of the next, ranged from 850 mm to 1000 mm during the study, with peaks in late spring and early summer.

#### FIELD METHODS

We monitored a total of 88 *C. candidum* and 721 *C. parviflorum* mature plants occupying eight study patches, separated by tallgrass areas without orchid growth. In each patch, we established one permanent stake and attempted to locate and map all individual plants, both flowering and vegetative. Every year during late anthesis, we recorded the location of each plant by marking its distance and direction from the permanent stake using a 50-m measuring tape and compass. Fruiting censuses were conducted roughly 1 month after the initial census each growing season. Experienced field crews were used in both censuses each year to maximize the probability of detection. Locating plants was relatively easy due to their low density and diffuse distribution (Shefferson *et al.* 2001).

In all surveys and across years, individual sprouts located within 20 cm of each other were considered to be part of the same plant to account for the likely spatial extent of each orchid without including offspring and other orchids (Shefferson 2006). This designation was based on knowledge of branching patterns and growth rates of *Cypripedium* rhizomes (Kull 1995, 1999). Thus, a plant is equivalent to a sprout or a clump of sprouts in close proximity to one another. Although this definition may lead to the inclusion of multiple individual plants per designated plant, plants were assumed to be spatially segregated and non-overlapping due to the low overall shoot density, which resulted in a low number of sprouts found per clump (50% of plants never consisted of more than one sprout through the study, while 77% never consisted of more than two sprouts). We included only mature plants defined as having at least one sprout with two or more leaves. Seedlings typically have one leaf, and the incidence of one-leaved sprouts in mature plants of this genus is rare (Curtis 1943; Cochran & Ellner 1992).

#### ESTIMATING COSTS OF REPRODUCTION

##### Mark-recapture analysis

We analysed census data via multistate mark-recapture modelling in program MARK (White & Burnham 1999). Open-population mark-recapture methods such as those used here estimate survival and other parameters under the assumption that all individuals share the same inherent demographic probabilities, and differ only in ways built into the model (see 'Mark-recapture analysis assumptions' below). Assuming this, failure to observe individuals for several monitoring occasions bounded between observation events can contribute to

the estimation of survival probability after an individual ceases to be sighted (Lebreton *et al.* 1992). For example, in a 6-year study, if some individuals are seen in years 1 and 6 but are not seen in years 2–5, then they must have been alive during that time interval. If other individuals are seen in years 1 and 2 but not 3–6, then the lack of observation of the former individuals during a period in which they must have been alive suggests that the latter individuals may have been alive after year 2. Through explicit modelling of the uncertainty in survival after an individual's last sighting, these methods allow survival to be estimated without requiring that any individual be designated as dead or dormant. Multistate mark-recapture methods further estimate survival among life-history states and transitions among states (Hestbeck *et al.* 1991; Brownie *et al.* 1993), but require the input of predetermined resighting probabilities for each state in order to estimate these parameters for unobservable life states such as dormancy (Kendall & Nichols 2002; Shefferson *et al.* 2003).

We estimated survival ( $S$ ) of, and survival-conditional transitions ( $\Psi$ ) among, flowering (Flo), non-flowering vegetative (Veg) and dormant (Dor) states, under the assumption of perfect and near-perfect re-detection ( $p$ ) of flowering and vegetative individuals, respectively, per previously published rates for the *C. parviflorum* population (i.e.  $p_{\text{Flo}} = 1$ ,  $p_{\text{Veg}} = 0.977$ ,  $p_{\text{Dor}} = 0$ ; Shefferson *et al.* 2003). Here, the probability of survival ( $S_j^i$ ) is defined as the probability that an individual alive and in state  $j$  in year  $i$  survives to year  $i + 1$ ; the probability of state-transition ( $\Psi_{i+1}^{jk}$ ) is defined as the probability that an individual surviving to year  $i + 1$  moves from state  $j$  in year  $i$  to state  $k$  in year  $i + 1$ ; and the probability of resighting ( $p_{i+1}^k$ ) is the probability of observing an individual at time  $i + 1$  assuming that it survived the interval between times  $i$  and  $i + 1$  and that it transitioned to state  $k$  in time  $i + 1$  (White *et al.* 2002).

The global model, defined as the most fully parameterized, biologically relevant model analysed, included species-time interactions in both survival and transitions (model  $S_{\text{state} \times \text{species} \times \text{time}}$ ,  $P_{\text{fixed}}$ ,  $\Psi_{\text{transition} \times \text{species} \times \text{time}}$ , abbreviated as  $S_{\text{str} \times \text{sp} \times \text{t}}$ ,  $P_{\text{fixed}}$ ,  $\Psi_{\text{m} \times \text{sp} \times \text{t}}$  in which subscripts refer to parameterizations for demographic parameters), from which further parameterizations were reduced until all possible models were tested. Factors tested include state (str, referring to variation among flowering, vegetative and dormant plants), state-transition (m, defined as the change from each state at time  $i$  to each state at time  $i + 1$ ), species (sp), size (sz), fruiting (fr), and time (t). Plant size (sz) was included as a series of individual covariates corresponding to each plant for each year of the study, calculated per plant as the number of sprouts per plant (0–8, with the few plants with more than eight sprouts classified as 8), divided by 8 to standardize to the interval 0–1 (Shefferson 2006). Similarly, fruiting (fr) was included as a series of individual covariates for each plant in each year, coded as '1' if even one fruit formed and '0' if either no fruits or

no flowers formed. To test whether size and fruiting influenced future survival and sprouting, both size and fruiting were included for the year before each transition. The influence of size and fruiting from further prior years was not investigated because multistate models as done in program MARK can only handle first-order Markovian transitions (i.e. the state at time  $i + 1$  is dependent only on the state at time  $i$ ), and thus cannot account for the influence of previous state transitions on current demography (Schaub *et al.* 2004). To assess whether size and fruiting exhibited negative relationships with future survival and state-transitions, we also tested separate models involving their complements as individual covariates. Fruiting was modelled only in survival for the interval immediately following flowering, and only in transitions from flowering. Our operational definition of size is determined by the number of above-ground sprouts, causing dormant plants to have a size of zero. Thus, size was modelled only in survival immediately following, and in transitions from, the vegetative and flowering states. The input file used in program MARK for this analysis has been provided in supplementary Appendix S1.

Model inference proceeded through the comparison of AICc (small sample size-corrected Akaike Information Criterion) values, in which

$$AIC_{c,i} = -2 \ln(L_i) + 2K + \frac{2K(K+1)}{n-K-1}$$

where  $L$  is the likelihood of model  $i$ ,  $K$  is the number of parameters and  $n$  is the sample size (Burnham & Anderson 1998). Here, the model with the lowest AICc was considered the best-fit model and models with values  $\leq 2.0$  units greater were equally parsimonious with it. We further estimated Akaike weights, which estimate the probability that a model is the correct model given that all relevant models have been parameterized (Burnham & Anderson 1998).

Not all parameters are separately identifiable in multistate models involving unobservable states (Schaub *et al.* 2004; Kéry *et al.* 2005). We assessed the identifiability of parameters by examining their estimates and associated standard errors. In this case, model parameters were considered likely to be non-identifiable if the standard errors were unusually high (i.e. 1.0 or greater), or if the parameter estimates themselves were boundary values with standard errors of zero. Though not as rigorous as recent methods designed to assess identifiability via symbolic linear algebra software (Gimenez *et al.* 2003, 2004), applications of such techniques to modelling scenarios involving individual covariates have not been developed, whereas our method can be easily used in situations with such complex models. Furthermore, we found that the number of identifiable parameters in the 10 best models agreed with predictions based on parameter identifiability in previously published multistate mark-recapture modelling exercises not using individual covariates, including Kéry *et al.* (2005).

### Mark-recapture analysis assumptions

Open population mark-recapture models derived from the Cormack-Jolly Seber framework assume independence of fates and identity of rates among individuals, often referred to as the 'iii assumption' (Lebreton *et al.* 1992). If individuals in the population vary non-randomly in the probability of being resighted in a way unaccounted for in the modelling exercise, then this assumption may be violated. No optimal methods exist to test the goodness-of-fit of the global model in multistate mark-recapture analysis, particularly when individual covariates are included. However, we conducted an overall goodness-of-fit analysis in U-CARE (Choquet *et al.* 2005) to test if our multistate data departed from the assumptions inherent in the Jolly Move model, in which survival varies by previous state and time, and state transitions and resighting vary by previous state, current state and time (Brownie *et al.* 1993; Pradel *et al.* 2003).

### Logistic regression of fruiting effects on future fruiting

The effects of fruiting on future fruiting could not be estimated with mark-recapture statistics because fruiting is conditional upon flowering, and because the annual numbers of fruiting plants were very low throughout the study. To estimate the effects of fruiting on future fruiting, we conducted a logistic regression in which fruiting in 2004 was assessed as a function of fruiting class in 2003, number of sprouts in 2003 and species, as well as all interactions among these terms. Only data for 2003 and 2004 were used as the year 2003 was the only year in which the number of fruiting plants of both species was high enough to examine fruiting effects without mark-recapture statistics. Plants in 2003 were grouped into fruiting and non-fruiting but flowering classes (non-flowering plants were excluded), while plants in 2004 were grouped into these classes plus a class denoting non-flowering but sprouting plants and a class denoting unobserved plants, which could have been dormant or dead. Logistic regression was conducted using the PROC GENMOD procedure in the SAS v.9.1 software package (SAS Institute 1999), using the cumulative logit link function for the multinomial distribution. Models were fit sequentially from the simplest to the most complex, and the significance of factors was assessed with likelihood ratio tests of models via the TYPE1 option.

## Results

### GENERAL POPULATION TRENDS

Non-flowering plants in both populations were smaller than flowering plants, and mean size differed by species. *Cypripedium candidum* plants, on average, developed  $3.77 \pm 0.39$  (mean  $\pm$  SE) sprouts throughout the study, while *C. parviflorum* grew  $1.65 \pm 0.02$  sprouts. On average,  $68.6 \pm 5.7\%$  of sprouting *C. candidum* plants

**Table 1** Best 10 mark–recapture models of survival and state transition probability for *Cypripedium parviflorum* and *C. candidum* censused at Gavin Prairie, Lake County, Illinois, USA, from 2000 to 2005. The  $\Delta\text{AICc}$  is calculated as  $\text{AICc}_i - \min(\text{AICc})$ , where  $i$  refers to the model, and  $w$  refers to the Akaike weight for each model using  $\text{AICc}$ .  $K$  refers to the number of identifiable parameters. Symbols denote variation by fruiting (fr), species (sp), state (str), stage-transition (m), size (sz) and year (t). Subscript pluses and minuses indicate positive and negative relationships to parameters. The best-fit and equally parsimonious models are shown in bold type

Model	Survival	State transition	$K$	Deviance	$\Delta\text{AICc}$	$w \leq$
<b>1</b>	<b>str <math>\times</math> (sz<sub>+</sub> + sp)</b>	<b>m <math>\times</math> (sz<sub>+</sub> + (sp + t) + fr<sub>-</sub>)</b>	<b>46</b>	<b>5622.04</b>	<b>0</b>	<b>0.588</b>
<b>2</b>	<b>str <math>\times</math> (sz<sub>+</sub> + sp + fr<sub>-</sub>)</b>	<b>m <math>\times</math> (sz<sub>+</sub> + (sp + t) + fr<sub>-</sub>)</b>	<b>47</b>	<b>5621.28</b>	<b>1.33</b>	<b>0.303</b>
3	str $\times$ (sz <sub>+</sub> + sp)	m $\times$ (sz <sub>+</sub> + (sp + t))	44	5632.11	5.89	0.031
4	str $\times$ (sz <sub>+</sub> + sp + fr <sub>+</sub> )	m $\times$ (sz <sub>+</sub> + (sp + t) + fr <sub>+</sub> )	47	5626.03	6.08	0.028
5	str $\times$ (sz <sub>+</sub> + sp + fr <sub>+</sub> )	m $\times$ (sz <sub>+</sub> + (sp + t) + fr <sub>-</sub> )	47	5627.80	7.86	0.012
6	str $\times$ sz <sub>+</sub>	m $\times$ (sz <sub>+</sub> + (sp + t) + fr <sub>-</sub> )	43	5637.11	8.80	0.007
7	str $\times$ (sz <sub>+</sub> + sp)	m $\times$ (sz <sub>+</sub> + (sp $\times$ t) + fr <sub>-</sub> )	70	5580.04	8.82	0.007
8	str $\times$ (sz <sub>+</sub> + fr <sub>+</sub> )	m $\times$ (sz <sub>+</sub> + (sp + t) + fr <sub>-</sub> )	44	5635.76	9.54	0.005
9	str $\times$ (sz <sub>+</sub> + sp + fr <sub>+</sub> )	m $\times$ (sz <sub>+</sub> + (sp $\times$ t) + fr <sub>-</sub> )	71	5578.73	9.65	0.005
10	str $\times$ (sz <sub>+</sub> + sp)	m $\times$ (sz <sub>+</sub> + (sp $\times$ t))	68	5585.36	9.86	0.004

flowered in any given year, while  $43.5 \pm 1.4\%$  of sprouting *C. parviflorum* flowered. Per plant, *C. candidum* individuals produced  $1.80 \pm 0.25$  flowers year<sup>-1</sup> on average, while *C. parviflorum* produced  $0.64 \pm 0.02$ . Per sprout, *C. candidum* produced  $0.47 \pm 0.03$  flowers year<sup>-1</sup> on average, while *C. parviflorum* produced  $0.39 \pm 0.02$ .

On average, less than half of the flowering plants in any year fruited, suggesting that fruiting in these populations may be pollen- or pollinator-limited (Fig. 1). Indeed, the proportion of flowering plants that fruited each year was generally equivalent between the two species ( $33.6 \pm 10.0\%$  and  $33.5 \pm 8.1\%$  for *C. candidum* and *C. parviflorum*, respectively), suggesting either a similar level of pollen limitation or similar levels of limitation in some other resource relevant to fruiting. Fruiting plants were on average larger than flowering plants in both species (Fig. 1), further suggesting that resource limitations may influence the chance of fruiting. Less than 23% of the flowering plants of *C. candidum* and *C. parviflorum* fruited from 2000 to 2002, while the proportion increased to  $52.4 \pm 8.1\%$  and  $50.0 \pm 7.9\%$  in the period from 2003 to 2005, respectively. Although fruiting decreased dramatically in 2002 and rose to new heights in 2003, this did not correspond to increased plant size in either species (Fig. 1). *C. candidum* plants produced  $0.20 \pm 0.13$  fruits per flower, and  $0.10 \pm 0.06$  fruits per sprout (both flowering and non-flowering), while *C. parviflorum* produced  $0.24 \pm 0.10$  fruits per flower and  $0.09 \pm 0.04$  fruits per sprout.

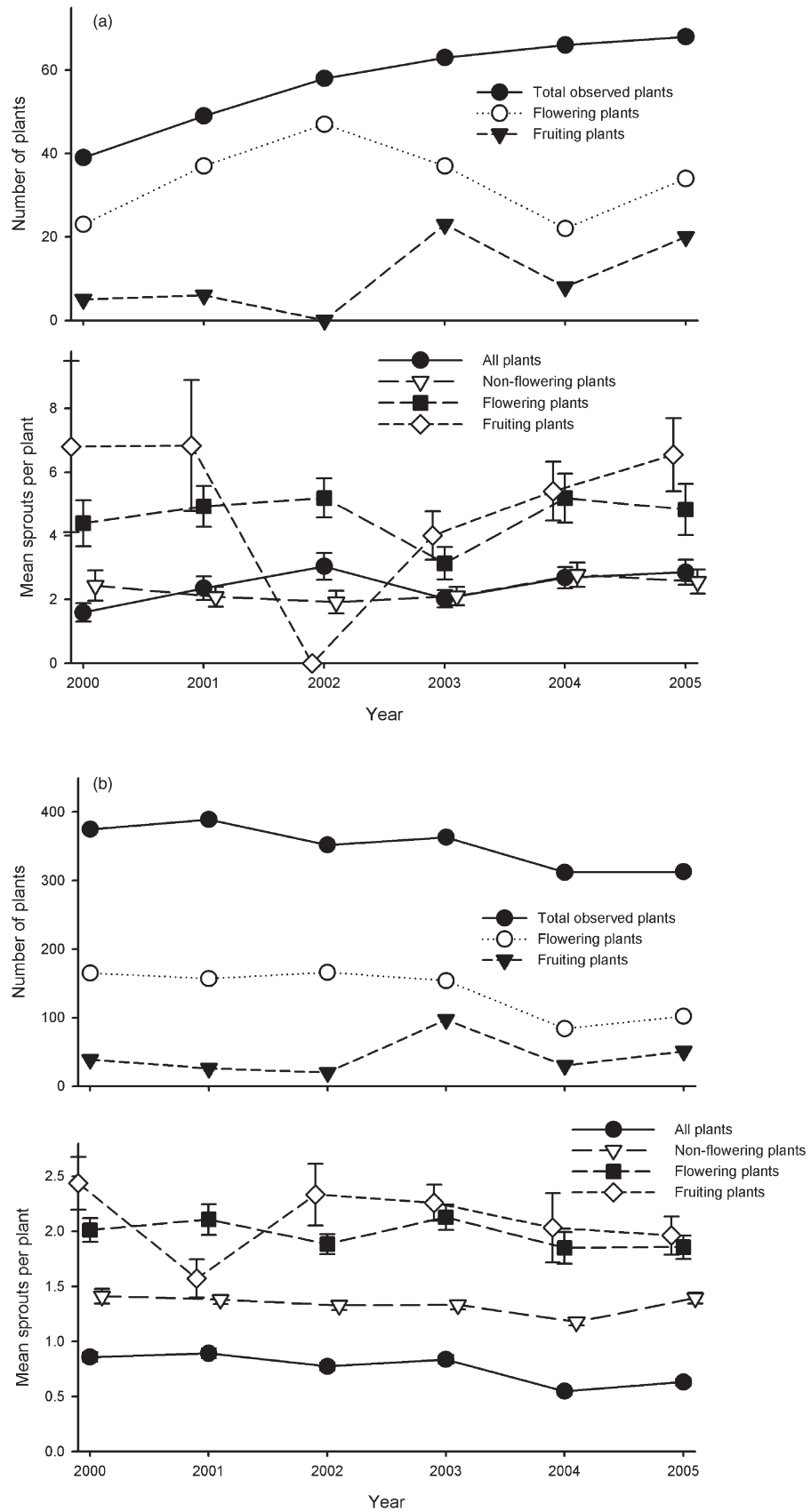
#### REPRODUCTIVE COSTS

Goodness-of-fit testing suggested some overdispersion may have occurred in the global model used in mark–recapture analysis. Our *C. parviflorum* data deviated significantly from the JMV model ( $\chi^2_{69} = 147.6$ ,  $P < 0.0001$ ), while our *C. candidum* data did not ( $\chi^2_{31} = 31.0$ ,  $P = 0.466$ ). As this goodness-of-fit test could not test for covariation with individual covariates, we suggest

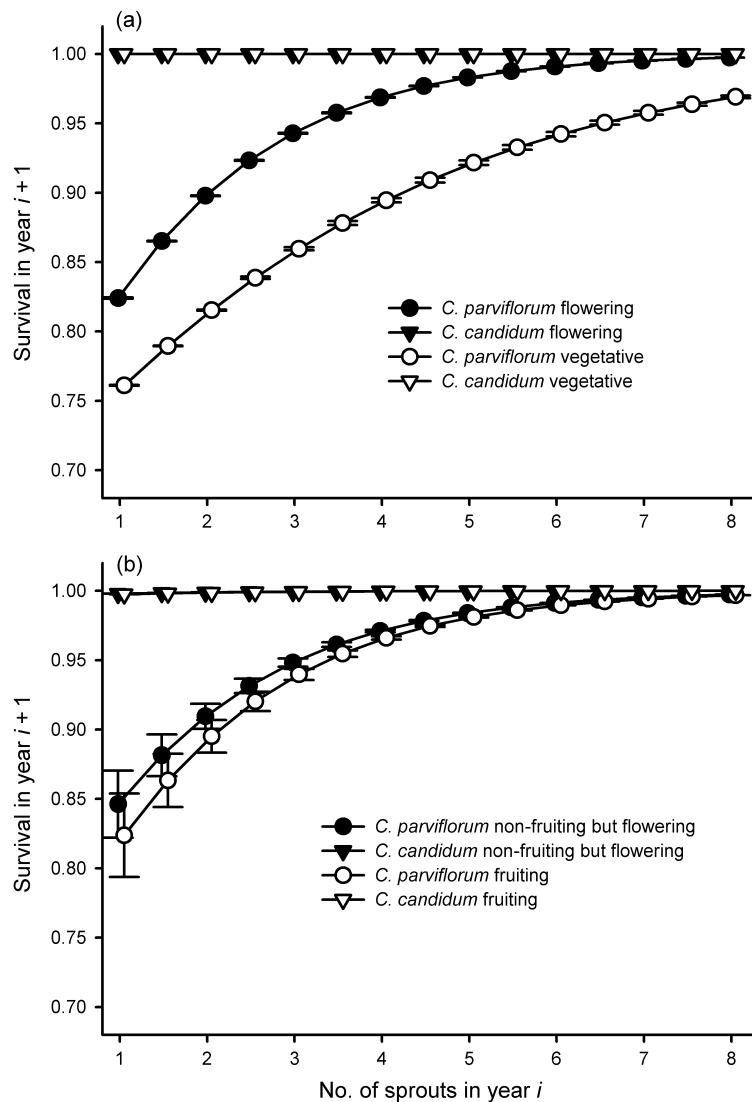
that the significant departure from expectation in the *C. parviflorum* population may be due to population parameters varying by plant size and the cumulative effects of previous fruiting.

Mark–recapture analysis suggested a possible, small cost of fruiting to survival. In the best-fit model, survival was unaffected by fruiting but varied positively with increasing plant size, suggesting that fruiting may be resource-limited (model 1, Table 1; Fig. 2a). However, an equally parsimonious model with 51.5% of the support of the best-fit model suggested a small negative influence on survival, particularly visible in *C. parviflorum* (model 2, Table 1; Fig. 2b). Furthermore, survival varied dramatically by species, with overall estimates approaching unity at all sizes in *C. candidum* while *C. parviflorum* ranged from 0.846 for plants with only one sprout to 0.997 for plants with eight or more sprouts (Fig. 2a).

Fruiting affected future reproduction, though not as expected via hypotheses of costs of reproduction and trade-offs. Fruiting did not result in a cost to future flowering. Instead, fruiting plants were more likely to flower in the following year than non-fruiting plants (Fig. 3a). The best-fit model suggested that state transitions in both species varied in parallel across time, with state transitions from flowering to dormancy and the vegetative state less likely in fruiting plants than in non-fruiting plants (model 1, Table 1; Fig. 3b,c). This model structure was repeated in the second-best model, accounting for approximately 90% of model support via Akaike weights (Table 1). Transitions from the vegetative state to flowering increased with increasing size. Furthermore, although fruiting plants appeared to be more likely to fruit in the next year than flowering, non-fruiting plants (17.4% and 19.6% chance of fruiting in previously fruiting and 0.0% and 8.5% in non-fruiting but flowering individuals of *C. candidum* and *C. parviflorum*, respectively; *C. candidum*:  $\chi^2_6 = 25.9$ ,  $P < 0.001$ ; *C. parviflorum*:  $\chi^2_6 = 67.2$ ,  $P < 0.0001$ ), this trend may have been due to size differences between fruiting vs.



**Fig. 1** Total numbers of plants, flowering plants and fruiting plants censused and mean number of sprouts per plant, per non-flowering plant, per flowering plant and per fruiting plant in sympatric populations of (a) *Cyripedium candidum* and (b) *C. parviflorum* from 2000 to 2005 in Gavin Prairie, Lake County, Illinois, USA.



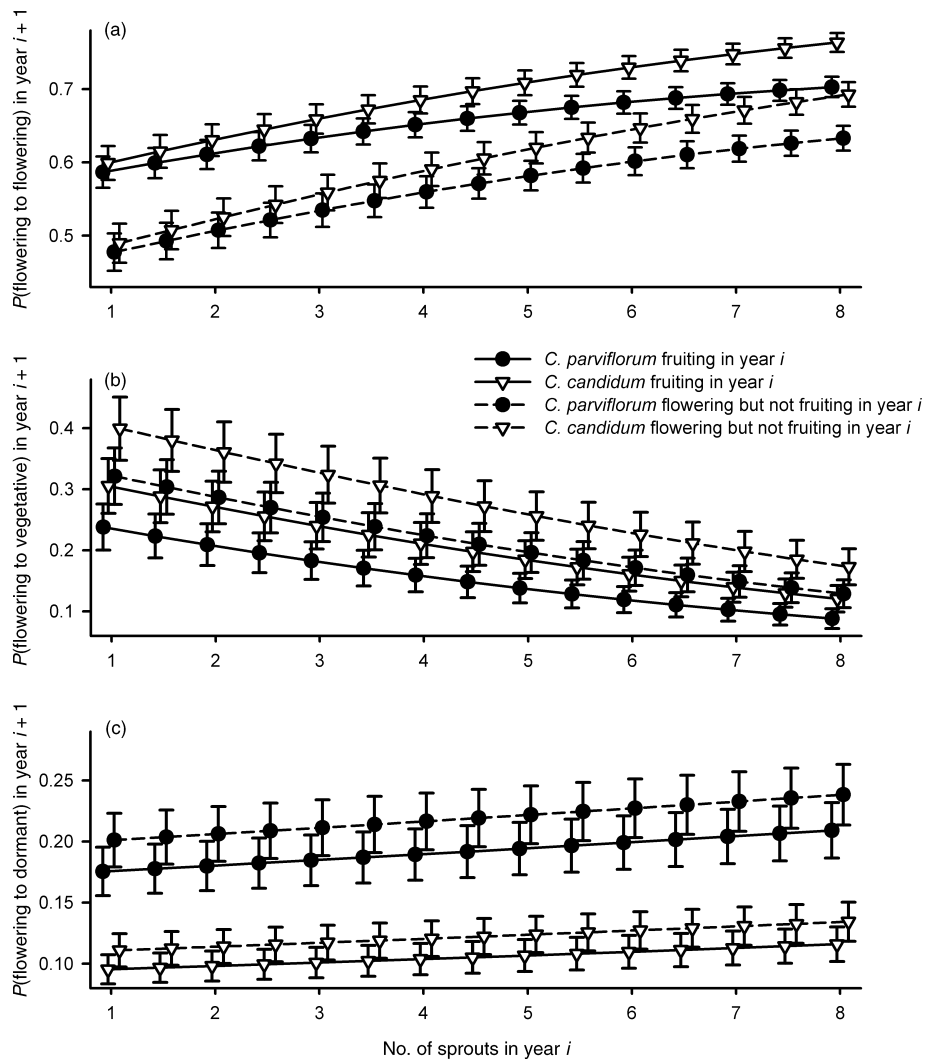
**Fig. 2** Survival of (a) flowering and vegetative individuals and (b) fruiting and non-fruiting but flowering individuals in sympatric populations of *Cypripedium parviflorum* and *C. candidum* from 2000 to 2005 in Gavin Prairie, Lake County, Illinois, USA. (a) Estimates derived from the best-fit model in multistate mark–recapture modelling (model 1, Table 1). (b) Estimates derived from the second-best model in multistate mark–recapture modelling (model 2, Table 1). Bars denote 1 SE.

non-fruiting but flowering plants in 2003. Logistic regression of fruiting probability against flowering class, number of sprouts and species found species to be the only significant factor (fruiting class in 2003:  $\chi_1^2 = 0.20$ ,  $P = 0.654$ ; number of sprouts in 2003:  $\chi_1^2 = 0.07$ ,  $P = 0.793$ ; species:  $\chi_1^2 = 9.90$ ,  $P = 0.002$ ; fruiting class  $\times$  number of sprouts in 2003:  $\chi_1^2 = 0.12$ ,  $P = 0.734$ ; fruiting class in 2003  $\times$  species:  $\chi_1^2 = 1.08$ ,  $P = 0.300$ ; number of sprouts in 2003  $\times$  species:  $\chi_1^2 = 0.29$ ,  $P = 0.592$ ; fruiting class  $\times$  number of sprouts in 2003  $\times$  species:  $\chi_1^2 = 2.59$ ,  $P = 0.107$ ).

## Discussion

Although fruiting is often costly to plant growth and flowering (Ackerman & Montalvo 1990; Calvo 1990; Primack *et al.* 1994; Wyka & Galen 2000; Rydgren & Økland 2003), the discovery of trade-offs between

reproduction and survival in observational life-history studies is somewhat rare (Horvitz & Schemske 1988; Reznick 1985). Trade-offs are generally not observed in natural populations when differential resource acquisition among individuals cannot be controlled (van Noordwijk & de Jong 1986; de Jong & van Noordwijk 1992). Clonal plants develop a continually growing root system with branching rhizomes and, as such, they can spread mortality risk (Pitelka & Ashman 1985), acquire resources and produce clonal offspring at higher rates each year. Variation in the size of the root system among individuals thus results in positive correlations among survival, plant size and reproduction (Verburg *et al.* 1996), and may result in high within-population variation in resource acquisition. Heterogeneous microsite conditions among individuals also have this effect, creating positive correlations among life-history traits rather than the expected negative correlations



**Fig. 3** The probabilities of transition from flowering in year  $i$  to (a) flowering, (b) vegetative sprouting and (c) dormant states in year  $i + 1$ , in sympatric populations of *Cypripedium parviflorum* and *C. candidum* from 2000 to 2005 in Gavin Prairie, Lake County, Illinois, USA. Transition probabilities were estimated via the best-fit model in multistate mark–recapture modelling (model 1, Table 1), are conditional upon survival and sum to 1.0 within each year (i.e.  $\Psi_{i+1}^{\text{Flo-Flo}} + \Psi_{i+1}^{\text{Flo-Veg}} + \Psi_{i+1}^{\text{Flo-Dor}} = 1.0$ ). Bars denote 1SE.

indicative of trade-offs (de Jong & van Noordwijk 1992; Reznick *et al.* 2000). Perhaps for these reasons, reproductive trade-offs have been difficult to detect in *Cypripedium* as well (Kull 1998; Shefferson *et al.* 2003).

Having accounted for the effects of clonal plant size, both increased flowering and fruiting following fruiting suggest the possibility that internal cues beyond those associated with pollination determine whether fruiting occurs in the future. Fruiting may be governed by a threshold plant size, which is often required for sexual reproduction to take place (Worley & Harder 1996; Greer & McCarthy 2000), including in *Cypripedium* (Kull 2002). However, resource acquisition and storage in one year may increase reproductive effort years later, even beyond the level expected from plant size (Worley *et al.* 2003). Furthermore, age- rather than size-related increases in flowering have been noted in some orchids (Hutchings 1987), and these may be

functions of variation in the cumulative acquisition of nutrients and resources more vital to reproduction than growth (Mendez & Karlsson 2005). Such ontogenetic variation in nutrient stoichiometry might also affect fruiting. Even the variance in allocation to reproductive vs. vegetative traits, in response to temporal and environmental variation in resource abundance and acquisition, may change with increasing age and size. Perhaps this occurs because larger resource-storing organs increase the ability to compensate for losses.

Adult survival may not be as all-important to fitness in these plants as previously thought. Fitness and population growth in long-lived organisms as a whole are generally more sensitive to changes in adult survival than in reproductive output, resulting in life histories in which adult survival is relatively invariant (Sæther & Bakke 2000). Such patterns have been noted in *Cypripedium parviflorum* (Shefferson *et al.* 2001),

and dormancy has been suggested to act as a buffer for survival against environmental stochasticity (Shefferson *et al.* 2005a). Low levels of flowering and high levels of dormancy further support the notion that survival is paramount to these plants, while sexual reproduction is not (Shefferson *et al.* 2003). Yet here, because future flowering increases after fruiting while future survival may suffer a small decrease, fruiting may actually have a slightly higher priority in the allocation of resources than previously thought. Furthermore, recent rethinking of the analyses used to determine sensitivities and elasticities suggest that relationships among survival, reproduction and fitness may vary considerably among organisms, even when they are long-lived (Doak *et al.* 2005). Because survival increases with growth, and growth and survival are thought to trade-off with sexual reproduction in clonal herbs (Silvertown *et al.* 1993, 2001), we suggest that sexual reproduction in long-lived, clonal herbs may indeed be more important to fitness than adult survival, and further research should flesh this out.

Long-lived, clonal herbs are often rare and have complex life histories with high age at maturity and low recruitment, making breeding studies and experimental manipulations difficult or altogether impractical. Dormancy further complicates the demonstration of trade-offs by introducing bias to survival estimates (Shefferson *et al.* 2001; Shefferson 2002). The use of mark–recapture statistics allowed us to assess basic reproductive trade-offs in a rare, long-lived, clonal and dormancy-prone herbaceous plant without using destructive methods. Our results support the hypothesis that reproductive effort correlates with plant size, but also suggests that fruiting has a large effect on future reproduction. Further efforts in modelling fruiting costs should include the estimation of the cumulative effects of fruiting over > 1 year on population dynamics. We also suggest that further research should explore the internal mechanisms determining fruiting beyond those associated with pollination, as well as physiological allocation of resources in dormancy-prone plants.

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### Supplementary material

The following supplementary material is available for this article:

**Appendix S1** Program MARK input file.

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2745.2007.01263.x>

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