Large population sizes mitigate negative effects of variable weather conditions on fruit set in two spring woodland orchids

Hans Jacquemyn^{1,*}, Rein Brys²
and Olivier Honnay¹

¹Division of Plant Ecology and Systematics, University of Leuven, Arenbergpark 31, 3001 Heverlee, Belgium

²Research Institute for Nature and Forest, Kliniekstraat 25, 1070 Brussels, Belgium

*Author for correspondence (hans.jacquemyn@bio.kuleuven.be).

Global circulation models predict increased climatic variability, which could increase variability in demographic rates and affect long-term population viability. In animal-pollinated species, pollination services, and thus fruit and seed set, may be highly variable among years and sites, and depend on both local environmental conditions and climatic variables. Orchid species may be particularly vulnerable to disruption of their pollination services, as most species depend on pollinators for successful fruit set and because seed germination and seedling recruitment are to some extent dependent on the amount of fruits and seeds produced. Better insights into the factors determining fruit and seed set are therefore indispensable for a better understanding of population dynamics and viability of orchid populations under changing climatic conditions. However, very few studies have investigated spatio-temporal variation in fruit set in orchids. Here, we quantified fruit production in eight populations of the orchid *Orchis purpurea* that does not reward pollinators and 13 populations of the rewarding *Neottia (Listera) ovata* during five consecutive years (2002–2006). Fruit production in large populations showed much higher stability than that in small populations and was less affected by extreme weather conditions. Our results highlight the potential vulnerability of small orchid populations to an increasingly variable climate through highly unpredictable fruit-set patterns.

Keywords: environmental stochasticity; female reproductive success; orchids; pollination; temporal variability

1. INTRODUCTION

Recent climate models have provided growing evidence that the degree of climatic variability among successive time periods is likely to change as a

consequence of human activities (Easterling *et al.* 2000; Giorgi *et al.* 2004; Rowell 2005). This is most likely to result in greater contrasts between dry and wet years or between hot and cold years. These changes could in turn increase variability in demographic rates and thus affect long-term population viability (Boyce *et al.* 2006). Because the population dynamics of plant species is a function of all the vital rates (i.e. birth, growth and death rates), understanding the effects of environmental variation on population dynamics will require understanding of how the environment affects each of these vital rates.

One of the vital rates that can be expected to be severely affected by climatic variability in plants is fecundity because pollination services may be strongly dependent on weather conditions (Piper *et al.* 1984; Price *et al.* 2005). As a result, fruit and seed set may be highly different among years (Piper *et al.* 1984). The effects of increasing interannual variability in environmental conditions on fruit set may be particularly harmful for orchid species, because most species depend on pollinators for successful fruit set and fruit set is, in general, low, particularly in species that deceive pollinators (Neiland & Wilcock 1998; Tremblay *et al.* 2005). Moreover, there is some evidence that a substantial amount of fruit set is indispensable for their long-term survival, because seed germination and seedling recruitment have been shown to be directly related to fruit and seed set (Ackerman *et al.* 1996). Increased interannual variability in weather conditions is therefore likely to translate into increased variability in fruit and seed set and, as a result, into variability in birth rates, thus resulting in demographic stochasticity.

Because obtaining insights into the magnitude of interannual variability in fruit set in orchid species requires long-term monitoring, the extent of and the precise factors that determine variation in fruit set among years are largely unknown (Tremblay *et al.* 2005). Deriving general patterns appears to be difficult because variation between populations may be confounded with patterns of interannual variation. It can therefore be expected that certain population characteristics buffer populations against the impact of environmental variability. It has, for example, been shown that patterns of fruit set depend to some extent on the size of the population (Fritz & Nilsson 1995; Jacquemyn *et al.* 2007; Brys *et al.* 2008) or density (Meléndez-Ackerman & Ackerman 2001), with large or dense populations often having higher fruit set than small or sparse populations. Large or dense populations may attract a higher number of pollinators and may therefore be better buffered against variation in climatic conditions than small and sparse populations, in which pollinator services may be more unpredictable. However, to our knowledge, no studies have been conducted that have related interannual variation in fruit set in orchid species to population size.

Here we monitored many populations, differing in size, during five consecutive years (2002–2006) to quantify the magnitude of interannual variation in fruit set and to relate this variation to population size. Two species (*Neottia ovata* and *Orchis purpurea*) with similar ecological requirements and that occur

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Figure 1. Average fruit set in small (less than 20 flowering individuals) and large (more than 20 flowering individuals) populations of the woodland orchids (a) *Neottia ovata* (filled circles, small populations; open circles, large populations) and (b) *Orchis purpurea* (filled squares, small populations; open squares, large populations) during five successive years (2002–2006).

at the same sites were selected, allowing for direct comparison between species independent of differences in local environmental conditions.

2. MATERIAL AND METHODS

Neottia (Listera) ovata and *O. purpurea* are both spring woodland orchids that can often be found growing together at the edge of oak-hornbeam forests. Flowering takes place at the same time (mid-May). Flowers of *N. ovata* secrete large amounts of nectar onto the labellum surface and have a distinct and somewhat sweet scent that attracts many insect species (Brys *et al.* 2008), whereas *O. purpurea* does not offer any reward to its pollinators. Although flowers of *N. ovata* are specifically adapted to ichneumonids for successful pollination (*Hymenoptera parasitica*, Ichneumonidae) (Brys *et al.* 2008), our observations indicate that both species are pollinated by a large suite of generalist pollinators.

Thirteen populations of *N. ovata* and eight populations of *O. purpurea*, all coincident with *N. ovata* populations, were surveyed in eastern Belgium in an area of ca 64 km² during five consecutive years (2002–2006). Population size varied between 2 and 2500 flowering individuals in *N. ovata* and between 2 and 140 in *O. purpurea*. In each population, 20 plants (or all plants when less than 20 plants were present) were randomly selected and marked. In May, the number of flowers was counted for each marked individual. In June (*N. ovata*) and July (*O. purpurea*), all populations were visited again, and for each marked individual, the number of fruits was counted. Fruit set was determined as the proportion of flowers setting fruit. For each population–year combination, we determined average fruit set as the mean fruit set of all individuals that were measured. Repeated-measures analysis of variance was used to study the effect of species, year and their interaction on fruit set. Both the between-subject main effect of species and the within-subject main effect of year and species–year interaction were determined. The average fruit set over all populations within a given year was also calculated and related to climatic data (average temperature and rainfall in May) that were taken from the nearest weather station (Bierset) using Spearman rank correlations.

To compare temporal variability in fruit set between populations, for each population, we calculated $S = \mu/\sigma$, where μ is the mean fruit set and σ is the standard deviation that results from temporal variation in fruit set. This measure has been previously used in studies of community stability (e.g. Lehman & Tilman 2000; Tilman *et al.* 2006). It is dimensionless and scale-invariant and its magnitude is larger for populations with smaller average temporal

variation, relative to the mean (Lehman & Tilman 2000). To compare variation in average annual fruit production among populations and to see whether this is affected by population size, we plotted S against population size. Ordinary least-squares regression was used to determine the functional relationship between annual variation in fruit production and population size. Population size was logarithmically transformed prior to analysis.

3. RESULTS

Average fruit production varied significantly ($F_{4,76} = 5.81$, $p < 0.001$) among years and was significantly ($F_{1,19} = 14.51$, $p = 0.001$) higher for *N. ovata* than for *O. purpurea*. For *N. ovata*, the mean number of fruits produced by an individual averaged over all populations within a given year varied between 3.9 in 2004 and 12.1 in 2003, corresponding to a fruit set (proportion of flowers setting fruit) of 12.3 and 32.2 per cent, respectively. In *O. purpurea*, the average number of fruits varied between 1.7 in 2004 and 3.7 in 2005, which corresponds to fruit-set levels of 3.7 and 8.5 per cent, respectively (appendix S1 in the electronic supplementary material). The interaction between species and year was also significant ($F_{4,76} = 3.56$, $p = 0.01$), indicating that species responded in a different way to the variation in weather conditions. However, in both species, average fruit set was very low in 2004, particularly in the smallest populations (figure 1). Average fruit set was significantly and positively ($r_s = 0.9$, $p < 0.05$) related to the average May temperature in *N. ovata*, but not in *O. purpurea* ($r_s = 0.2$, $p > 0.05$).

When averaged over years, the mean number of fruits per plant increased linearly with increasing population size in *O. purpurea*, but showed a unimodal pattern in *N. ovata* (figure 2a,b). Fruit number was low in populations containing less than 20 individuals, increased for populations with a size between 20 and 300

Figure 2. Relationship between population size (number of flowering individuals) and (a and b) fruit set averaged over years ($\pm$ s.e.) and (c and d) temporal variation of average annual fruit production, calculated as $S = \mu/\sigma$, in the woodland orchids *Neottia ovata* ((a): $y = -0.07 + 0.35x - 0.09x^2$, $r^2 = 0.68$, $p = 0.01$; (c): $y = 0.86 + 0.84x$, $r^2 = 0.62$, $p = 0.002$) and *Orchis purpurea* ((b): $y = -0.005 + 0.047x$, $r^2 = 0.53$, $p = 0.04$; (d): $y = 0.13 + 1.26x$, $r^2 = 0.67$, $p = 0.01$).

flowering individuals and decreased again once populations contained more than 300 individuals (figure 2a). Patterns of variation in fruit set, on the other hand, were consistent between species (figure 2c,d). Small populations showed very high variation in fruit set relative to the average number of fruits produced (denoted by low S -values), whereas large populations showed very high stability in fruit set. In both species, temporal stability in fruit set (S) increased linearly with the logarithm of population size.

4. DISCUSSION

Fruit production in the rewarding *N. ovata* was significantly higher than that of the deceptive *O. purpurea* and confirms earlier findings that fruit set in rewarding species is generally much higher than that of non-rewarding species (Neiland & Wilcock 1998; Tremblay *et al.* 2005). Although species responded differently to the observed variation in weather conditions, in both species, fruit production was very low in 2004, particularly in the smallest populations. From the climatic data (appendix S2 in the electronic supplementary material), it is clear that May 2004 was abnormally cold compared with the other years (on average 1.65°C colder). It can be hypothesized that pollination of these orchid species is temperature related, as warmer months are generally associated with high pollination success, whereas cold months are related to

low fruit production. Since both species are primarily pollinated by bees and wasps, it is plausible that cold weather delays emergence and/or reduces foraging activity of these insects.

Small populations had a lower fruit production and showed much more variation in fruit production relative to the mean than large populations, suggesting that large populations are better buffered against variable weather conditions. This is most likely a consequence of inadequate pollinator visitation in small populations, resulting in strong pollinator limitation. Pollination experiments have indeed shown that fruit set increases to more than 80 per cent when plants are hand-pollinated with supplemental pollinaria (Jacquemyn *et al.* 2007; Brys *et al.* 2008). Bees and wasps often forage in a local range and can therefore be expected to avoid small unprofitable patches. Low visitation rates in small populations can become particularly manifest when weather conditions are unfavourable, so that small populations are completely overlooked by foraging insects in bad years, resulting in the observed variance in fruit production.

Because extinction risks in natural populations depend on various factors that contribute to stochasticity (Melbourne & Hastings 2008), the observed high variation in fruit production in small populations can be expected to contribute to the overall variance in population growth and thus to the risk of stochastic extinction. Given that the degree of climatic variability

among successive time periods is most likely to change in the coming decades, small orchid populations may become extremely vulnerable to extinction in the future. Understanding the contribution of highly variable fruit production caused by changing environmental conditions to population growth therefore deserves more attention.

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