The relationship between reproductive success and demographic structure in remnant populations of *Primula veris*

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Abstract

Plants often suffer reductions in fecundity due to fragmentation, degradation and destruction of populations and their sites. Whether this decrease in seed production has population-level consequences is generally unknown. Here, we aimed to determine the current status of remnant populations in the perennial herb *Primula veris* in Belgium. Furthermore, we investigated the effects of reduced population size and morph bias on reproductive success and explored if changes in demographic structure could be associated with population fecundity. We studied 69 populations that differed in population size from three to nearly 1500 flowering plants. Three different population types could be distinguished: (a) "dynamic" populations, characterized by high densities of 1 year old juveniles, (b) "normal" populations with adult age-stages prevailing, but still a considerable number of juveniles, and (c) "regressive" populations, in which only flowering adults dominate and rejuvenation hardly occurs. The three population types differed with respect to population size and morph frequency. Dynamic populations were significantly larger and showed a weaker morph bias compared to the intermediate normal and the small regressive populations. Reproductive success, studied the previous year in 26 populations, decreased significantly with decreasing population size and was significantly associated with the demographic structure of the populations. Coefficients of variation for the proportion of flowers setting fruit, the number of seeds per fruit and the total number of seeds per plant decreased significantly with increasing population size. Hence, the observed variability in seed set may be one of the causal factors affecting the observed types of population demographic structure. © 2003 Editions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Fecundity; Population structure; Seed limitation; Habitat fragmentation

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1 Introduction

Habitat fragmentation, degradation and destruction caused by human activities have become a serious threat to the maintenance of biodiversity in many terrestrial ecosystems (Saunders et al., 1991). As a consequence, populations of many plant species became small and isolated, factors believed to increase their local extinction risk (Lennartsson, 2002), even when local environmental conditions remain unchanged (Fischer and Stöcklin, 1997). It is frequently hypothesized that plant populations reduced in size exhibit altered population structures, due to disrupted demographic processes (Tomimatsu and Ohara, 2002). Moreover, a population's current age-stage structure may reflect prior variation in recruitment and mortality (Harper, 1977), and can be the result of factors such as reproductive success (e.g. Maron and Gardner, 2000; Tomimatsu and Ohara, 2002), environmental variability (e.g. Kephart and Paladino, 1997), and disturbance (e.g. Bullock et al., 1994). Since vital demographic rates in plants are usually stage-dependent (Harper, 1977), the structure of a population may be indicative for its

demographic future. Furthermore, in heterostylous plants with strict intramorph incompatibility, morph frequencies have been frequently found to deviate more strongly from equality with decreasing population size (Byers, 1995; Endels et al., 2002). Recently, attention to the possible consequences of habitat fragmentation on plant fitness and overall population fecundity has increased (e.g. Van Treuren et al., 1993; Oostermeijer et al., 1994; Ouborg and Van Treuren, 1995; Jacquemyn et al., 2003). In general, it has been widely regarded that population size is the most important factor influencing reproductive success, although the exact mechanisms remain often unclear. Furthermore, in heterostylous species, biased morph frequencies may interact with limited pollinator attraction in small populations to further decrease population fecundity (Byers, 1995; Kéry et al., 2003). It may be clear that reduction in seed set is likely to have both short-term and longer term negative consequences for a species population dynamics and persistence (Eriksson and Ehrlén, 1992; Lennartsson, 2002). Moreover, lowered seed output is one of the major threats to plant life-history processes influencing the population age-stage structure directly, and may increase the probability of extinction of populations and species in the long-run (e.g. Lennartsson, 2002). Despite this widespread assumption, however, few studies have explicitly investigated the relationship between population fecundity and the demographic structure of plants in fragmented landscapes (e.g. Tomimatsu and Ohara, 2002). One group of plant species that exhibit a dramatic decline in population numbers and size are species typical of nutrient poor semi-natural grasslands (Ratcliffe, 1984; Hillier et al., 1990;Willems et al., 1993). Changes in agricultural land use during the last decades caused a considerable decrease of this once common type of grassland and a corresponding dramatic decline of many of their characteristic species (e.g. Weeda et al., 1990; Fischer and Stöcklin, 1997). As a consequence, many grassland species are forced to survive in small landscape elements, such as road verges, ditch banks and hedgerows (e.g. Tikka et al., 2000). However, intensification of agricultural land use has led to the removal of many hedgerows and ditch banks and destruction of many field boundaries, and to an increased application of herbicides and fertilizers (Endels et al., 2002). One of these grassland species that suffered greatly in Western Europe during the last decades, is the perennial distylous herb *Primula veris* (Zoller and Wagner, 1986; Kéry et al., 2000). In Flanders, this species mainly occurs in verges of intensively used landscapes and shows a highly scattered distribution (Jacquemyn et al., 2002a). This paper has three objectives: (1) to analyse whether the age-stage structure of populations is related to population size and morph bias, (2) to determine whether different reproductive characteristics are influenced by population size and/or morph bias, and (3) to investigate whether there is a relationship between reproductive success and demographic structure. We hypothesized that populations of *P. veris* can be classified into different types that vary in the relative proportions of life stages.

2 Methods

2.1 Species and study area

The cowslip, *P. veris* (Primulacea) is a small perennial rosette herb, typical of nutrient poor grasslands from Spain to Eastern Asia (Hegi, 1975). It normally has one or two (up to seven) inflorescence stalks, each with 5–15 yellow flowers. Flowering usually begins in April and ends 3–4 weeks later in May, the seeds ripen in June–July. Like other species of *Primula, P. veris* is

distylous and strictly self-incompatible (Wedderburn and Richards, 1990). Only cross-pollination between the two genetically determined long (pin) and short styled (thrum) flowers results in successful seed set (Richards, 1986). Flowers are mainly pollinated by Hymenoptera and Diptera (Woodell, 1960, and personal observation). The seeds of this species have no special features to facilitate dispersal and the plant is, therefore, considered as a barochorous species. A large fraction of the seeds stays viable in the seed bank for more then 3 years (Milberg, 1994). Seedling emergence and survival is generally low, but adults can live for several decades under favourable conditions (Tamm, 1972). Vegetative propagation of adult individuals can occur, in which the number of daughter rosettes increases (Tamm, 1972; M. Kéry, personal communication). The study was carried out in a polder landscape, in the vicinity of Veurne in the northern part of Belgium (Fig. 1). The study area comprises 260 km2, in which a total of 69 *P. veris* populations are located.

2.2 Population characteristics and demographic structure

In April 2001, the total number of vegetative and reproductive adult individuals and 1 year old juveniles were determined in all 69 populations. The number of 1 year old juveniles as used as a measure of population recruitment instead of the number of seedlings, because counting the number of seedlings was difficult due to their small size, the surrounding grassland vegetation and because *P. veris* seeds germinate over a relatively long time period of several weeks with great variation between sites (personal observation). To avoid an overestimation of the number of 1 year old juveniles, population surveys were made in April, before *P. veris* seeds germinate (mid-May). An individual was determined as a juvenile when leaves were smaller than 3 cm and/or the length of the largest leaf was smaller than 2 cm. Six populations were omitted from further analysis because they were severely damaged before the moment of survey. To describe the demographic structure of each population, we used the relatively simple measure of determining the relative proportions of these different "age-stages" (cf. Gatsuk et al., 1980; Rabotnov, 1985) or "life-stages". This concept has proven to be useful to describe the current status or demographic viability of populations in relation to environmental features, such as the surrounding vegetation structure and composition, soil conditions and management in several studies, e.g. *Gentiana pneumonanthe* (Oostermeijer et al., 1994), and recently on *Succisa pratensis* and *Salvia pratensis* (see Bühler and Schmid, 2001; Hegland et al., 2001, respectively). The total number of pin and thrum individuals was counted in 2001 for all populations. Morph bias was calculated for each population as the absolute value of the difference between the number of individuals of the two morphs, divided by the total number of flowering plants. It can vary from 0 (both morph types in equal frequency) to 1 (only one of the two morph types present). If groups of individuals were separated by at least 250 m from each other, they were considered as different populations. Most populations, however, were separated by at least several hundred metres (mean = 769 m, minimum = 265 m and maximum = 1889 m).
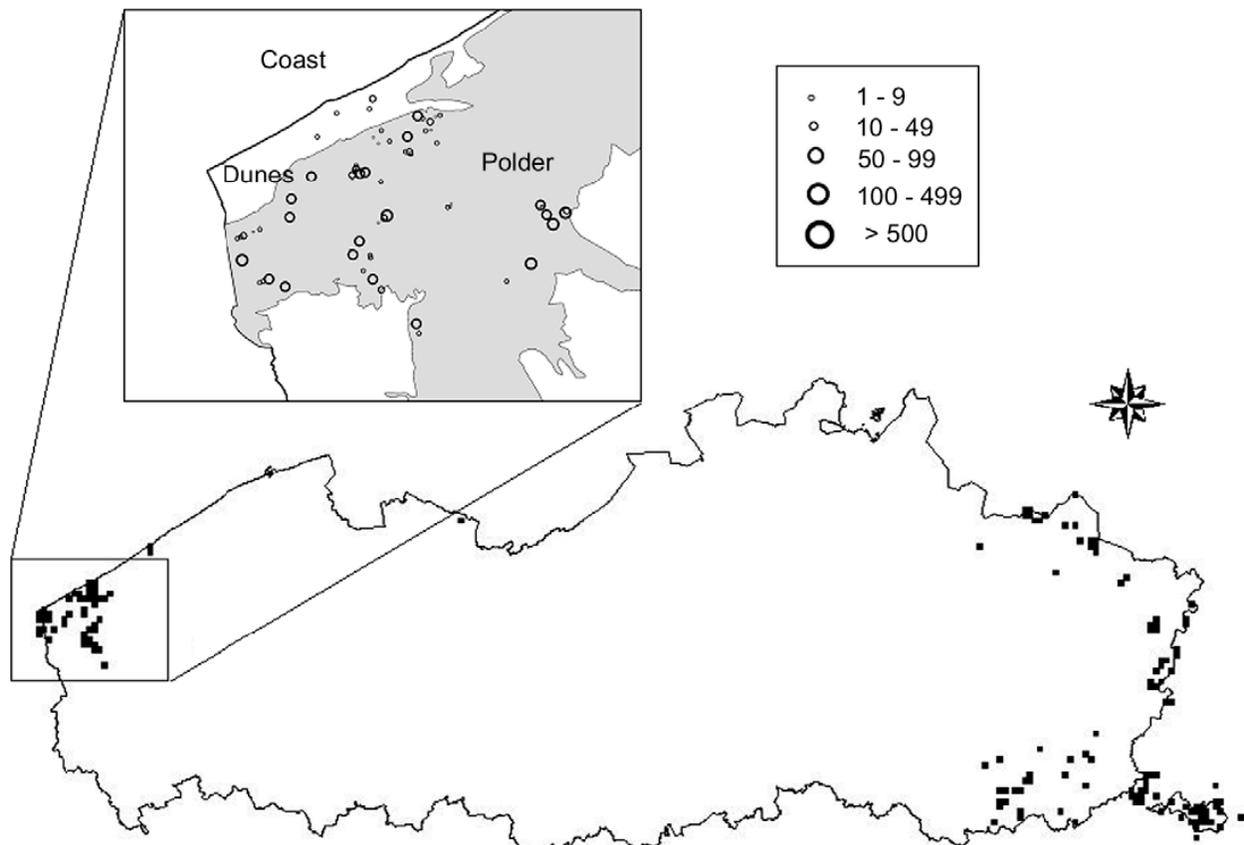
Fig. 1 Distribution of *P. veris* in Belgium: results of the 2001 survey with location of the different populations

2.3 *Sampling procedure for the reproductive characteristics*

A sub-sample of 26 *P. veris* populations was selected to investigate the relationship between population size and different reproductive characteristics. In each population, 20 flowering rosettes were randomly chosen and individually marked. When population size was less than 20 flowering individuals, all individuals were selected. At flowering time, the number of flowers was counted for each selected plant. All the locations were visited again at fruiting time (July), and the number of initiated fruits (capsules) was counted for each individual. Only 340 of the 373 marked plants in the 26 populations could be relocated. The rest had been lost to cattle grazing and/or anthropogenic factors. For each plant five mature, unopened fruits were collected, and their seeds were counted. Total number of seeds per plantwas calculated as the average number of seeds per fruit multiplied by the total number of fruits per plant Reproductive success was determined as the proportion of flowers that developed successfully into fruits (capsules), number of seeds per fruit, and total number of seeds per plant.

3   Data analysis

To distinguish populations with similar demographic structures a K-means clustering (Hartigan, 1975) was applied to the demographic structure data (i.e. the proportion of each age-state in a population). This method clusters samples in a pre-selected number of groups by maximizing

between relative to within-group variation. Overall statistical significance of the groups (population types) obtained by the K-means clustering was tested using analysis of variance (see Hartigan, 1975). To evaluate appropriateness of the classification, the within-cluster variability was compared with the between-cluster variability. In other words, the number of groups in which the within-cluster variability was smallest and the between-cluster variability was largest, was used for further analysis. Analysis of variance was also used to investigate differences in population size and morph frequency among the different population demographic structures. To investigate a possible relationship between population size and biased morph frequency, and the proportion of flowers setting fruit, the number of seeds per fruit, number of seeds per plant, and the (un)certainty estimates, multiple regression analysis was used. An estimate of the (un)certainty of reproduction in the populations was obtained by calculating the coefficient of variation (CV sensu Oostermeijer et al., 1998) for the proportion of flowers setting fruit, number of seeds per fruit and number of seeds per plant of each population. Variables were transformed (log or square root) if necessary to achieve normality and homoscedasticity of residuals.

4 Results

In 2001, all populations in the study area ($n = 69$, Fig. 1) ranged in reproductive size from 1 to 1470 (mean = 147; median = 36), while the selected populations ($n = 26$) used for the determination of the reproductive success the previous year, ranged in size from 3 to 987. Sixty-eight percent of all the populations ($n = 43$) contained fewer than 100 flowering individuals, and from this group 19% contained fewer than 10 flowering individuals (Fig. 2). Three clearly distinct types of population structure could be distinguished with more or less equal number of samples ("dynamic" = 13; "normal" = 25; "regressive" = 25; Fig. 3). Moreover, the proportion of juveniles, vegetatives and generatives differed significantly among the three population types ($F_{2,60} = 142.94$; $P < 0.001$; $F_{2,60} = 14.54$; $P < 0.001$ and $F_{2,60} = 169.03$; $P < 0.001$, respectively). The "dynamic" type comprises more than 50% of juveniles and a fair number of generatives. In the "normal" type, juveniles were present, but most plants were adult flowering individuals. The "regressive" population type only comprised flowering plants and rejuvenation hardly occurred. The total population size and morph bias also differed significantly among population types ($F_{2,62} = 11.53$; $P < 0.001$ and $F_{2,62} = 4.74$; $P = 0.012$, respectively). Populations of the "dynamic" type were significantly larger and contained more equal morph frequencies compared to populations of the "normal" and "regressive" type. The multiple regression analysis in which the impact of population size and morph bias was investigated on different reproductive characteristics revealed that population size had a significant and positive influence on the proportion of flowers setting fruit ($t_{pop. size} = 2.45$; $P = 0.027$), the number of seeds per fruit ($t_{pop. size} = 3.68$; $P = 0.001$), and total seed production per plant ($t_{pop. size} = 2.94$; $P = 0.07$) (Fig. 4). Plants in large populations produced up to 1000 seeds each, while plants in some of the very small populations produced fewer than 50 seeds per plant. On the other hand, no significant relationship was found in the same analysis between the proportion of flowers setting fruit ($t_{morph bias} = -1.70$; $P = 0.109$), the number of seeds per fruit ($t_{morph bias} = -1.01$; $P = 0.322$), and total seed production per plant ($t_{morph bias} = -1.52$; $P = 0.141$), and morph bias. The CV for the proportion of flowers setting fruit decreased with increasing population size ($t_{pop. size} = -2.19$; $P = 0.034$), while no relationship was found with morph bias ($t_{morph bias} = -0.24$; $P = 0.815$). Similarly, the CVs for the number of seeds per fruit and the number of seeds per plant decreased with increasing population size ($t_{pop. size} = -2.87$; $P = 0.009$ and $t_{pop. size}$

= –2.51; *P* = 0.020, respectively), while no association was found with morph bias (*t*morph bias = –0.81; *P* = 0.412 and *t*morph bias = –0.84; *P* = 0.410, respectively).

5 Discussion

In the study area, *P. veris* populations showed a skewed distribution to a large number of very small populations. It appeared that population size underlies the reduced reproductive success in the studied populations. Similar relationships have been reported elsewhere for fragmented populations of *P. veris* (Kéry et al., 2000) and the related *P. elatior* (Jacquemyn et al., 2002b). In several cases, population size has been shown to be directly related to the number of pollinators (Sih and Baltus, 1987; Ågren, 1996). Moreover, bumblebees, which are important pollinators for *P. veris* (Woodell, 1960), have been shown to fertilize higher proportions of flowers in large and dense populations compared to small and sparse populations (Jennersten, 1988; Ågren, 1996). This is in agreement with our findings in which plants growing in small populations are likely to experience reproductive difficulties. In small populations, on average fewer than 60% of flowers per plant set fruit, whereas in large populations on average more than 75% of flowers per plant set fruit. The increased variability (CV) in the proportion of flowers setting fruit further suggest that pollination probability decreases, and more flowers remain unvisited in smaller populations (Oostermeijer et al., 1998).
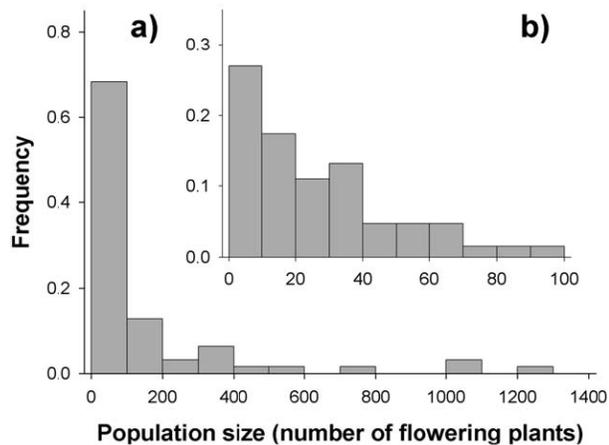

Fig. 2. Frequency distribution of population size (number of flowering plants) for (a) the 69 recorded populations and (b) those populations with up to 100 individuals of *P. veris* within the study area.
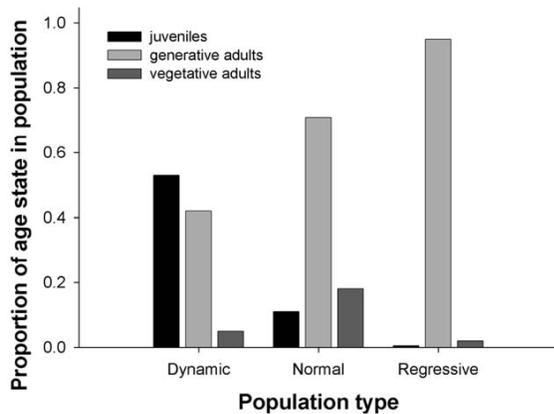
Fig. 3. Population-structure (proportions of life stages) of the three population types of *P. veris* that emerged from the K-means clustering in 63 populations.

Similar results of higher variance for a reproductive characteristic among plants from the same population were also observed by Jacquemyn et al. (2002b) in the related *P. elatior*. Besides reduced pollinator visitation services, due to reduced attractiveness, the breeding system of *P. veris* may also have contributed to the observed results. Moreover, the probability that flowers will receive compatible pollen will decrease for individuals of the morph type in majority if the morph frequency becomes more biased (Byers, 1995; Kéry et al., 2003). The higher variability (CV) in the number of seeds per fruit and total number of seeds per plant in small populations give some indications that insufficient mate availability may strengthen the reduced reproductive output. However, due to the strong co-linearity between population size and morph bias, it is not possible to determine the impact of both factors separately on the process of reproduction in this study. Therefore, further research is needed in a more experimental design to investigate the main effect of population size, morph bias and the interaction of both on the reproductive output of a population. Habitat quality, although not studied here, may have affected these results too in a direct or indirect way. For example, it appeared that reductions in the number of insectpollinated plants affect pollinator densities negatively (Kwak et al., 1998). Finally, the genetic status of a population may contribute to the process of reduced fitness in small populations due to reduced gene flow and inbreeding depression (Barrett and Kohn, 1991; Husband and Schemske, 1996). In contrast to the results found for several self-compatible species (e.g. Van Treuren et al., 1993; Fischer and Matthies, 1998), lower fecundity as a result of an increased frequency of close inbreeding is unlikely to appear in *P. veris*. First, the cowslip is an obligate out-crosser, and self-pollination cannot occur or is very restricted in pin morphs (Woodell, 1960;Wedderburn and Richards, 1990). This most extreme form of inbreeding is probably a very rare event, and only biparental inbreeding is likely to occur in the field. Although, many studies have shown similar relationships between population size and reproductive success (e.g. Van Treuren et al., 1993; Oostermeijer et al., 1994; Ouborg and Van Treuren, 1995), the impact of such reductions in fecundity on demographic structure or plant abundance is almost entirely unknown (cf. Maron and Gardner, 2000). Nevertheless, changes in population fecundity may have large implications for seedling recruitment and the demographic structure of a population (Eriksson and Ehrlén, 1992; Juenger and Bergelson, 2000). Simulation results of Maron and Gardner (2000) revealed that reductions in seed production had surprisingly large impacts on plant abundances, even in perennial plants with a persistent seed-bank, and under conditions thought to minimize the population effects of reduced seed set. Furthermore, the number of seeds progressing into recruits

was found to be surprisingly low. Moreover, a 4 year study on population dynamics of *P. veris* in two large (*n* > 5000) and viable populations, growing in calcareous grasslands in the Eastern part of Belgium, revealed that only 0.01% of the total seed set germinated successfully (Brys and Jacquemyn, unpublished data). If at least 10 000 seeds are needed to ensure one successful recruit, the smallest populations (*n* < 30 flowering individuals) producing fewer than 10 000 seeds per population may not be viable in the medium to long-term. In spite of the 15–30 year lifespan of *P. veris* (Tamm, 1972), local extinction of those small populations is a likely scenario within the next decades if recruitment remains unsuccessful.
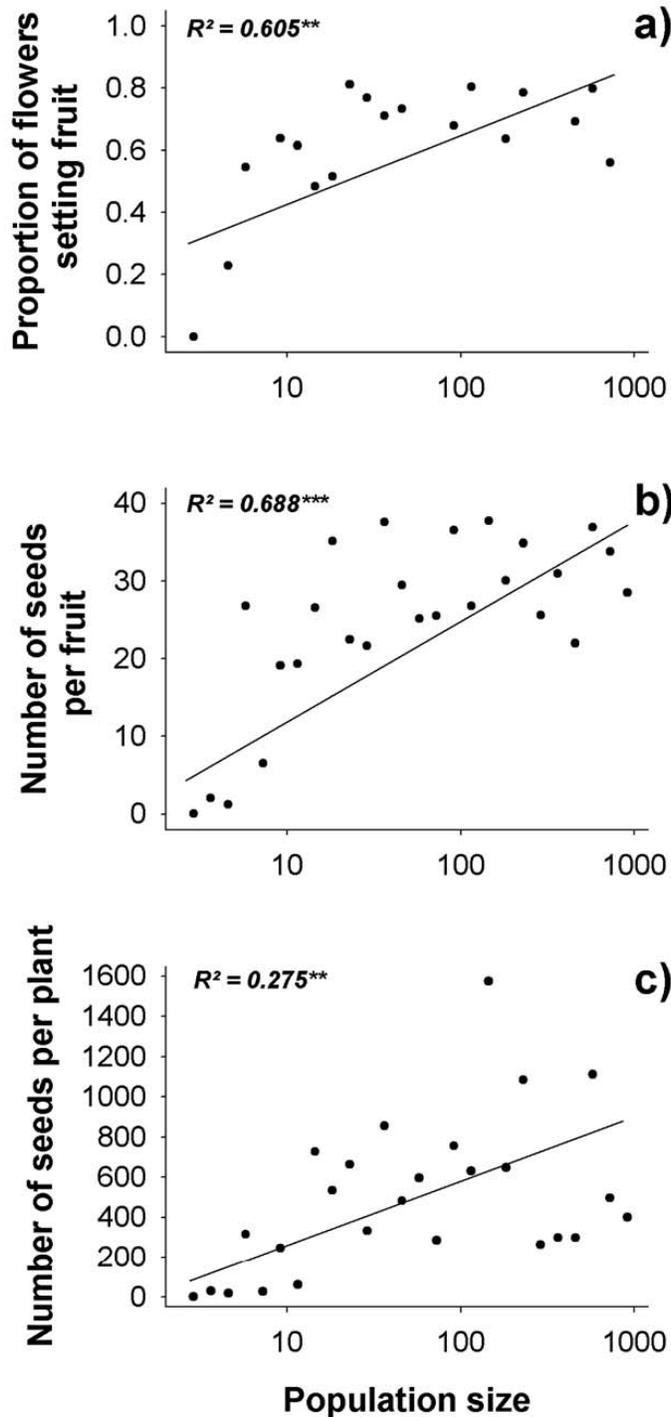

Fig. 4. Relationship between population size (number of flowering individuals) and (a) the proportion of flowers setting fruit, (b) mean number of seeds per fruit and (c) mean number of seeds per plant in *P. veris* (in 2000).
** $P < 0.01$; *** $P < 0.001$. Note the log-scale in the *X*-axis.

Hence, the observed variability in seed set may be one of the causal factors affecting recruitment rates and the observed types of population demographic structure. Finally, and however not investigated in this work, recruitment is not only a function of seed density, but of suitable micro-site availability too (Eriksson and Ehrlén, 1992). Indeed, ostermeijer et al. (1994) and Hegland et al. (2001) showed that presence of bare soil was critical for successful seedling recruitment in *Gentiana pneumonanthe* and *Salvia pratensis*. Therefore, further empirical research is needed to determine the extent to which recruitment into populations is likely to be limited by seed density or micro-site availability. Hence, we may conclude that the skewed distribution of *P. veris* populations to a large fraction of very small and senescent populations, probably as a result of reduced seed set, decreasing recruitment and higher mortality rates, makes them more vulnerable to size fluctuations and extinction due to environmental and demographic stochasticity (Holsinger, 2000).

5.1 Implications for conservation

The first and essential component of any conservation strategy is habitat preservation. However, in case of *P. veris*, this requirement is not fulfilled for many sites in the studied area due to the intensification of the agricultural land use of the surrounding matrix. Therefore, a stand still principle in which any further destruction of the remaining habitat patches with their populations is prevented, is one of the most pressing objectives for the long-term conservation of the remnant populations. On the other hand, preservation of habitat patches alone may not be enough to conserve small populations in the long-run, if their growth rates are below the level of population maintenance or increase. Consequently, one should intend measures to increase population growth rates by promoting recruitment and decreasing mortality rates. Since we found that plants, even in the smallest populations, are still able to produce a certain amount of seed yield, this suggest that germination and seedling development are still possible if gaps and optimal conditions for establishment are created. Similar to other grassland species (e.g. Klinkhamer and de Jong, 1988; Oostermeijer et al., 1998), seedling establishment of *P. veris* is positively influenced by a low and open vegetation cover (personal observation). Thus, all types of management leading to a more open vegetation structure, but ensuring the presence of at least some seed-producing individuals, will be favourable for the regeneration of the small "regressive" populations. Therefore, management practices creating a certain degree of disturbance, such as gradual grazing, mowing and cleaning ditches (after the species has shed its seeds), are suitable options for conserving *P. veris* populations. The strongly enhanced initial seedling establishment in small gaps (personal observation) suggests that disturbance, e.g. by grazing animals, could even be beneficial for populations at sites that are otherwise mown. Grazing animals could have the additional advantage of increasing seed dispersal (Ellenberg, 1996), especially within sites. In the smallest populations, a more intensive management option is small-scale sod-cutting in the vicinity of the remaining reproductive individuals. This may be a reasonable option to help those populations over a threshold level, if the environmental conditions fulfil the species" ecological requirements. At the same time, by promoting recruitment, the number of flowering plants will grow. This may restore in turn pollination processes as populations may become more conspicuous to pollinators.

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