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# Genetic Rescue in an Isolated Metapopulation of a Naturally Fragmented Plant Species, *Parnassia palustris*

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**Abstract:** *The loss of habitat of naturally fragmented species may result in isolated metapopulations: small groups of populations that are still connected by gene flow but have become isolated from other metapopulations. Genetic isolation may result in genetic differentiation between metapopulations and lowered genetic diversity within the metapopulation. Gene input from outside the metapopulation can hence be expected to enhance crosscompatibility and seed viability. Simultaneously, due to genetic drift, inbreeding, and pollen limitation in the small populations, fitness is likely to increase with population size within a metapopulation. I tested these hypotheses in three isolated metapopulations of the endangered plant species Parnassia palustris L. in a study area of 15 × 0.5 km along the coast of western Belgium and northwestern France. Seed and fruit weight, measured in the 27 populations distributed over the three metapopulations, significantly differed among the metapopulations, which suggests genetic differentiation. These fitness variables also increased with population size within two metapopulations. With a pollination experiment, I investigated whether genetic rescue with pollen from another metapopulation enhanced seed set. Outside-metapopulation crosses led to a significantly higher seed set than within-metapopulation crosses, and this effect was more pronounced in small populations. This means pollen from outside the metapopulation was more compatible than pollen from within the metapopulation, due to a lowered genetic diversity within the metapopulation. The seed set of naturally pollinated flowers was at least equal to that of hand-pollinated flowers, which can be explained by a compensation effect of pollen quantity and donor diversity for pollen quality. One can assume that the loss of genetic diversity at the level of the metapopulation affected loci not just related to cross compatibility. Reconnection of metapopulations by stepping stones may be necessary to prevent further genetic erosion and assure the viability of the Parnassia populations in the study area over the long term.*

**Keywords:** genetic drift, habitat fragmentation, plant fitness, pollen limitation, pollination, self-incompatibility

Rescate Genético en una Metapoblación Aislada de una Especie de Planta Fragmentada Naturalmente, *Parnassia palustris*

**Resumen:** *La pérdida del hábitat de especies fragmentadas naturalmente puede resultar en metapoblaciones aisladas: pequeños grupos de poblaciones que están conectadas mediante flujo de genes pero que están aislados de otras metapoblaciones. El aislamiento genético puede resultar en la diferenciación genética entre metapoblaciones y la reducción de la diversidad genética dentro de la población. Por lo tanto, se puede esperar que la llegada de genes del exterior de la población incremente la compatibilidad de las cruces y la viabilidad de semillas. Simultáneamente, debido a la deriva génica, la endogamia y la limitación de polen en las poblaciones pequeñas, es probable que la adaptabilidad incremente con el tamaño poblacional dentro de la metapoblación. Probé estas hipótesis en tres metapoblaciones aisladas de la especie de planta en peligro Parnassia palustris L. en un área de estudio de 15 × 0.5 km a lo largo de la costa de Bélgica occidental y Francia noroeste. El peso de las semillas y de frutos, medidas en las 27 poblaciones distribuidas en las tres*

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metapoblaciones, varió significativamente entre las metapoblaciones, lo que sugiere diferenciación genética. Estas variables de adaptabilidad también incrementaron con el tamaño poblacional en dos metapoblaciones. Mediante un experimento de polinización, investigué si el rescate genético con polen de otra metapoblación mejoraba el conjunto de semillas. Cruzas fuera de la metapoblación condujeron a un conjunto de semillas significativamente mayor que las cruas dentro de la metapoblación, y este efecto fue más pronunciado en poblaciones pequeñas. Esto significa que el polen proveniente del exterior de la metapoblación fue más compatible que el polen proveniente del interior de la metapoblación, debido a una diversidad genética disminuida. El conjunto de semillas de flores polinizadas naturalmente fue por lo menos igual al de las flores polinizadas manualmente, lo que puede explicarse por un efecto compensatorio entre la cantidad de polen y diversidad de donantes con la calidad del polen. Se puede asumir que la pérdida de diversidad genética a nivel de la metapoblación no solo afectó a loci relacionados con la compatibilidad. La reconexión de metapoblaciones mediante piedras de paso puede ser necesaria para prevenir mayor erosión genética y asegurar la viabilidad a largo plazo de las poblaciones *Parnassia* en el área de estudio

**Palabras Clave:** adaptabilidad de plantas, autoincompatibilidad, deriva génica, fragmentación de hábitat, limitación de polen, polinización

## Introduction

It has been demonstrated extensively in a wide range of habitats that small and isolated plant populations have a lower fitness and probability of survival compared with large populations (e.g., Jacquemyn et al. 2001; Kéry & Matthies 2004; Hensen & Oberprieler 2005). One cause of this Allee effect—reduced reproduction with decreasing population size (Stephens et al. 1999)—is genetic. Genetic drift results in a random loss of genetic diversity and an accumulation of deleterious mutations. Higher levels of inbreeding, caused by increased selfing and matings among closely related individuals, lead to an increased proportion of homozygotes so that recessive genetic load becomes phenotypically expressed, so-called inbreeding depression (e.g., Keller & Waller 2002; Culley & Grubb 2003; Pluess & Stocklin 2004). A lower within-population genetic diversity of alleles that determine pollen compatibility results in a lower availability of compatible pollen within the population (decreased pollen quality) (e.g., Byers 1995; Kéry et al. 2003; Kolb 2005). On the other hand, smaller, less dense, and more isolated populations experience fewer pollinator visitations and have lower pollen loads (decreased pollen quantity) (e.g., Kunin 1997; Wilcock & Neiland 2002; Waites & Ågren 2004).

Some plant species, such as *Parnassia palustris* L., occur in naturally fragmented habitats (Bonnin et al. 2002; Leimu & Mutikainen 2005), which means they exhibit adaptations to cope with population isolation. These adaptations may increase gene flow through large resource investments in pollen and seed dispersal or may be direct adaptations to low gene flow between populations (e.g., the development of self-compatibility) (Larson & Barrett 1998; Goodwillie 2001; Moeller & Geber 2005). During the last century, however, the naturally fragmented habitat of these species has undergone additional fragmentation as a result of human activities. This has resulted in isolated metapopulations: small groups

of naturally fragmented populations connected by seed and/or pollen flow that have no gene exchange between the groups because distance between the metapopulations is too high to allow systematic pollen or seed dispersal (Bonnin et al. 2002). Thus, isolated metapopulations genetically diverge from each other.

Simultaneously with genetic diversification among the metapopulations, genetic diversity within the metapopulation decreases and individuals of the same metapopulation may become less compatible with each other. This may result in decreased quality of the available pollen deposited on the stigma and, thus, reduced seed set. This lack of compatible pollen may be more pronounced in small than in large populations of the metapopulation, resulting in increasing reproduction with increasing population size within the metapopulation (Kolb 2005; Ward & Johnson 2005; Willi & Fischer 2005).

Several researchers have found that between-population crosses result in genetic rescue (Heschel & Paige 1995; Tallmon et al. 2004), expressed by higher seed set or offspring vigor than after within-population crosses (Byers 1998; Sheridan & Karowe 2000; Colling et al. 2004). Similarly, but at the metapopulation level, it can hence be expected that cross-pollination with pollen from another metapopulation will increase reproduction in comparison with crossings from the same metapopulation. On the other hand, when gene input from other populations breaks local adaptation or isostasis, offspring from cross-pollinations may have a lower fitness, so-called outbreeding depression (Newman & Tallmon 2001; Paland & Schmid 2003).

In three isolated metapopulations of *Parnassia palustris*, I investigated (1) differences in fitness between metapopulations and the relationship of fitness and population size within metapopulations and (2) whether outside-metapopulation crosses lead to genetic rescue expressed by a higher seed production and viability. I first tested whether there were systematic differences in

fitness among the three metapopulations, whether there was an increase of fitness with increasing population size within the metapopulation, and whether the relationship between population size and fitness differed among the metapopulations. Second, I executed a pollination experiment to examine whether gene input in the form of pollen from another metapopulation resulted in a higher seed set than pollination with pollen of individuals of the same metapopulation and whether the effect of pollen supplementation differed between small and large populations within the same metapopulation. Because the mating system of a species determines the sensitivity of a species to inbreeding depression (Leimu & Mutikainen 2005) and the response to decreased pollen quality (Aizen et al. 2002; Colling et al. 2004; Kolb 2005) and *Parnassia palustris* has a mixed mating system (Sandvik & Totland 2003), I also tested for the degree of self-compatibility of *Parnassia palustris* in the study area.

## Methods

### Study Area

The study area was a sandy dune area along the western Belgian and northwestern French coast, which has been extensively fragmented by human activities since the beginning of the twentieth century. The remaining dune areas are protected as nature reserves. In the dune landscape dune slacks are formed by wind erosion down to the level of the groundwater. These dune slacks, with a calcareous-rich and nutrient-poor soil, flood in winter and dry out in summer, and are the natural habitat for *Parnassia palustris* at the coast. Due to the natural fragmented nature of the dune slacks, which occur as islands in an ocean of dry dune habitat (Bossuyt et al. 2003; Bossuyt & Honnay 2006), *P. palustris* forms natural metapopulations. Nevertheless, due to increased human activity, these natural metapopulations have been further fragmented, resulting in isolated metapopulations.

In this study area there were 27 populations of *Parnassia palustris* within three metapopulations (Fig. 1). One metapopulation (Ter Yde, 260 ha) contained 10 populations, ranging in size from 3 to more than 10,000 individuals, and were considered strictly genetically isolated from the other metapopulations because they were separated by a densely built area of 10 km, where no individuals of *P. palustris* occurred. The other two metapopulations (Westhoek, 340 ha, and Perroquet, 225 ha) were separated by a road and a camping site at the border between Belgium and France, so there may have been some exchange of pollen, and seeds may have been dispersed by wind between the two populations. These two metapopulations contained 7 and 10 *P. palustris* populations, respectively, ranging in size between 7 and more than 10,000 individuals.

### Study Species

*Parnassia palustris* (Saxifragaceae) is a perennial herb with a circumboreal distribution (Bonnin et al. 2002; Borgen & Hultgård 2003). Each plant has basal leaves and 1–30 flowering stems, each with one terminal flower. The species is hermaphroditic and protandrous. The five stamens usually discharge their pollen before the stigma begins maturation. It is usually cross-pollinated and rarely autogamous (Martens 1936). Sandvik and Totland (2003) report that *P. palustris* populations in Sweden produce fruit after autogamous pollination, although with drastically reduced seed set. They concluded that the species is mainly outcrossing and strongly dependent on pollinators for optimum pollination and maximum seed set. *P. palustris* is insect pollinated, mainly by Diptera, particularly hoverflies (Syrphidae), but other insects contribute to pollen distribution (Sandvik & Totland 2003 and references therein). It flowers in August through September and fruits containing several hundreds of seeds ripen in September through October. The small, light seeds are dispersed by water and wind. *P. palustris* is considered endangered in northern France, Luxembourg, Belgium, and the Netherlands (Bonnin et al. 2002).

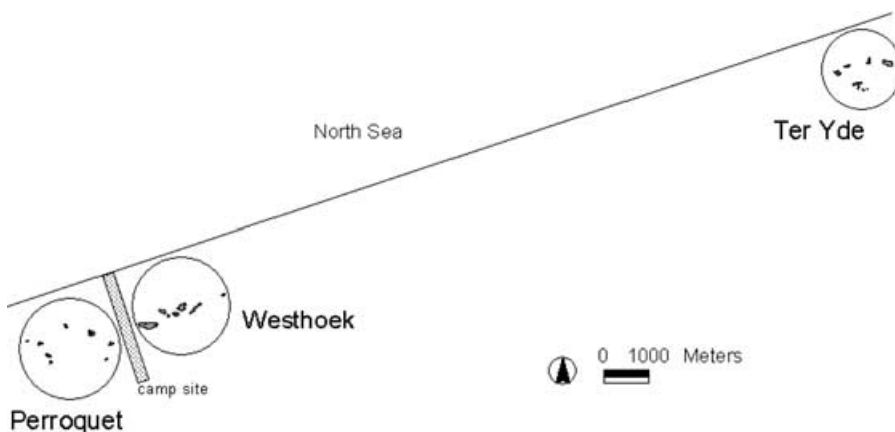


Figure 1. Map of the study area showing the populations of *Parnassia palustris* in the three metapopulations.

## Data Collection

I collected fitness data in the summer of 2004. First, I determined population size by counting the number of flowering individuals in each of the 27 populations. When the population was large, I counted the number of individuals in a subsample and extrapolated this value. In each population of <1000 individuals, I randomly selected 20 individuals. If there were <20 individuals in the population, I selected all individuals. If there were more than 1000 individuals, I selected 5% of the population. For each selected individual I counted the number of flower stems, measured the plant height, and collected ripe fruits. A total of 648 individuals were sampled. Fruits and the total amount of seeds within one fruit were weighed in the laboratory. Seed size was determined by measuring 15 randomly selected seeds of each fruit under a microscope and averaging these values per individual.

To test whether seed set increased with increasing pollen quantity (through hand pollination with pollen from the same metapopulation) or with increasing pollen quality (through hand pollination with pollen from outside the metapopulation), I executed a pollination experiment on two large (>1000 individuals) and four small (<100 individuals) populations in the isolated metapopulation in Ter Yde. In the two large populations I randomly selected 20 individuals. In the small populations I randomly selected 5 or 10 individuals, depending on population size. In August 2005 I marked four flowers of each selected individual. One flower was bagged before it opened to exclude pollinators (self-pollination), and one flower was left untouched (control). Two other flowers were emasculated and bagged before the five anthers could ripen. Four days later, when the pistil was ripe, I pollinated one of these two flowers with pollen from one randomly selected flower collected from Westhoek (outside-metapopulation pollination) and the other with pollen from one randomly selected flower from Ter Yde (within-metapopulation pollination). The pollen donors for the within-metapopulation pollination were selected both in large and small populations. If the individual had fewer than four flower stems, only one individual was emasculated and attributed randomly to one of the two hand-pollination treatments. To avoid an effect of systematic fitness differences between the two parents in the outside-metapopulation pollination and to test only the effect of increased pollen compatibility, I collected flowers from Westhoek because there were no differences in average fitness between the populations in Ter Yde and Westhoek (see below). Pollination was executed by carefully rubbing the flowers against each other, and pollination success was assured by visually checking the presence of pollen on the pistil. After hand pollination, the flowers were bagged again to avoid additional pollination by pollinators.

In September 2005 fruits were collected when they were ripe but not yet open. The weight of the fruit and

the total amount of seeds in the fruit were determined in the laboratory. For a random selection of 15 seeds per fruit, I measured seed and embryo size and determined the percentage of seeds without a viable embryo under a microscope. Seed and embryo size values were averaged per fruit.

## Data Analysis

I tested the effect of metapopulation and population size on plant fitness with a multilevel linear mixed model (Singer 1998) with Satterthwaite's (1946) corrections on the degrees of freedom. Population size, metapopulation, and their interaction were entered as fixed effects, an intercept was incorporated as random effect, and population was entered as subject. Dependent variables were consecutively the number of flower stems per plant, plant height, fruit weight, seed weight, and seed size. Population size was  $\log_{10}$  transformed and the number of flowers was square-root transformed before analysis to meet normality assumptions. Estimated marginal means were compared between the metapopulations with Bonferroni pairwise comparisons. Because the results indicated there was, at least for some fitness variables, a strong interaction between metapopulation and population size, I executed the mixed-model analysis for each metapopulation separately, incorporating population size as a fixed factor, intercept as a random factor, and population as a subject variable.

The effect of pollination treatment and the potential interaction with population size was also tested with a multilevel linear mixed model with Satterthwaite's corrections on the degrees of freedom. Fruit weight, seed weight, seed size, embryo size, and the percentage of empty seeds were dependent variables. Population size class (<100 vs. >1000), pollination treatment (self-pollination, within-metapopulation pollination, outside-metapopulation pollination, and control), and their interaction were entered as fixed factors. Intercept was entered as a random factor and population as a subject. The percentage of empty seeds was transformed to the arc-sin square root to meet normality assumptions. Differences in estimated marginal means between the treatments were assessed with Bonferroni pairwise comparisons. We used SPSS 11.0 (SPSS, Chicago, Illinois) for all data analyses.

## Results

Fruit weight, seed weight, and seed size significantly increased with increasing population size over the three metapopulations (Table 1). Nevertheless, there was a significant interaction between population size and metapopulation for fruit and seed weight, indicating that the relationship between population size and fitness differed between the metapopulations. The number of

**Table 1.** Results of the mixed-model analyses,<sup>a</sup> which tested for the effects of population size and metapopulation and their interaction for the whole data set and for the effect of population size for each metapopulation separately on fitness characteristics of *Parnassia palustris*.<sup>b</sup>

Fitness characteristic	Population size <sup>c</sup>			Interaction with metapopulation		
	df (num./denom.)	F	p	df (num./denom.)	F	p
Total data set						
fruit weight	1/20.6	8.23	0.009	2/20.6	3.92	0.036
seed weight	1/21.1	8.72	0.008	2/21.2	4.41	0.025
seed size	1/18.2	4.63	0.045			
Ter Yde						
weight	1/7.42	4.50	0.069			
seed size	1/6.60	5.01	0.063			
Perroquet						
weight	1/9.67	7.79	0.020			
seed weight	1/9.49	7.74	0.020			

<sup>a</sup>For details on the mixed models, see text.

<sup>b</sup>Only at least marginally significant effects ( $p < 0.10$ ) are shown.

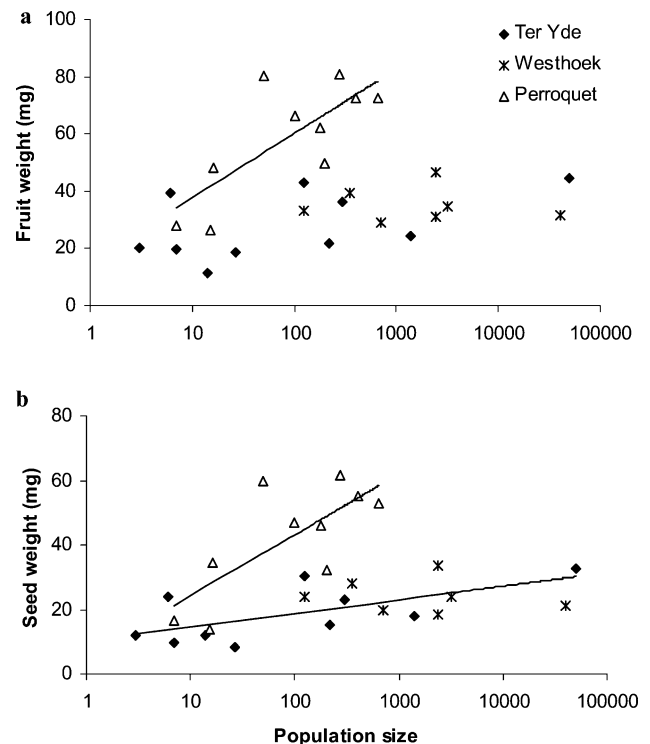
<sup>c</sup>Log<sub>10</sub> transformed.

flowers and plant height were not related to population size ( $F = 0.17$  and  $0.34$ ,  $p = 0.69$  and  $0.47$ , respectively), but plant height was the only variable that significantly differed among the metapopulations ( $F = 3.93$ ,  $p = 0.035$  for plant height,  $F < 1$ ,  $p > 0.50$  for all other variables).

When the analysis was executed within each metapopulation separately, fruit and seed weight increased significantly with population size for Perroquet, and there was a marginally significant increase of seed weight and size with population size for Ter Yde (Table 1, Figs. 2a–b). The slope of the relationship was higher for the Perroquet than for Ter Yde (Figs. 2a–2b), as indicated by the significant interaction term (Table 1). This resulted in a higher fruit and seed weight in populations of medium size (100–1000 individuals) in Perroquet than in the populations of equal size in the two other metapopulations (Figs. 2a–b). Overall, fruit weight, seed weight, and seed size were significantly larger in Perroquet than in the other two populations (simple effect  $p < 0.001$  for fruit and seed weight and  $p = 0.015$  for seed size). I did not find a significant relationship among the three remaining fitness variables and population size in Perroquet ( $F = 0.17$ ,  $2.20$ , and  $3.19$ ;  $p = 0.69$ ,  $0.17$ , and  $0.11$  for number of flowers, plant height, and seed size, respectively) and Ter Yde ( $F = 0.15$ ,  $0.10$ , and  $2.60$ ;  $p = 0.71$ ,  $0.76$ , and  $0.15$  for number of flowers, plant height, and fruit weight, respectively). In Westhoek, none of the fitness variables were related to population size (in all cases  $F < 1$ ,  $p > 0.3$ ).

Pollination treatment had a strong effect on all fruit and seed characteristics (Table 2). Overall, fruit weight, seed weight, seed size, and embryo size were higher and the percentage of empty seeds was the lowest for the outside-metapopulation treatment compared with the within-metapopulation treatment (Table 3). Self-pollination resulted in a significantly lower seed weight and size and a higher percentage of empty seeds than outside-metapopulation crossing and natural pollination. Seed set

after natural pollination was for all variables as high as after outside-metapopulation pollination. Population size class was not significant for any of the variables ( $F = 1.4$ ,  $3.8$ ,  $3.2$ ,  $0.7$ , and  $13.4$ ;  $p = 0.31$ ,  $0.15$ ,  $0.17$ ,  $0.46$ , and  $0.13$



**Figure 2.** Population average for (a) fruit weight and (b) seed weight as a function of population size for the three metapopulations of *Parnassia palustris*: Ter Yde ( $n = 10$ ), metapopulation with high degree of isolation; Westhoek ( $n = 7$ ) and Perroquet ( $n = 10$ ), metapopulations with low degree of isolation. A trend line is fitted for the significant relationships (see Table 1).

**Table 2.** Results of the mixed-model analysis\* of the effect of pollination treatment, population size class (< and > than 100 individuals), and their interaction on fruit and seed characteristics of *Parnassia palustris*.

Seed characteristic	df (num./denom.)	F	p
Fruit weight			
treatment	3/237.9	28.05	<0.0001
interaction with population size	3/237.9	6.3	<0.0001
Seed weight			
treatment	3/237.6	33.4	<0.0001
interaction with population size	3/237.6	5.8	0.001
Seed size			
treatment	3/242.5	37.1	<0.0001
interaction with population size	3/242.5	2.7	0.049
Embryo size			
treatment	3/227.8	10.5	<0.0001
Percentage of empty seeds			
treatment	3/237.1	33.5	<0.0001

\*For details on the mixed model, see text. Only significant results are shown.

for fruit weight, seed weight, seed size, embryo size, and percentage of empty seeds, respectively).

There was, however, a significant interaction effect of treatment and population size for fruit weight, seed weight, and seed size (Table 2). In large populations fruit weight, seed weight, and seed size after both hand-pollination treatments were smaller than for the control flowers, whereas this was not the case in small populations (Figs. 3a–c). As a result, control flowers had a higher reproduction in large than in small populations, whereas there were no differences in reproduction between large and small populations for the hand-pollinated treatments. For embryo size and percentage of empty seeds, the interaction between treatment and population size was not significant ( $F = 1.8$  and  $0.1$ ,  $p = 0.14$  and  $0.93$ , respectively).

## Discussion

### Relationship between Fitness and Population Size

There were large differences in fitness between the metapopulations, which accounted for most of the variation (Table 1). Plant fitness in Ter Yde and Westhoek was, for populations of a similar size, lower than in Le Perroquet (Fig. 2). Because the distance between Ter Yde and the other regions was >10 km (Fig. 1), it is reasonable to assume that gene flow by seed or pollen from other metapopulations does not occur (Bonnin et al. 2002), which may result in decreased genetic diversity and heterozygosity within the metapopulation. The results of the pollination experiment, with outside-metapopulation crosses resulting in a higher reproduction capacity than within-metapopulation crosses (Table 3, Figs. 3a–c), indeed indicated genetic differentiation between the metapopulations. Consequently, the lower fitness in Ter Yde may at least partly be attributed to genetic causes, although the contribution of other factors (e.g., differences in habitat conditions) cannot be excluded.

In contrast, individuals from Westhoek are more likely to receive regular pollen or seed flow from populations in Le Perroquet, which had on average a much higher fitness (Fig. 2). Instead, the lower fitness in Westhoek could be due to decreased habitat quality or recent changes in population size rather than to genetic isolation. Most dune slacks in Westhoek are grazed to prevent succession, whereas the dune slacks in Le Perroquet are mowed. I observed that grazing had a negative effect on fitness of *Parnassia* in the study area. On the other hand, dune slack habitat in Westhoek has increased in the last 10 years as a result of restoration projects. Consequently, most *Parnassia* populations increased in size very recently so that their lower fitness in Westhoek may be due to recent genetic bottlenecks. This is confirmed by the absence of a relationship between plant fitness and population size within this metapopulation, which may reflect different unknown histories of populations that are not correlated

**Table 3.** Estimated marginal means of the seed characteristics of *Parnassia palustris* for the four treatments.\*

Seed characteristic	Treatment			
	self-pollination (n = 66)	within metapopulation (n = 59)	outside metapopulation (n = 60)	control (n = 70)
Fruit weight (mg)	23a	32b	40c	46c
Seed weight (mg)	9a	15a	22b	29c
Seed size (mm)	6.2a	6.9a	8.5b	9.2b
Embryo size (mm)	4.3a	4.2a	5.1b	5.1b
Empty seeds (%)	64a	48b	28c	19c

\*The  $p$  of the test of simple effect was <0.0001 for all seed characteristics. Significant differences in Bonferroni pairwise comparisons are indicated with different letters.

with current population sizes (Bonnin et al. 2002; Keller & Waller 2002; Leimu & Mutikainen 2005).

Within Ter Yde and Le Perroquet there was an increased fitness in large populations, in particular concerning the reproductive fitness characteristics of fruit weight and seed weight, compared with small populations (Figs. 2a–b). For several species, reproduction increases with increasing population size (e.g., Jacquemyn et al. 2001; Aguilar & Galetto 2004; Hensen et al. 2005). This is attributed to a higher level of genetic diversity, including heterozygosity, and/or a higher pollination success rate due to higher pollen quantity or quality in large populations.

### Genetic Rescue after Outside-Metapopulation Crosses

In the pollination experiment self-pollination resulted in significantly lower seed production; between-metapopulation crossings led to a higher seed production than within-metapopulation crossings; control, open-pollinated flowers had higher seed production than hand-pollinated flowers; and the effect of pollen supplementation was more pronounced in small than in large populations (Tables 2 & 3, Figs. 3a–c). A significantly lower seed production after self-pollination indicated that there was at least some degree of self-incompatibility. Other researchers investigating the breeding system of *Parnassia* found the species to be self-compatible (Bonnin et al. 2002) or concluded, similar to our conclusions, that there is some, but strongly reduced, seed production after self-pollination (Sandvik & Totland 2003).

Flowers pollinated with pollen from outside the metapopulation—the genetic rescue treatment—produced a higher amount of and larger and more viable seeds than flowers pollinated with pollen from inside the metapopulation (Table 3, Figs. 3a–c). Because populations of pollen donor plants had on average no higher fitness than populations in the metapopulation where the pollination experiment was executed, a systematic parental effect of the pollen donor can be excluded. This indicates that a lack of compatible pollen was responsible for the lower seed production after the within-metapopulation treatment and that genetic rescue with pollen from outside the metapopulation restored cross-compatibility. Although some researchers have found no differences (Paschke et al. 2002; Kolb 2005), most concluded that between-population crosses increase reproduction or offspring vigor in comparison to within-population crosses (Byers 1998; Sheridan & Karowe 2000; Colling et al. 2004; Willi & Fischer 2005), which they attribute to the input of compatible alleles. Similarly, gene flow from outside an isolated metapopulation is not likely to occur frequently, and the resulting lower genetic diversity within the metapopulation may reduce cross-compatibility.

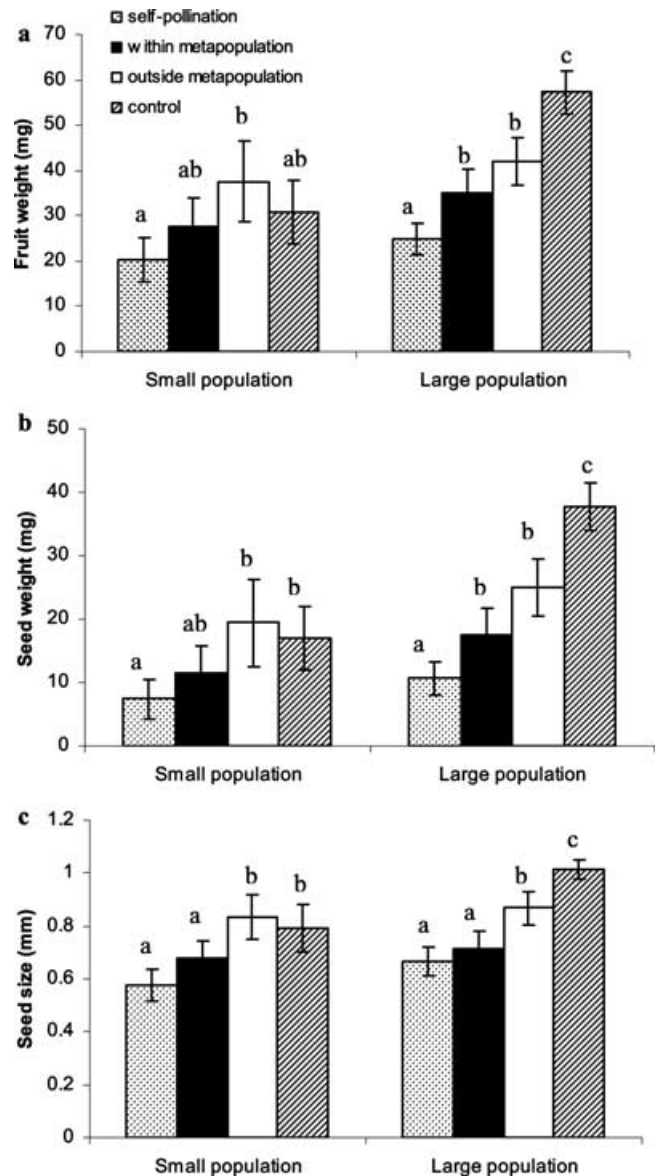


Figure 3. Average (a) fruit weight, (b) seed weight, and (c) seed size after each of the four treatments for small populations (<100 individuals,  $n = 30$ ) and large populations (>1000 individuals,  $n = 40$ ) of *Parnassia palustris*. Error bars indicate 95% confidence interval of the subsample. Different letters indicate significant differences between the treatments within each population class.

The lower seed production and fewer viable seeds after within-metapopulation pollination also indicated that pollination with pollen from another population within the metapopulation did not increase pollen quality. This means there may be less genetic differentiation between the populations within the metapopulation than between the metapopulations because gene flow between the populations is more likely. Control flowers had a similar seed weight and size than hand-pollinated flowers, in

particular in the large populations, despite the fact that natural pollinations of control flowers likely deposited pollen that was primarily from inside the population. This indicates that fruit set in the populations of *Parnassia palustris* was not pollen limited. Most supplemental hand-pollination experiments show an increase in seed production after hand pollination and conclude that reproduction of the studied populations is hampered by decreased ovule fertilization due to restricted pollinator activity or a lack of compatible pollen (Brown & Kephart 1999; Colling et al. 2004; Waites & Ågren 2004 but see Paschke et al. 2002; Kolb 2005; Ward & Johnson 2005).

Besides pragmatic explanations (a negative effect of bagging on seed production due to an altered microclimate [Paschke et al. 2002]) or the risk of pollen clogging on the stigma due to the less gradual pollen release when hand-pollinating flowers [Ashman et al. 2004], the quantity of pollen supplied by hand pollination might be too low to allow full seed set and/or pollen competition, despite a visual control of pollen load. When there are no possibilities for pollen competition, selection for maximal fitness within the time frame between pollen deposition and fertilization is hampered, which may result in a lower seed set or less viable seeds (Winson et al. 2000). Moreover, in the hand pollination I added pollen of only one pollen donor, whereas natural pollination probably occurs from several visits of pollinators carrying pollen from many pollen donors (Kolb 2005), and the latter has been found to have a positive effect on reproductive success (Niesenbaum 1999; Paschke et al. 2002). This is related to a higher probability of receiving compatible pollen and to differences in pollen-tube growth rates, which allows pollen competition (Brown & Kephart 1999). My results indicate that although natural pollination deposits pollen of lower quality of the same (meta)population, this may be compensated for by a higher pollen quantity and donor diversity.

The effect of pollen supplementation was more pronounced in small populations compared with large populations (Table 2, Figs. 3a–c). Some researchers have found no interaction between pollination limitation and population size (Paschke et al. 2002), whereas others (Ågren 1996; Waites & Ågren 2004; Kolb 2005; Ward & Johnson 2005; Willi & Fischer 2005; Willi et al. 2005) conclude that the degree of pollen limitation decreases with increasing population size. I found that seed production of open-pollinated flowers was higher in large populations. Because seed production of flowers receiving supplemental pollination did not differ between small and large populations, individuals in small populations were not systematically more resource limited and the lower seed production of naturally pollinated flowers can be attributed to pollen limitation caused by a lower rate of pollinator visitation (pollen quantity) or a lack of compatible pollen (pollen quality) (Ashman et al. 2004).

Small populations with a low population density are less attractive to pollinators, so flowers are less visited by pollinators and visits occur more within the same individual (Kunin 1997; Roll et al. 1997; Ashman et al. 2004; Kircher et al. 2005), resulting in lower pollen quantity and quality in open-pollinated flowers in small populations, compared with large populations. Sandvik and Totland (2003) found pollen limitation in alpine *Parnassia palustris* populations but not in lowland populations, which they attributed to differences in pollinator abundance. A lower pollinator visitation rate in small and/or less-dense populations occurs in other species (e.g., Kunin 1997; Bosch & Waser 1999; Hendrix & Kyhl 2000) and results in a lower pollen load (Groom 1998; Aguilar & Galetto 2004; Waites & Ågren 2004), so there may not be sufficient pollen available to fertilize all ovules in control flowers in small populations (Kircher et al. 2005) and/or to allow pollen competition (Winson et al. 2000; Kolb 2005).

Pollinators preferentially move between two neighboring flowers, so they mainly carry pollen from flowers of the same population (Colling et al. 2004). Because there is a low genetic diversity of alleles in a small population for purely numerical reasons, this results in both less compatible pollen and a higher inbreeding level within small populations, which in turn lowers genetic diversity. Thus, a feedback loop is generated. Lower pollen quality may also have been responsible for the lower seed production in the control flowers and the larger effect of pollen supplementation in small populations (Willi et al. 2005; Willi & Fischer 2005).

## Conclusions

One can assume that genetic isolation of the metapopulation also results in loss of alleles on other loci not directly related to cross-compatibility. Reconnection of the metapopulations by creating stepping stones of suitable habitat for *Parnassia* in between may enhance gene exchange between the metapopulations, prevent further genetic erosion, and lead to an increased viability of *Parnassia palustris* populations in the study area over the long term. Because rare alleles brought in by genetic rescue have a strong comparative advantage, they will spread rapidly over the metapopulation (Willi & Fischer 2005). On the other hand, further research on offspring fitness after outside-metapopulation crossing is necessary to determine whether reduced offspring fitness due to outbreeding depression will not neutralize the positive effect of genetic rescue on reproduction.

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