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Effects of population size on plant reproduction and pollinator abundance in a specialized pollination system

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Summary

1. Many plant species are currently experiencing negative consequences of habitat fragmentation as a result of reductions in population size and disruptions in pollination services. Plants in specialized pollination systems might be especially vulnerable to changes in plant population size and density resulting from land-use changes.

2. Representing such a system, we chose the globeflower *Trollius europaeus* L. with its pollinating fly *Chiastocheta*, which also acts as a seed predator at its larval stage, to investigate the effects of small plant population size on reproductive success and pollinator abundance.

3. Reproductive output of *T. europaeus* declined with increasing plant population size, while *Chiastocheta* abundance within *T. europaeus* flowers was independent of plant population size. However, at the local level, *Chiastocheta* numbers within flowers were inversely correlated to local *T. europaeus* flower density. We further found that increasing floral densities increased plant reproductive success at the population level.

4. *Chiastocheta* abundance was the main driver of reproductive output of *T. europaeus* through its dual role as an obligate pollinator and seed predator: at least some *Chiastocheta* flies were needed to secure pollination, but a continued increase in *Chiastocheta* flies within a flower incurred seed predation costs that greatly reduced reproductive success. Thus, high local flower density contributed positively to per capita reproductive output by diluting *Chiastocheta* abundance within flowers independently of overall population size.

5. *Synthesis.* Our findings highlight that plant population size is not always the main determinant of reproductive success for populations, but that other factors such as plant density and the specific ecology of a pollinator and its interplay with other population parameters can be more important in determining the fate of a population. Furthermore, the effects of plant population size and floral density on pollinator visitation in *T. europaeus* vary across scales, with implications for plant fitness. It is therefore important not to focus solely on pure plant population size in determining population viability. Thus, from a conservation perspective, even small and isolated *T. europaeus* populations may be viable and resistant to pollination-associated vulnerabilities depending on plant density at local (subpopulation) scales.

Key-words: *Chiastocheta*, habitat fragmentation, obligate mutualism, pollination, population size, reproductive ecology, reproductive success, *Trollius europaeus*

Introduction

During the last century human activities have greatly changed Earth's ecosystems and landscapes. In consequence, regional and local plant extinctions have been caused by direct habitat loss and by altered ecosystem processes within and across

recently fragmented, small populations (Drayton & Primack 1996; Fischer & Stocklin 1997; Thompson & Jones 1999; Stehlik *et al.* 2007). Mechanisms driving these changes are diverse. Habitat fragmentation directly reduces plant population size and increases population isolation, which makes plant populations more susceptible to environmental stochasticity (Kery, Matthies & Spillmann 2000; Hobbs & Yates 2003) and disrupts ecological processes that underlie fecundity and

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recruitment (Fischer & Matthies 1998; Ghazoul 2005; Leimu *et al.* 2006; Bowman *et al.* 2008; Schleuning *et al.* 2009). Specifically, negative effects of habitat fragmentation on plant–pollinator interactions have been widely documented (Aizen & Feinsinger 2003; Harris & Johnson 2004; Ghazoul 2005; Aguilar *et al.* 2006). Such negative effects may arise from disruptions affecting either the pollinator assemblage or pollinator behaviour (Steffan-Dewenter & Tschardt 1999; Harris & Johnson 2004; Steffan-Dewenter & Westphal 2008; Gonzalez-Varo, Arroyo & Aparicio 2009; Jakobsson, Lazaro & Totland 2009), both of which might alter the quantity and quality of pollination events, thereby decreasing plant fitness (Goverde *et al.* 2002; Peterson, Bartish & Peterson 2008).

Further factors influencing pollination rates are population and subpopulation plant densities. High densities might enhance pollination by attracting larger numbers of pollinators (Bernhardt, Mitchell & Michaels 2008; Hegland, Grytnes & Totland 2009). On the other hand, high flower densities might increase competition for a limited number of pollinators, hence reduce per capita pollination rates and reproductive success (Campbell & Husband 2007; Gunton & Kunin 2009; Spigler & Chang 2009). The outcome of plant–pollinator interactions with respect to plant density, population size and population isolation may vary across spatial scales depending on the scale at which particular pollinators respond to floral resources (Gunton & Kunin 2009; Dauber *et al.* 2010).

Plants that have highly specialized and obligate interactions with their pollinators are thought to be most susceptible to habitat fragmentation effects (Bond 1994; Johnson & Steiner 2000; Ghazoul 2005), as any impact on the pollinator would directly affect the plant and *vice versa*. Although specialized plant–pollinator systems are relatively rare (Waser *et al.* 1996), they may nevertheless provide insight and early warnings of wider changes at the community level. For these reasons, this study focussed on globeflower (*Trollius europaeus*) and its pollinating flies *Chiastocheta* spp. (Diptera: Anthomyiidae), an example of a specialized pollination mutualism that could be reproductively affected by habitat fragmentation, which has negatively affected population levels of *T. europaeus* across much of its European range. While still common in many alpine areas of Switzerland, *T. europaeus* has experienced substantial reductions in the Swiss lowlands due to drainage and agricultural intensification.

The tightly closed, globose flower of *T. europaeus* effectively excludes access to the inner flower for insects other than *Chiastocheta*, rendering the plant almost entirely dependent on up to six *Chiastocheta* species for pollination (Pellmyr 1989; Jaeger & Després 1998). The flies forage, mate, shelter and oviposit within the globe-shaped flower, effecting pollination in the process. Their larvae feed and develop on the ripening seeds during fruit maturation (3–4 weeks) and then, after completion of larval development, fall to the ground where they pupate and overwinter to emerge the following spring (Pellmyr 1989, 1992; Jaeger & Després 1998).

This double role of *Chiastocheta* as the main pollinator during its adult stage and seed predator during larval development adds further complexity to the reproductive success of

T. europaeus in terms of possible trade-offs between pollination services and predation costs.

We thus hypothesized that (i) *Chiastocheta* abundance increases with *T. europaeus* population sizes and that (ii) reproductive success is positively related to *T. europaeus* population size. Specifically, we seek to (i) determine the effects of plant population size and local plant density on *Chiastocheta* fly abundance in *T. europaeus* flowers, (ii) determine the reproductive output of *T. europaeus* across a range of plant population sizes, and (iii) to investigate the reproductive success of *T. europaeus* in the absence of *Chiastocheta* flies.

Material and methods

STUDY SPECIES

Trollius europaeus L. (Ranunculaceae) is a perennial, arctic-alpine, hermaphroditic plant, distributed throughout Europe, Asia and North America, occurring in moist habitats throughout northern and central Europe (Lauber & Wagner 2001). It usually has one yellow flower per stem (Doroszewska 1974), with sometimes two or more flowers branching off a stem. Flowering commences simultaneously within a population (Pellmyr 1989; Jaeger & Després 1998; Lauber & Wagner 2001). The self-incompatible flowers have c. 12 ovules per carpel and a mean lifespan of 7 days (Pellmyr 1989; Jaeger & Després 1998; Jaeger, Pompanon & Després 2001). All seventeen species of *Chiastocheta* (Anthomyiidae) flies, distributed throughout Europe and Asia (Pellmyr 1992), are associated with flowers of the genus *Trollius* (Collin 1954; Pellmyr 1992). Pollination systems vary from facultative to obligate, depending on the *Trollius* species (Després *et al.* 2002). In Europe, six species (*C. inermella*, *C. dentifera*, *C. macrophyga*, *C. setifera*, *C. rotundiventris* and *C. trollii*) have been described, and all are associated obligately with *T. europaeus* (Jaeger & Després 1998).

DATA COLLECTION

From 2006 to 2008, 19 *T. europaeus* populations were studied in northeast Switzerland. Eight populations were sampled over three consecutive years (2006, 2007, 2008), and an additional 11 populations in 2007 and 2008. Elevations ranged from 537 to 1250 m a.s.l. Seventeen populations were situated in the greater area around Lake Zurich, while two populations were situated further away (Fig. 1). All but one of the populations were located in small nature protection areas, in regions which have been subject to considerable changes in land use over the last century. It is therefore very likely that these populations are a subset of populations that once occurred in the region. To determine plant population size, exhaustive counts of all *T. europaeus* flowers were done in the ten smallest populations at the end of the flowering season (July–August). For the nine larger populations the population size was extrapolated based on the number of flowers within 70–74 randomly selected 5-m radius plots recorded in 2007 and 2008 (Table 1). Numbers of flowers were converted to density as flowers m⁻² for analysis. Population-level plant density as flowers m⁻² was calculated by dividing population size (total number of flowers) by the population area.

POLLINATOR ABUNDANCE

To estimate the abundance of the pollinating flies in a population we recorded the number of *Chiastocheta* in all flowers for the ten small

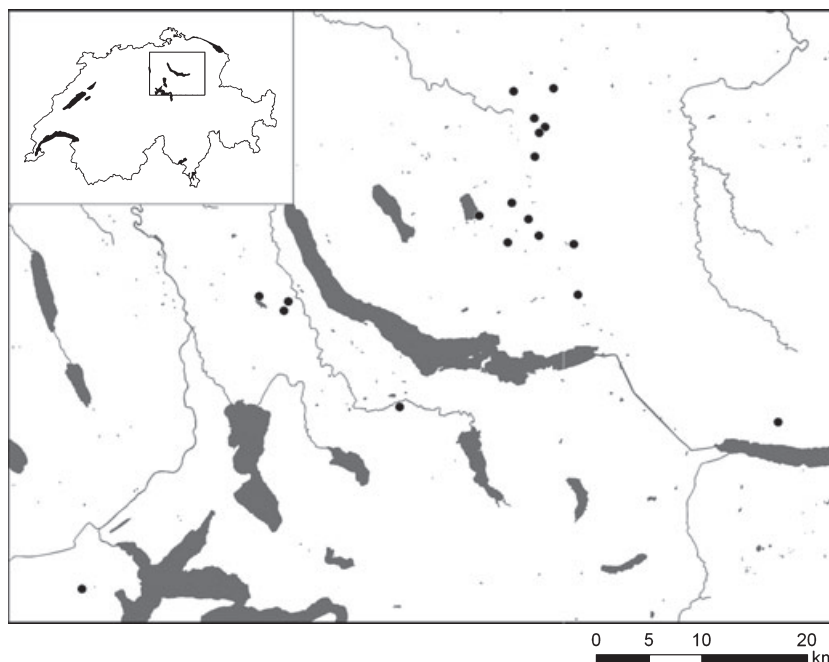


Fig. 1. Location of the 19 *Trollius europaeus* populations in Switzerland. The grey areas are lakes; the largest lake in the centre is Lake Zurich. Reproduced with the permission of swisstopo (JA100120).

Table 1. Characteristics of the 19 *Trollius europaeus* populations sampled in this study

Population	Elevation	Population area	No. of sampling areas*	Area sampled [m ²]	% of population sampled	Total no. flowers	Density (flowers m ⁻²)
Kafer	740	940	NA	935	100	140	0.15
B11	678	720	NA	718	100	230	0.32
F02	745	1290	NA	1291	100	230	0.18
F15	712	250	NA	250	100	310	1.24
F11	612	80	NA	52	100	800	10.00
B15	656	7570	NA	7567	100	840	0.11
F25_27	612	650	NA	651	100	1120	1.72
F13	764	950	NA	950	100	1140	1.20
F24	732	1370	NA	1374	100	1920	1.40
F01	639	750	NA	750	100	3930	5.24
F30	771	1820	NA	1822	100	4260	2.34
F07	852	4440	70	2198	49.50	12 110	2.73
F23	703	11 520	70	2198	19.08	24 350	2.11
F29	800	6810	70	2198	32.28	27 120	3.98
F34	597	15 650	70	2198	14.04	43 360	2.77
B03	537	32 260	70	2198	6.81	57 780	1.79
F41	752	17 520	71	2229	12.72	86 460	4.93
Kriens	1050	34 500	74	2324	6.74	238 040	6.90
Arvenbuel	1250	56 840	70	2198	3.87	820 690	14.44

*Each sampling area is 31.42 m².

populations, and in at least 200 flowers for large populations. Counts were done once during the flowering season in 2006 and 2007, and twice in 2008. For later inclusion in the analysis of reproductive ability we calculated fly abundance per flower in each population for each year, using the mean across all sampled flowers. In 2007 and 2008, we also determined *Chiastocheta* abundance in relation to local *T. europaeus* density by scoring *Chiastocheta* numbers in a focal plant together with the number of *T. europaeus* flowers within a 5-m radius of that plant. In the smallest population, which covered an area of 80 m², we were only able to sample six focal flowers in this way, while avoiding overlapping 5-m radius circles. At other populations

we used a larger focal flower sample size of up to a maximum of 51 flowers per population.

REPRODUCTIVE SUCCESS AND SEED PREDATION IN NATURAL POPULATIONS

Reproductive success and seed predation were quantified by counting the number of carpels, intact seeds and seeds with signs of predation in developed seed heads. For each population 35 seed heads were bagged after flowering with fine mesh during each season and collected after seed maturation. As a measure of reproductive success

and seed predation the proportion of seeds (intact or predated) was calculated by dividing the number of seeds by the maximum number of seeds that could have been produced by one flower. To obtain the maximum number of seeds, we multiplied the number of carpels per seed head by the mean number of ovules per carpel. We justify this on account of the highly variable number of carpels across globe-flowers (range 4–78 from 1575 flower heads) which contrasts with the marked consistency of ovules per carpel across flowers and populations (mean of 11.5 ± 1.5 , range 5–18, based on a sample of five carpels per seed head from 10 individuals each drawn from five populations; no significant differences between populations). These values are almost identical to those obtained by Jaeger & Després (1998) and Després *et al.* (2002): range of 10–69 carpels sampled across 1710 flower heads, with mean 11.6 ± 1.6 ovules per carpel.

REPRODUCTIVE SUCCESS AND SEED PREDATION IN THE ABSENCE OF *CHIASTOCHETA*

To account for possible pollination of *T. europaeus* by other insects in the absence of *Chiastocheta*, we placed groups of around 20 *T. europaeus* plants bought from nurseries in locations with no naturally occurring populations in the vicinity that might act as *Chiastocheta* sources. In 2007 plants were placed at six locations, in 2008 at two locations. To ensure that no *Chiastocheta* flies were present, plants were checked regularly during the flowering season. Seed heads were bagged and numbers of carpels and seeds were counted as in the natural populations.

To verify self incompatibility of *T. europaeus*, plants reared from seeds collected from natural populations were kept in a greenhouse. A total of 89 plants were used to test for selfing. Developing flower buds were bagged with fine mesh to prevent insect access to flowers. Of the 89 plants, 28 were tested for apomixis by removing the developing anthers, 30 flowers received no treatment, and 31 flowers were self-pollinated by hand.

STATISTICAL DATA ANALYSIS

All statistical analyses were carried out in R, version 2.10.1 (R Development Core Team, 2009). All data sets were analysed with generalized linear mixed-effects models (GLMM) to allow for random effects caused by the repetition of years and population, fitting them as populations nested in years in the random-effect term, following the advice in Bolker *et al.* (2008) and O'Hara (2009). We applied the lmer function from the lme4 package (Bates 2005); fixed effects showing strong outliers were log-transformed before fitting the model and those showing a pairwise correlation coefficient above 0.8 were centred (Zuur *et al.* 2009). Data on pollinator abundance was analysed with a Poisson error family. On the population level fixed explanatory variables were plant population size (log-transformed) and population-level plant density. Data on fly abundance in relation to plant densities in a 5-m radius were analysed separately, with plant density at the 5-m scale being log-transformed. Data sets on reproductive success and seed predation were analysed using a binomial error family to compare proportions. The fixed explanatory variables were plant population size (log-transformed), population-level plant density, and fly abundance. We also included two interaction terms in the model, average fly abundance : plant population density and average fly abundance : plant population size. Using the Akaike Information Criterion (AIC), model reduction and selection was tested based on significant differences (chi-square test) between models, selecting the model with the lowest AIC. To analyse whether *Chiastocheta*-free flowers produced significantly different proportions of intact or

predated seed than plants in natural populations, a binomial test to compare proportions was carried out (Crawley 2007).

Results

The 19 populations measured in this study varied greatly in plant population size (Table 1). The smallest population produced a total of 140 flowers during the recorded flowering season, the largest population had an estimated 820 690 flowers. Average plant density for each population ranged from 0.11 to 14.44 plants m^{-2} (Table 1).

POLLINATOR ABUNDANCE

Estimates of the abundance of *Chiastocheta* spp. showed that flies were present in all years and populations except 2006, when no *Chiastocheta* flies were recorded in one population (F30). Fly numbers differed between years (Kruskal–Wallis test: $\chi^2 = 570$, d.f. = 2, $P < 0.001$) and populations (Kruskal–Wallis test: $\chi^2 = 489$, d.f. = 18, $P < 0.001$). In 2006, no *Chiastocheta* were found in 92.5% of the 2042 flowers sampled, with a maximum of four flies found in the rest; in 2007, from a total of 3684 flowers 75.6% lacked *Chiastocheta*, with a maximum of six flies per flower; and in 2008, 89.6% of 11 141 flowers lacked *Chiastocheta*, with a maximum of eight flies per flower. Neither plant population size nor population-level plant density had a significant effect on the number of *Chiastocheta* found per flower (Table 2a).

Chiastocheta abundance in relation to the 5-m scale *T. europaeus* density (Fig. 2) decreased with increasing flower density around the focal plant ($P = 0.0003$, Table 2b), which varied between 0 and 17.88 flowers m^{-2} .

Table 2. *Chiastocheta* abundance per flower in relation to a) population size and density of *Trollius europaeus* on the population level and b) the number of *T. europaeus* in a 5-m radius around the focal flower. Both data sets were analysed using a generalized linear mixed-effects model with Poisson error distribution and year and population fitted as random effects

Random effects	SD			
a) Large scale effects ($n = 16867$)				
Year	0.6202			
Population:year	0.6966			
Fixed effects	Estimate	SE	Z-value	P-value
Intercept	-2.1949	0.5627	-3.901	<0.001
Log(plant population size)	0.0395	0.0553	0.714	0.48
Plant population density	-0.0600	0.0400	-1.498	0.13
b) Small scale effect ($n = 998$)				
Year	0.4442			
Population:year	0.4701			
Fixed effects	estimate	SE	Z-value	P-value
Intercept	-1.0144	0.3294	-3.080	0.0021
Log(local density + 0.1)	-0.1991	0.0543	-3.666	0.0003

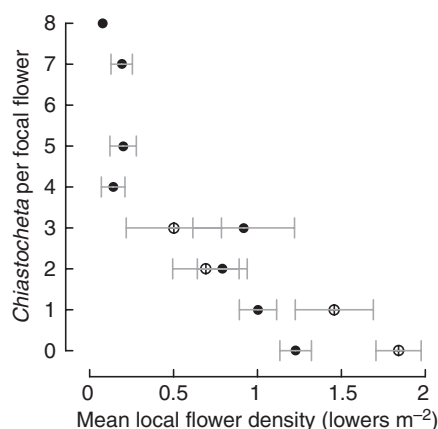


Fig. 2. Mean local flower density versus number of *Chiasiocheta* found in a focal flower, averaged for each level of *Chiasiocheta* for each year (2007: filled circle, 2008: crossed circle). Error bars represent the standard error of the local density. Sample size exceeded 50 except for fly presence of 3 ($n = 17$), 4 ($n = 4$), 5 ($n = 3$), 6 ($n = 0$), 7 ($n = 2$) and 8 ($n = 1$).

REPRODUCTIVE SUCCESS

The median proportion of intact seeds over all years was 0.369 (inter quantile range (IQR) 0.288) and varied significantly between years (Kruskal–Wallis test: $\chi^2 = 99.5$, d.f. = 2, $P < 0.001$) and populations (Kruskal–Wallis test: $\chi^2 = 209.7$, d.f. = 18, $P < 0.001$). Median values were 0.424 in 2006 (IQR 0.281), 0.304 in 2007 (IQR 0.284) and 0.412 in 2008 (IQR 0.276). Out of the 1575 seed heads collected, only 18 samples did not produce intact seeds. Including an interaction of fly abundance with either population-level plant density (model selection: $\chi^2 = 2.817$, d.f. = 1, $P = 0.09$) or plant population size (model selection: $\chi^2 = 2.156$, d.f. = 1, $P = 0.14$) did not significantly improve the model based on the AIC value. We thus used the simple GLMM without interactions. We found a significantly negative relationship between reproductive success and plant population size averaged over all years ($P = 0.0032$, Table 3), although there were differences between years (Fig. 3a) with a strong effect only in 2007.

Table 3. Effects of plant population size, fly abundance and population-level plant density on reproductive success of *Trollius europaeus* derived from a binomial generalized linear mixed-effects model with year and population fitted as random effects

Random effects	SD			
Year	0.0000			
Population:year	0.3312			
Fixed effects	Estimate	SE	Z-value	P-value
Intercept	-0.3634	0.0946	-3.840	< 0.001
Log (plant population size)*	-0.0744	0.0253	-2.944	0.0032
Fly abundance	-1.5144	0.2334	-6.490	< 0.001
Plant population density	0.0554	0.0183	3.022	0.0025

*log (population size) was centred.

Reproductive success was also negatively associated with *Chiasiocheta* abundance over all years ($P < 0.001$; Table 3), with each year showing negative slopes (Fig. 3b). Population-level plant density was the only variable showing a positive correlation with reproductive success ($P = 0.0025$, Table 3, Fig. 3c).

SEED PREDATION

Seed predation had a median of 0.102 (IQR 0.137) and was significantly different between years (Kruskal–Wallis test: $\chi^2 = 72.1$, d.f. = 2, $P < 0.001$) and populations (Kruskal–Wallis test: $\chi^2 = 416.8$, d.f. = 18, $P < 0.001$). We used a simple GLMM without interaction terms, as including interactions between fly abundance and population-level plant density (model selection: $\chi^2 = 2.8665$, d.f. = 1, $P = 0.09$) or plant population size (model selection: $\chi^2 = 0.7783$, d.f. = 1, $P = 0.38$) did not significantly improve the model. *Chiasiocheta* abundance was positively correlated with seed predation ($P < 0.001$, Table 4) in all years (Fig. 4a). Population-level plant density showed a marginally significant decreasing effect ($P = 0.052$; Table 4, Fig. 4b) on seed predation.

REPRODUCTIVE SUCCESS AND SEED PREDATION IN THE ABSENCE OF *CHIASIOCHETA*

Plants placed in artificial groups at a total of eight *Chiasiocheta*-free locations in 2007 and 2008 produced a total of 278 flowers, of which 46 (16.5%) produced no seeds. The proportion of intact seeds (2.6%) was significantly lower than in the natural populations in this study ($\chi^2 = 19488.45$, d.f. = 1, $P < 0.001$). Predated seeds were only found in 8 of the 278 samples (2.9%), which was also much lower than in natural populations ($\chi^2 = 10293.96$, d.f. = 1, $P < 0.001$). No seeds were produced by apomixis. Seeds were produced in 10.9% of artificially selfed flowers and in 4.2% of flowers that received no treatment (i.e. self-pollination in the absence of a pollen vector).

Discussion

Trollius europaeus within small populations was expected to have reduced reproductive ability compared to large populations on account of a reduced ability to support sufficiently large numbers of the obligate *Chiasiocheta* pollinator. Contrary to our expectation, we found no direct effects of plant population size or population-level plant density on the abundance of *Chiasiocheta* at the population level (Table 2a). Furthermore, there was a decline in seed production with increasing plant population size, contrary to our expectation that seed production would decline in smaller plant populations. *Chiasiocheta*-free plants in both natural and greenhouse settings did not produce substantial numbers of intact seeds, confirming the dependency of *T. europaeus* on *Chiasiocheta* and the limited availability of alternative pollinators (see also Ibanez, Dujardin & Despres 2009).

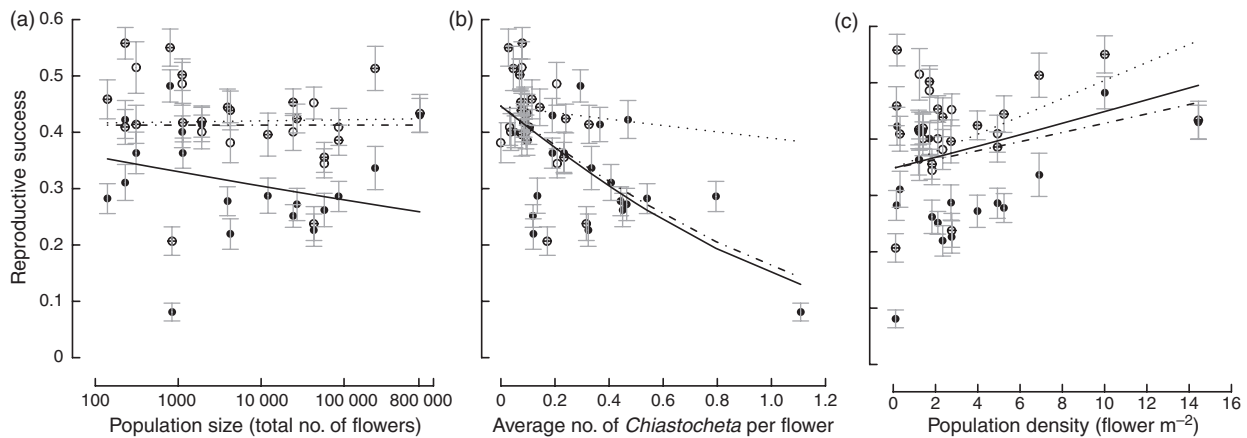


Fig. 3. Reproductive success (proportion of intact seeds, mean per population year) as a function of (a) *Trollius europaeus* population size, (b) *Chiastocheta* abundance and (c) plant population density (2006: open circle and dotted line, 2007: filled circle and solid line, 2008: crossed circle and dashed line). Error bars represent the standard error of the mean.

Table 4. Effects of plant population size, fly abundance and population-level plant density on seed predation of *Trollius europaeus* derived from a binomial generalized linear mixed-effects model with year and population fitted as random effects

Random effects	SD			
Year	0.0000			
Population:year	0.5060			
Fixed effects	Estimate	SE	Z-value	P-value
Intercept	-2.2457	0.1446	-15.956	< 0.001
Log(plant population size)*	0.0673	0.0386	1.739	0.082
Fly abundance	1.4724	0.3561	4.133	< 0.001
Plant population density	-0.0546	0.0280	-1.946	0.052

*log (population size) was centred.

We further found that reproductive success (seed set per flower) decreased when fly abundance per flower was high, on account of higher seed predation (Fig. 4a), a result that is similar to that found for figs and fig wasps, another nursery pollination system (Herre & West 1997). As fly abundance per flower was negatively associated with 5-m scale plant density (Table 2b), a local dilution effect of flies among flowers in high-plant-density patches appears to reduce the cost to flowers in terms of predated ovules. Thus, while *Chiastocheta* abundance was not impacted by overall population size, plants growing in high-plant-density patches appear to benefit from the availability of sufficient flies for pollination while avoiding high costs associated with seed predation. A study of the senita cactus–senita moth reproductive mutualism (another example of a nursery pollination system similar to that in *T. europaeus*) revealed no consistent relationship of either fruit or seed consumption by senita moth larvae or fruit set with variation in cactus population density (Holland & Fleming 1999). This study was, however, conducted at only five populations distributed across two widely separated regions. Further, density was scored only at the population scale and possible local density effects were not investigated.

Despite the many reported instances of declining reproductive output in smaller populations (e.g. Fischer & Matthies 1998; Kery, Matthies & Spillmann 2000; Wolf & Harrison 2001; and reviews by Hobbs & Yates 2003; Johnson *et al.* 2004; Ghazoul 2005; Aguilar *et al.* 2006; Leimu *et al.* 2006; Winter, Lehmann & Diekmann 2008; Schleuning *et al.* 2009), other recent studies have found no clear association between population size and reproductive output, or even declines in per capita seed set in large populations (Brys, Jacquemyn & Hermy 2008; Spigler & Chang 2008, 2009; Rabasa, Gutierrez & Escudero 2009; Tsaliki & Diekmann 2009). Intraspecific resource competition and, alternatively, pollinator limitation, have been proposed as explanations for limited or declining seed set within large populations. In our study, pollen limitation is unlikely because we did not find a relationship between fly abundance per flower and plant population size. *Trollius* distributions within a population are, however, often variable and patchy and flies might respond to smaller scales which could turn individual patches into relatively distinct subpopulations.

The negative effect of plant population size on reproductive success can be explained by the double role of *Chiastocheta* as the main pollinator and pre-dispersal seed predator. As fly abundance per flower increased, reproductive success declined due to seed predation. A similar effect has been suggested by Morris, Bronstein & Wilson (2003) as a regulatory mechanism for coexistence between mutualistic partners, whereby high pollinator densities depress plant reproduction, thus imposing a negative feedback. *Trollius europaeus* therefore benefited from modest *Chiastocheta* visitation, but as visitation continued to increase, the cost to seed predation increased more rapidly than the gains from pollination. This outcome further provides empirical support for the unimodal functional response model developed for the senita cactus–senita moth nursery pollination system where modelled cactus populations decreased when moth populations were either too low (insufficient pollination) or too high (high fruit consumption by larvae) (Holland, DeAngelis & Bronstein 2002). In contrast to

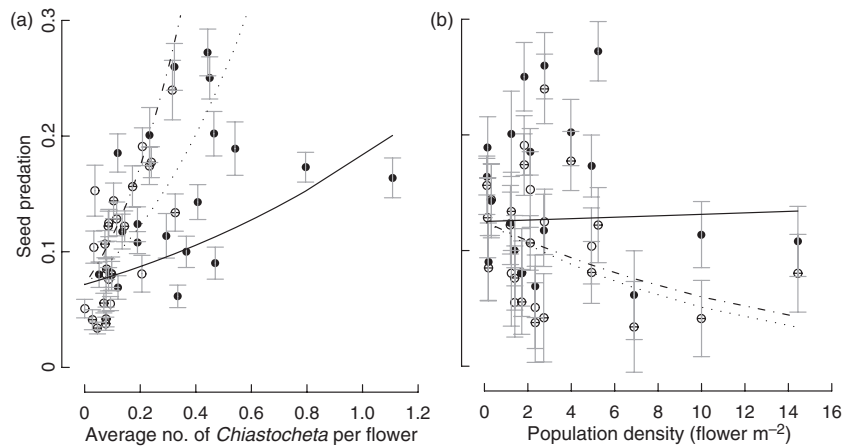


Fig. 4. Seed predation (proportion of intact seeds, mean per population year) as a function of (a) *ChIAstocheta* abundance and (b) *Trollius europaeus* population density (2006: open circle and dotted line, 2007: filled circle and solid line, 2008: crossed circle and dashed line). Error bars represent the standard error of the mean.

our study, Despres *et al.* (2007) did not find a relationship between fly density and seed production across 38 globeflower populations. These populations were, however, sampled from different years and across wide altitudinal (400–2500 m a.s.l.) and latitudinal ranges (Swedish Lapland and French Alps). Substantial inter-year differences (see Figs 3a, 3b and 4b) and much variation among populations are found in our own results, despite the populations being from similar geographical and altitudinal localities. Thus, differences in year, altitude and locality across populations might, possibly, confound the detection of such responses in the Despres *et al.* (2007) study. On the other hand, their data for higher predation at high fly densities (i.e. four or more eggs per flower head) confirmed our own results.

Fly abundance per flower was itself an inverse function of the density of flowers within local patches (i.e. at the 5-m radius scale). Local flower density therefore had an important mitigating effect on seed predation as the probability of harbouring large numbers of *ChIAstocheta* adults (and therefore presumably larvae) per flower decreased at higher flower densities. Our data do not allow us to test this directly, but a similar dilution effect (a form of predator satiation) has been proposed for dipterocarp trees in Asian rain forests, where high densities of seed increase per capita seed survival through the dilution of seed predation (Ghazoul & Satake 2009). In the *Trollius*–*ChIAstocheta* system the process unfolds with respect to local small-scale flower (rather than seed) density, but the mechanism remains the same.

Overall, even though *T. europaeus* relies on a highly specialized pollination system, no evidence was found to suggest that the relatively small populations in the Swiss lowlands are experiencing negative consequences of habitat fragmentation and reductions in plant population size. Other nursery pollination mutualisms have been suggested to be vulnerable to Allee effects at small population sizes. Anstett, HossaertMcKey & McKey (1997), Anstett, Michaloud & Kjellberg (1995) note that at low densities asynchronously flowering figs may not be able to sustain viable pollinator populations and suggest that fig–wasp systems are especially sensitive on account of the highly specialized pollination systems. In our system even the smallest *Trollius* populations showed no sign of Allee effects –

if anything the opposite was true in that reproductive ability in small populations might be enhanced due to a more optimal trade-off between pollination services and seed predation, mediated through per capita *ChIAstocheta* abundance, which itself is a function of *T. europaeus* flower density at local scales (5-m scale). While some flies are needed to ensure pollination of *T. europaeus*, seed predation by *ChIAstocheta* larvae can greatly decrease seed set as abundance per flower increases (Fig. 3b).

While *T. europaeus* populations are still large and continuous at higher altitudes, other populations in the Swiss lowlands only occur on relatively small nature protection areas that are often surrounded by farmland or urban areas. Our results indicate that these small and isolated populations remain viable, but are more likely to be successful if the plants are distributed in locally dense patches, rather than being uniformly distributed across the habitat. Conservation attention should therefore prioritize populations with high local plant densities of *T. europaeus*, as it is these that are likely to be most reproductively secure. Further, the protection of even small pockets of suitable habitat with existing *T. europaeus* populations, as is often currently the case, appears both feasible and even desirable from a plant reproductive perspective.

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