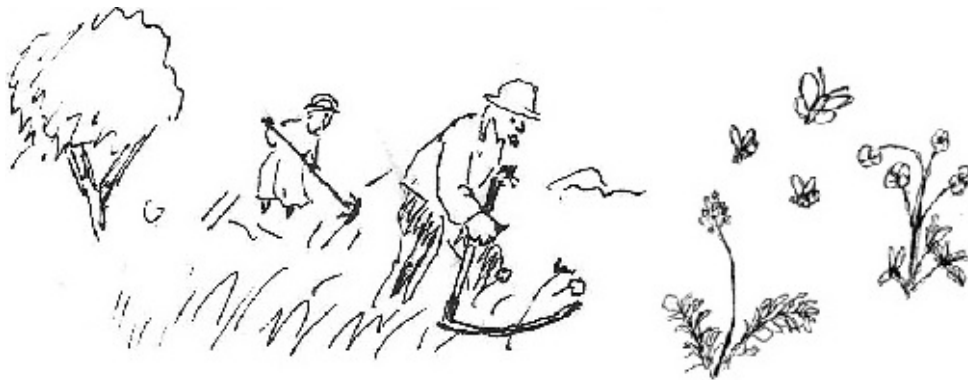




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Pollination failure in traditionally managed hay meadows of low quality

Comparing two different pollination strategies



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Abstract

Today traditionally managed wooded hay meadows only exist in small fractions of their former distributions. Because of the fragmentation and degeneration of hay meadows and the fact that pollinating insect diversity and abundance also are declining, pollination services in these habitats requires attention. To examine the pollination services in traditionally managed hay meadows I collected *Ranunculus acris* (Buttercup) in 20 meadows of varying quality on Gotland and evaluated the mean seed set and mean number of produced seeds per plant. I also collected *Filipendula vulgaris* (Dropwort) in 18 meadows and evaluated the mean seed set to be able to compare the pollination success of the insect pollinated *R. acris* with the wind pollinated *F. vulgaris*. A range of habitat variables were collected in the meadows and in older surveys to examine their relative impact on seed set. I found significantly higher seed set for *R. acris* in the meadows with higher habitat quality, than in meadows with lower quality. In contrast seed set in *F. vulgaris* was not related to habitat quality. The population density also seemed to play an important role in fertilization rate for *R. acris*, through increased seed set in high density areas, while plant height was positively correlated with number of produced seeds. For *F. vulgaris* seed set was positively correlated with moss cover, and number of seeds per plant was positively correlated with population density. These results suggest that reproductive success among insect pollinated plants are more sensitive to habitat degeneration than among wind pollinated plants. The status of pollination services in traditionally managed wooded hay meadows should be evaluated further.

Introduction

The future status of our traditionally managed wooded hay meadows and other semi-natural grasslands is uncertain. Since the beginning of the agricultural revolution about 150 years ago these habitats have diminished dramatically. Alongside this retreat, pollinating insects has also been subject to drastic declines in species and individual numbers. The species-rich wooded hay meadows and their inherent insects are nowadays to be found in small isolated fragments in the landscape. Pollen limitation is a reality facing a large fraction of the worlds insect pollinated plant species.

Serious threats to pollinating species and thus plant-pollinator interactions have become a growing concern during the last decades (Kearns *et al.* 1998). Typical causes for these threats are in the forms of pesticide and herbicide use, changes in land use and agricultural practices and habitat fragmentation and degeneration. While chemicals used in agriculture are directly harmful for insects, fragmentation affects them in more indistinct ways by controlling their movements and affecting population sizes. Substantial diversity declines in taxa important for plant pollination has occurred (Thomas *et al.* 2004) and pollen limitation is a common phenomenon (Ashman *et al.* 2004) that may reduce plant population growth.

If a habitat is too degenerated and fragmented to sustain a particular pollinating species it would likely have drastic consequences for the flowering plants dependent on that pollinator exclusively (Pauw 2007). But plants that are pollination generalists and are visited by several pollinators may perform well even though one or a few pollinating insect species disappears. They would of course suffer from declines in insect abundance or activity but may not be as sensitive as very specialized plant species. Species that is pollinated by wind or a combination of wind and insects would naturally be least affected by the absence of pollinating insects. Habitat degeneration and fragmentation is also likely to have evolutionary consequences for species inhabiting these landscapes e.g. by affecting selection on phenotypic traits associated with pollinator attraction (Weber and Kolb 2011). The population structure of plant species is known to have a large impact on the pollination and reproductive success of individual plants (e.g. Dauber *et al.* 2010; de Jong *et al.* 2005) but the outcomes differs a great deal depending on the system studied. This highlights the complexity of plant-insect interactions, especially in degenerated and fragmented landscapes which is an area where we still have a lot to learn and where the theoretical models often are ahead of empirical studies (reviewed by Tscharrntke and Brandl 2004).

Small or sparse populations are likely to be subjected to Allee effects because of low pollination services (Forsyth 2003; Ågren 1996; Groom 1998). The consequence for this may result in a decreased seed set with even smaller populations and fewer potential mates and possible negative growth rate as a result. Ultimately fragmentation and habitat degeneration may lead to local population extinctions and lower rates of colonization (Hanski and Ovaskainen 2003). This would result in a reduction in local species richness. In spite of a lack of empirical support it is usually assumed that these effects are not as apparent in wind-pollinated plants (Friedman and Barrett 2009; Davis *et al.* 2004). Wind pollination is believed to have evolved as a way to assure pollination when pollinator visits are infrequent (Friedman and Barrett 2009). This may led to the conclusion that pollen limitation may not be as commonly occurring in wind pollinated plants as it is in insect pollinated ones. Few studies have actually examined pollen limitation in wind pollinated species but Friedman and Barrett's study (Friedman and Barrett 2009) confirms this view. Putting these patterns together one may suspect that reduced number of pollinating insects in degenerated habitats would suppress population growth in insect pollinated plant species whereas wind pollinated plant species may be less affected.

In semi-natural grasslands local habitat quality should influence the intensity of plant-pollinator interactions. A hay meadow of low quality has by definition higher vegetation, more litter, higher moss and tree cover and is richer in nutrients compared to high quality habitats. A habitat dominated by tall grass and other species pollinated by wind should be less attractive to pollinating insects than habitats with high diversity of flowering forbs. Ehrlén *et al.* (2002) found factors that affect a plants visibility to also affect seed set in an insect pollinated species. But whether plants in a certain population experience pollen limitation or not may depend on factors other than pollinator visitation rates. Plants in one habitat may not be pollen limited the same time as another population experience high levels of pollen limitation even if plants in the latter are more often visited by pollinators (Totland 2001). In this case the plants in the first population were constrained

by abiotic factors and did not respond to pollen supplementation i.e. increase in pollination services through hand pollination. Totland and Birks (1996) found differences in seed set between populations of *Ranunculus acris* and concluded that local pH-conditions and latitude was the most determining factors.

Agriculture became established in southern Sweden already some 6000 years ago (Ekstam et al. 1988). The earliest agricultural practices were of a slash- and burn type which later, during the Iron Age, developed into more permanent systems (Widgren 1983). An increased need for winter fodder during this time led to the practice of cutting and storing hay in the summer. Many of these hay meadows have since then been continuously mowed for hundreds, sometimes thousands of years, which has resulted in extraordinary species-rich communities that still are important for biodiversity today (Cousins and Eriksson 2002). The traditionally managed species-rich grasslands of Europe have shown a declining existence as a result of reformatations and intensifications in agricultural practices (Gustavsson *et al.* 2007; MacDonald 2000). In Sweden this has occurred gradually since the mid 19th century and onwards (Gustavsson *et al.* 2007; Hultgren *et al.* 2006). The area reduction of semi-natural grasslands in Europe during the last decade is estimated to round 90 % (referenced in Eriksson *et al.* 2007) and the reductions appear to be most severe in easy accessible low land areas (Hodgson *et al.* 2005). On the Baltic island of Gotland less than 1 % of the 40 000 ha of wooded hay meadows existed in the 18th century remains today (Petersson 2010). These trends in agricultural has caused substantial declines in plant diversity (Mykelstad and Saetersdal 2004; Niedrist *et al.* 2009).

In this study I tested two essential hypotheses related to pollination that may have important implications for understanding the present and future status of traditionally managed wooded hay meadows and other semi-natural grasslands. I tested whether there are indications of pollen limitation in traditionally managed hay meadows that varies in habitat quality. I hypothesize that habitats of higher quality are more attractive to pollinators and thus increases the reproductive success of insect pollinated plant species. Plants of a certain species may therefore have lower seed set in a habitat less attractive to pollinators even if their population sizes are comparable to plants from high quality sites. I also tested whether the reproductive success in one wind pollinated plant species is related to habitat quality. I hypothesize that wind pollinated plants are not affected to the same extent by habitat quality as their reproductive success not is dependent on insects. I define a habitat of low quality as having higher vegetation, more litter, higher moss cover, higher tree cover and be more nutrition rich compared with the high quality habitats. The species chosen for this study are the insect pollinated Buttercup (*Ranunculus acris*) and the Dropwort (*Filipendula vulgaris*). The latter is capable both to wind and insect pollination (Weidema *et al.* 2000).

Specifically I tested the predictions that:

1. Seed set in the insect pollinated plant *R. acris* is positively related to habitat quality as defined above.
2. Seed set in the wind pollinated plant *F. vulgaris* is not related to habitat quality.

3. *R. acris* growing in low quality habitats respond more to pollen supplementation than those growing in higher quality meadows.

Methods

Study site

The study took place at the Baltic island of Gotland from mid June to mid July 2011. Gotland is located east of the Swedish mainland and belongs to the hemi-boreal vegetation zone. The island normally has a mild maritime climate with an annual precipitation of ca. 500-600mm for the years 2000-2010 and a monthly mean temperature in June ranging from 12 to 16 °C during the years 2004-2011 (SMHI, Norrköping Sweden, <http://www.smhi.se> Nov. 9th 2011). During the year when the study was conducted the island had a mean temperature of 16 °C in June. The mild climate and calcareous bedrock gives Gotland a distinctive flora. Although drastic declines in numbers of traditionally managed hay meadows occurred on Gotland during the 20th century they are still to be found in concentrations hard to find elsewhere in the rest of Sweden (Hultgren *et al.* 2006). They are scattered in isolated patches connected to the cultural landscapes in a total area of ca 380 ha (Jordbruksverket 2005). Although this is comparable high numbers this represents only ca. 1 % of the total areas that existed before the agricultural revolution (Petersson 2010). Most meadows range from just below 1 ha to a few ha in size and are seldom larger than 3 ha, with a few exceptions (Croneborg 2001).

Study species

Ranunculus acris ssp. *acris* (Ranunculaceae) is a self-incompatible, hermaphroditic, perennial herb (Richards 1997). It has an erect stem that branches above the middle and flowers with yellow flowers from May to June. The species is insect-pollinated, mainly by Diptera, Hymenoptera and Coleoptera species (Hegland and Totland 2008; Clapham *et al.* 1986; Steinbach and Gottsberger 1994). Each flower has several ovaries that may develop into a dry one-seeded fruit (achene). In the studied populations the number of ovaries per flower ranged between 18 and 29. The species grows in damp meadows and pastures on calcareous soils and occurs in most of Europe and have become naturalized in North America, South Africa and New Zealand (Clapham *et al.* 1986). Pollen limitation has previously been studied in *R. acris* and the result differs between populations studied. Jakobsson *et al.* (2009) and Hegland and Totland (2007) did not find pollen limitation on seed-set whereas the latter showed pollen limitation on progeny vigor. Jakobsson *et al.* (2009) studied within-population variation in seed set, Hegland and Totland (2007) studied pollen limitation in a species rich temperate grassland in western Norway. Totland (2001) found pollen limitation on seed set in habitats from low altitude but not in high altitude habitats.

Filipendula vulgaris (Rosaceae) is a perennial, hermaphroditic herb that is capable of both wind- and insect pollination and has shown high outcrossing rates (0.96) (Weidema

et al. 2000). The plant has inflorescences with many cream-white flowers arranged in a brome like structure and flowers from May to June. The species is native to calcareous grasslands in most of Europe (Clapham *et al.* 1987). To my knowledge no earlier studies has examined pollen limitation in *F. vulgaris*.

Data collection

Specimens from 22 meadows were collected from five randomly placed 0.5x0.5 m sample quadrats, if possible in an area where both species were present. Within each sample quadrat I counted the number of individuals of both species and measured their height. Thereafter I collected every individual's seeds and placed them in different envelopes for each species and saved for further examinations. If it was not possible to collect 5 or more individual plants from the quadrats e.g. if the species grew in sparse or small populations I collected additional material in a haphazardly manner. In some meadows though I could only find very few individuals. Due to the sampling approach the total number of individuals collected from each meadow varied from 2 to 22 for *R. acris* (9 in average) and 3 to 21 for *F. vulgaris* (7 in average). *R. acris* was collected in 20 meadows and *F. vulgaris* in 18 meadows.

I estimated seed set as the proportion developed seeds : total ovules in *R. acris*. I counted number of seed heads (number of flowers), number of filled (fertilized large and swollen nuts) and unfilled (not fertilized shriveled ovules) seeds from each sample respectively. A few of the filled seeds showed signs of predation but these were easily distinguished from the unfilled ones. These numbers were later pooled for each meadow and mean seed set for each meadow was calculated as the number of filled seeds divided with the sum of filled and unfilled seeds. Mean seed production per plant and mean seed set was calculated for every meadow and used in the statistical analyses.

In *F. vulgaris* I counted the number of developed seeds (fertilized ovaries) and the number of seed heads (number of flowers) collected from each meadow as for *R. acris*. Due to the large number of seeds in *F. vulgaris* I used the software imageJ (Abramoff *et al.* 2004) in this procedure. ImageJ is a program for image processing and analyzing that for instance enables you to count the number of particles on a photograph. I evaluated the accuracy with different settings on photos with a known number of seeds and used the settings where the result was within 5% of the true number on photos with 1050 seeds (i.e. particle size: 1-10 mm² and circularity: 0.3-0.8). For *F. vulgaris* it was not possible to calculate the number of unfertilized ovaries. Therefore the measure used for seed set in this species is expressed as the mean number of developed seeds per flower. The seed heads were counted manually and here I included withered flowers without seeds (i.e. all flowers per plant were calculated). Mean seed production per plant and mean seed set was calculated for every meadow and used in the statistical analysis.

For each meadow I also collected information on litter height, vegetation height and moss cover because these are all features that in low values indicate a meadow of high quality (e.g. Ekstam *et al.* 1988). This was done by haphazardly toss a one meter long wooden ruler and vertically lift it up from the same end every time and then note the highest point

where vegetation and litter touched the ruler. Moss was recorded as either present or absent on the spot where the ruler landed e.g. if any moss touched the ruler or not. This procedure was repeated 20 times in each meadow. Litter and vegetation height were later calculated as the mean of the 20 samples taken and moss cover as the proportion of samples where moss touched the ruler. I also walked through the meadow and recorded if nitrophilous species were present, as these indicate a more nutrient-rich and therefore a less managed meadow or a meadow somehow affected by anthropogenic effects e.g. fertilized. Data on tree cover, management intensity (continuity in the management) and a classification of the conservation value of the meadows were taken from Anonymous (1992). A well managed meadow would have low tree cover and uninterrupted management regime. A meadow could have a tree cover of the following ranges: 0-25% (0%), 25-50% (25%), 50-75% (50%) or 75-100% (75%). The values inside the brackets were the ones I used in the analysis. For the classification of conservation value, integer values number between 1 and 4 were given. Four main criteria were considered: 1; continuity in the management (the vegetation should give the impression of being managed without interruption), 2; biological function (the meadow should have a function so that genetic diversity can be preserved), 3; management intensity (the whole object should be well managed in traditional ways) and 4; negative impact or interventions (at least 75 % of the meadow should be unaffected by humans in ways that not represent the traditional management e.g. fertilized or planted with forest). A class 1 meadow should fulfill all four criteria, a class 2 meadow should fulfill criteria 1, 2 together with one of the others, a class 3 meadow should fulfill criteria 2 and two optional criteria, a class 4 meadow should fulfill at least one of the criteria. I visited seven class 1 meadows, five class 2 meadows and ten class 3 meadows. I did not have any class 4 meadow in my dataset. For three meadows without classifications in the literature I added my own by comparing their qualities with the quality of the already classified ones. From here on I will use this classification as a measure on habitat quality and name it thereafter.

Pollen supplementation experiment

To examine whether pollen supplementation would increase seed set I experimentally pollen-supplemented *R. acris* flowers and compared their seed set with flowers in naturally pollinated control plants. I performed hand pollinations in 15 of the meadows included in the study, starting from the 20th of June. In each meadow I haphazardly chose up to 20 individuals and randomly assigned them to either the hand pollination or control treatment. If enough individual plants were available, ten individuals were hand pollinated or left as control plants respectively. However if the populations were small fewer individuals were used but I always tried to keep the ratio between number of treatments and controls as 1:1. On the individuals selected for treatment, I brushed the stigmas on all open flowers with pollen-filled anthers from pollen donors located in the same meadow. A fairly low number of flowers from each individual received the treatment as flowering started earlier than expected. Flowers receiving pollen supplementation were marked with white cotton thread and both treatment and control individuals were marked with white sticks. During the second visit to the meadows when the seeds had matured, the marked seed heads were collected and placed in envelopes and

the height of individual plants were measured. Later in the lab I counted the number of filled and unfilled seeds for treatments and controls respectively and calculated the seed set as the proportion of filled seeds as explained for *R. acris* above.

Statistical analysis

I examined if seed set and number of seeds produced per plant in naturally pollinated plants differed between meadows of different quality with an ANCOVA. I used the habitat quality as a fixed factor and plant mean height as covariant. This made it possible to evaluate the variance in seed set and number of produced seeds accounted for by habitat quality against potential background variations caused by plant height. I analyzed the seed set and number of produced seeds for both species in separate models. A posteriori Tukey and Fischer comparisons were performed to examine in detail how seed set and seed production differed between habitat qualities.

I used 2-sample *t*-tests to analyze potential differences in seed set and number of seeds produced per plant for plants growing in meadows inhabited by either nitrophilous plants or meadows not inhabited by nitrophilous plants and meadows with intact management regime or meadows with interrupted management. The *t*-test compares the means of two samples e.g. seed set in meadows with intact management and meadows with interrupted management and tests the null hypothesis that the means of the two samples are equal. This was done for seed set and number of seeds produced for both species in the study.

Stepwise regression analysis was carried out to examine which of the environmental variables collected that best predicts seed set and number of seeds per plant. A stepwise regression analysis is used to find a model that fit a response variable from several predictor variables. This procedure starts by choosing the predictor variable with the highest R^2 among the variables of interest (Zar 2010). Then each of the other variables is added to the model one by one and the one that causes the highest increase in R^2 is added if the P-value in increase is below or equals the chosen confidence level (0.15) (Zar 2010). When a new predictor variable is added the effect of removing one of the others is evaluated. This procedure continues until adding predictors does not cause a significant increase in R^2 or removing predictors does not significantly decrease it. With this method I examined the contributions of meadow area, plant height, population density, litter depth and vegetation height to the variation in seed set and seed number. Since litter depth and vegetation height were correlated I used them individually in two different models together with the other predictors.

The data set for moss cover and tree cover were smaller than the ones used in the stepwise regression analysis and thus I performed single regression analyses on them, both for seed set and seed number in both species.

I used 2-way ANOVA to analyze the effects of pollen supplementation in *R. acris* from meadows of different qualities and potential interactions with plant height. Habitat quality and plant heights were used as independent factors. I only used data from class 1 and 3 meadows in this analysis due to low dataset from class 2 meadows.

All statistical analyses were performed in the software Minitab 15 (Minitab 15 Statistical Software 2007).

Results

Seed set and number of seeds in relation to habitat quality

Habitat quality influenced seed set in *R. acris*. Seed set in *R. acris* showed significant differences between meadows of different habitat quality (Table 1). *R. acris* in meadows with highest habitat quality (class 1 and 2) had higher seed set than those from class 3 meadows ($P < 0.05$, Fischer, Fig. 1). The number of seeds produced did not differ between meadows (Table 1).

Table 1. The effect of habitat quality on seed set and number of produced seeds per plant in the studied species. The analyses were performed using ANCOVAs with habitat quality as fixed factor and plant height as covariant.

Response variable	Source of variation	d.f.	MS	F	P
Seed set in <i>R. acris</i>	Plant height	1	0.0285	3.82	0.068
	Habitat quality	2	0.0432	5.79	0.013
	Error	16	0.0075		
	Total	19			
Number of seeds in <i>R. acris</i>	Plant height	1	3860.2	8.58	0.01
	Habitat quality	2	57.9	0.13	0.88
	Error	16	450		
	Total	19			
Seed set in <i>F. vulgaris</i>	Plant height	1	0.779	0.52	0.482
	Habitat quality	2	0.733	0.49	0.622
	Error	14	1.49		
	Total	17			
Number of seeds in <i>F. vulgaris</i>	Plant height	1	847	0.07	0.788
	Habitat quality	2	12751	1.13	0.352
	Error	14	11318		
	Total	17			

Seed set and number of seeds for *F. vulgaris* did not differ between meadows of different habitat quality (Table 1).

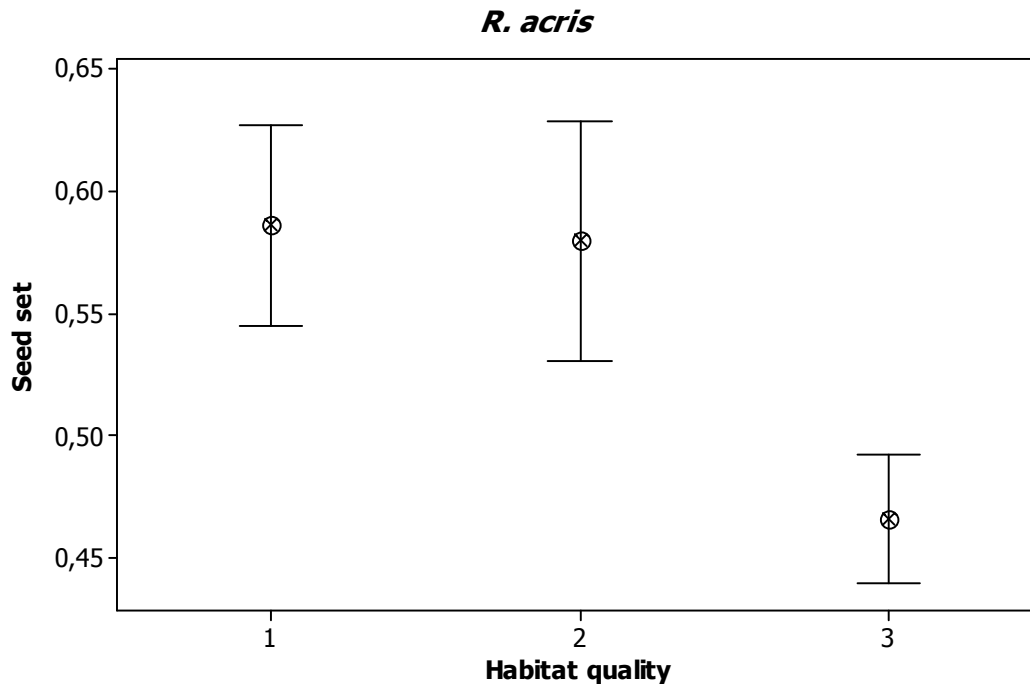


Fig. 1. Mean seed set (\pm one SD) for *R. acris* in meadows of different habitat quality. A habitat with the value 1 has the highest quality while 3 represent a poorer habitat.

Seed set and number of seeds in relation to nitrogen content and management intensity

The seed set in *R. acris* was not related to presence of nitrophilous species or management intensity (Table 2). Nor was the number of seeds produced per plant related to these factors (Table 2).

For *F. vulgaris* neither the presence of nitrophilous species nor management intensity explained the variation in seed set (Table 2). Likewise, seed production per plant was not related to these variables (Table 2).

Seed set and number of seeds vs. plant height, meadow area, population density and litter depth or vegetation height

The stepwise models for seed set in *R. acris* returned population density as the best predictor both when litter dept ($t_{19} = 1.78$, $P = 0.093$) and vegetation height ($t_{19} = 1.78$, $P = 0.093$) were used respectively. Seed set was higher in high density populations. The number of seeds produced were positively correlated with plant height ($t_{19} = 3.14$, $P = 0.006$).

The models with *F. vulgaris* returned no significant predictor variables for seed set in the model with litter dept or the one with vegetation height. The number of seeds produced correlated positively with population density ($t_{19} = 2.85, P = 0.012$).

Table 2. Mean seed set and mean number of seeds tested against nitrogen and management intensity using 2 sample t-tests. None of the analyses showed significant results.

Response variable	Predictor variable	t	d.f	P
Seed set in <i>R. acris</i>	Nitrogen	0.51	17	0.614
	Management intensity	1.50	14	0.155
Number of seeds in <i>R. acris</i>	Nitrogen	-0.95	13	0.359
	Management intensity	-0.19	11	0.849
Seed set in <i>F. vulgaris</i>	Nitrogen	-0.30	15	0.770
	Management intensity	-1.05	12	0.313
Number of seeds in <i>F. vulgaris</i>	Nitrogen	-1.17	15	0.262
	Management intensity	-1.48	11	0.167

Seed set and number of seeds vs. moss and tree cover

Seed set in *R. acris* did not correlate with moss cover (t-value = 0.79, $P = 0.444$) or tree cover (t-value = -0.39, $P = 0.703$, Fig. 2). Number of seeds produced did not correlate with the predictors (moss cover: t-value = -1.37, $P = 0.195$; tree cover: t-value = -0.50, $P = 0.623$).

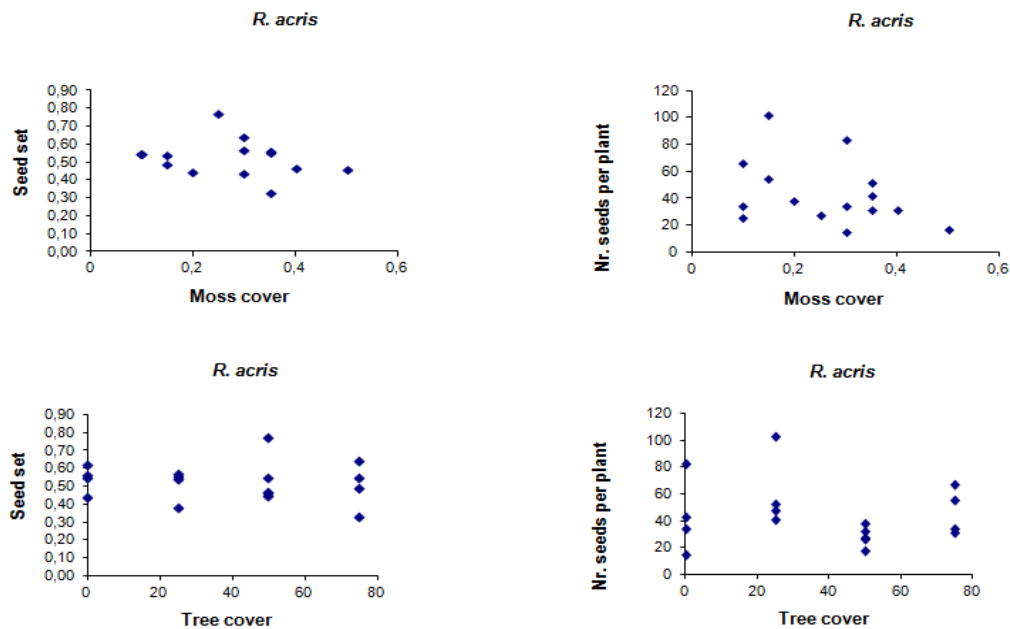


Fig. 2. Relationships between seed set and number of produced seeds per plant and moss and tree cover in *R. acris*. Possible relationships between the dependent (seed set or number of produced seeds) and independent (moss cover or tree cover) were analyzed using linear correlation and regression. Data on moss cover are based on means from 15 populations and data on tree cover are based on means from 17 populations. None of the relationships are significant.

Seed set in *F. vulgaris* showed significant positive correlation with moss cover (t-value = 2.33, $P = 0.042$) but not with tree cover (t-value = -0.58, $P = 0.569$, Fig. 3). Number of seeds produced did not show any significant relations with the predictors (moss cover: t-value = 0.90, $P = 0.389$; tree cover: t-value = 0.49 $P = 0.635$).

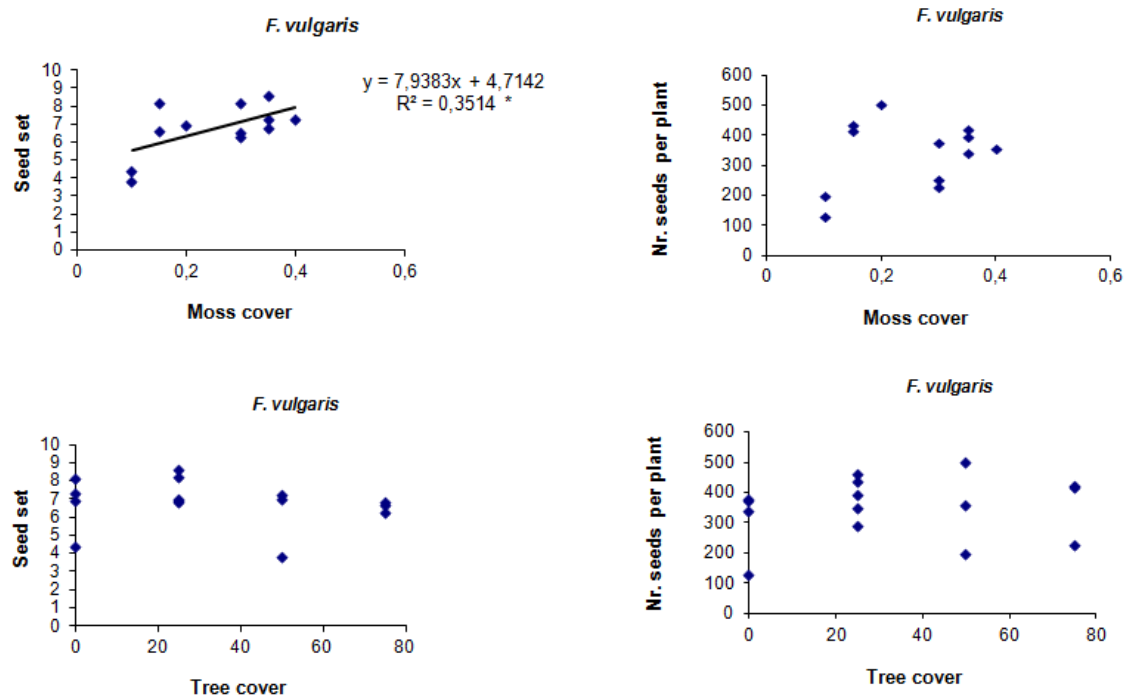


Fig. 3. Relationships between seed set and number of produced seeds per plant and moss and tree cover in *F. vulgaris*. Relationships were analyzed with linear correlation and regression based on means from 12 populations for moss cover and 15 populations for tree cover. *The relationship between seed set and moss cover is significant (P -value = 0.042).

Pollen supplementation experiment

Seed set did not vary significantly between hand pollinated and control plants, independent of habitat quality (treatment: $F = 0.01$, $P = 0.904$; habitat quality: $F = 0.43$, $P = 0.521$; interaction: $F = 0.08$, $P = 0.783$ (Fig. 3)).

Average seed set and seed production

On average *R. acris* produced 47.9 ± 24.4 (mean \pm S.D, $n = 177$). Seed set in *R. acris* calculated as the proportion of developed seeds and total number of ovules was 0.54 ± 0.11 .

On average *F. vulgaris* produced 357.3 ± 104.2 (mean \pm S.D, $n = 134$) seeds. Seed set in *F. vulgaris* calculated as the average number of seeds produced per flower was 6.8 ± 1.2 .

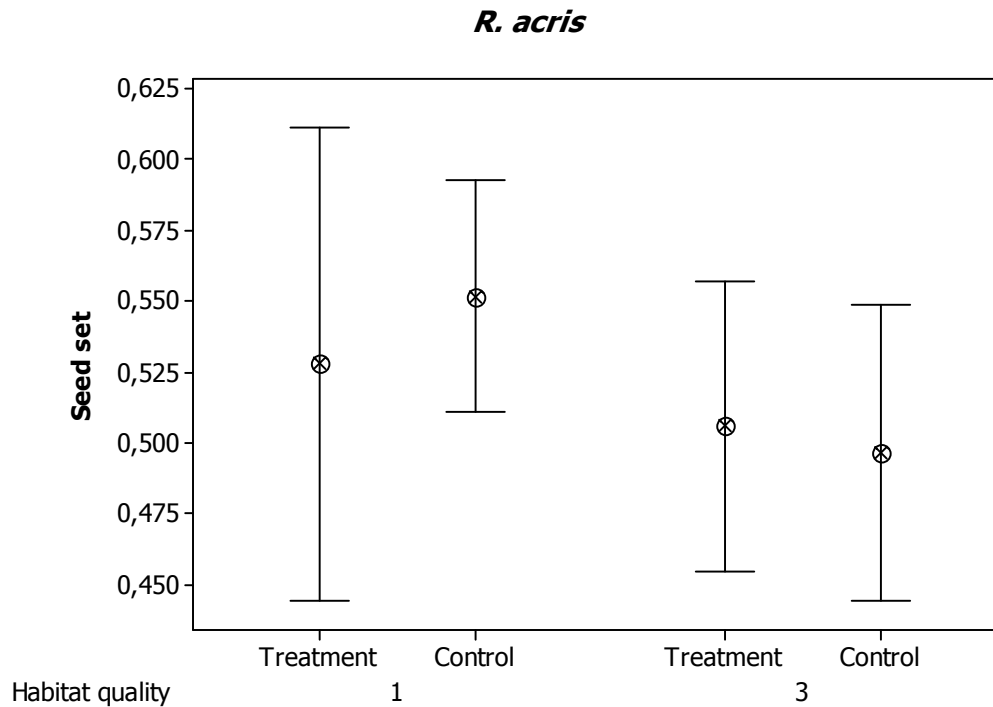


Fig. 4. Mean seed set (\pm one SD) for *R. acris* in hand pollinated individuals (treatment) and control individuals. Means and variation were analyzed with a two-way ANOVA with treatment and habitat quality as independent variables and seed set as dependent. The analysis revealed no significant results.

Discussion

I found higher seed set in high quality habitats in the insect pollinated *Ranunculus acris* but not in *Filipendula vulgaris* which has capabilities for wind pollination. The population structure appears to play a role for the result in *R. acris*.

Seeds set, seed production and habitat quality

Seed set, measured as the proportion of developed seeds and total ovules, for *R. acris* was positively related to habitat quality. Seed set was higher in class 1 and 2 meadows than in class 3 meadows. One possible reason behind this result could be higher pollinator visitation rates in meadows of higher quality. When taking into account that mean number of seeds developed by individual plants did not vary among meadows it gets more obvious that this is may be a relevant measure on the amount of insect-plant interactions. For this to happen individuals from low quality habitats must produce more flowers to compensate for the reduced seed set. Since low nutrition soils are associated with high quality meadows due to their management e.g hay removal, pollarding, after-math grazing (Tallowin and Jefferason 1999) a higher nutritional status may allow plants in less managed meadows to develop extra flowers when not constrained by modest amounts of nutrition. It is thus unlikely that the high seed set in high quality meadows is a

result of nutrition status. This suggests that pollinators might be more active in habitats of higher quality. *R. acris* is mostly visited by pollination generalists e.g. Diptera, Hymenoptera and Coleoptera species. Steinbach and Gottsberger (1994) observed that *R. acris* was visited by as many as 54 different species in Germany. Thus if the reduced seed set in low quality habitats is caused by low pollinating services this reduction is also likely to affect other species dependent on pollination from these non specialized species.

This does not mean that *R. acris* has a higher fitness in these meadows since seed set is not directly translated into fitness (e.g. Marques *et al.* 2011). The number of seeds each plant produce is commonly used as fitness measure (Leimu *et al.* 2006) and did not differ between meadows. This result may however, as discussed above, be heavily altered by among meadow differences in nutritional status. An experiment where the amount of available nutrition is manipulated would reveal these interactions.

For *F. vulgaris* mean seed set did not vary with the habitat quality of the meadows. This suggests that they are not as sensitive to habitat degeneration as *R. acris* and is in line with my second hypothesis. *F. vulgaris* is frequently wind pollinated and may therefore have sufficient pollination services also in low quality habitats where pollinating insects may be infrequent. Number of produced seeds per plant in *F. vulgaris* did not differ between meadows of different quality indicating that *F. vulgaris* cope equally well, independent of the habitat quality.

Using seed set when comparing pollen limitation is common practice (e.g. Becker *et al.* 2011; Schleuning *et al.* 2011; Gómez *et al.* 2010). In my study I used the ratio between number of developed seeds and empty ovules in open pollinated plants as a measure of seed set. Most studies on pollen limitation compare the success of open pollinated flowers with pollen supplemented flowers (e.g. Jakobsson *et al.* 2009; Totland 2001). I only did this in small scale and my experiment revealed no significant differences between seed set in *R. acris* between pollen supplemented and control plants. In the first analysis of the experiment I did, data from all meadows were pooled according to the treatment e.g. pollen supplement and control. This approach was not relevant though, since the result likely would be blurred by differences in how the plants from different habitat qualities respond to the treatment and of course this approach does not mirror the biology behind my third prediction. Therefore I examined the effect of pollen supplementation in plants from different habitat qualities respectively. The problem with this new approach was that the power of the analysis became extremely low due to small sample sizes. Therefore it would not be wise to conclude anything from this experiment but rather let the question whether *R. acris* respond different to pollen supplement in meadows of different quality still be open. If it could have been proved that plants from low quality habitats would respond to pollen supplementation with an increase in seed set it could be claimed with greater certainty that the differences between seed set found between habitat qualities is due to pollen limitation.

Effects of environmental variables and population characteristics on seed set and seed production

The stepwise regression analysis returned population density as the main factor influencing seed set in *R. acris*. I hypothesized that seed set may be higher in high quality meadows independent of population density. This could not be concluded from my data since population densities seems to have a role in this system. Since many studies deals with pollen limitation in relation to population densities and population sizes (e.g. Ågren 1996; Groom 1998; but see Bosch and Waser (2001) for a study where population densities were manipulated) it would also be interesting to know whether pollen limitation may occur in denser populations. By this approach we might discover pollen limitation in an early state of succession and also whether pollen limitation leads to smaller populations or of pollen limitation occurs after populations has decreased by any other reason. A reason that none of the other variables collected became included as predictors of seed set during the stepwise regression analysis may be because the data in this study was too limited to disentangle the contributions from several different quality indicators. It should also be stressed that the measure of habitat quality used in this study is a good representative of the different environmental variables collected. A meadow with high habitat quality in general has low nutrition status, low litter and vegetation heights, low moss and tree cover, and long time of continuous management. Although it would be valuable to know the exact contributions from each of these variables to seed set, the fact that seed set is lower in low quality meadows is an important result. Factors not examined in this study that also may play important roles may for instance be connectivity and distance to other meadows. It has been shown that the density of pollinating species declines with distance to semi-natural grasslands (e.g. Jauker *et al.* 2009; Öckinger and Smith 2007). Therefore species living in low quality habitats may show high seed set if the connectivity to meadows of higher quality is sufficient. Density of conspecific flowers would also be a likely candidate influencing seed set for *R. acris*, especially since it is a species pollinated by generalists. If certain factors were found to affect seed set more than others it would be easier to target conservation efforts to enhance seed set in meadows where it is believed to be critical low. The number of seeds produced correlated positively with plant height, reflecting the fact that higher plants may produce more flowers.

Seed set in *F. vulgaris* showed positive correlation with moss cover. This is a somewhat surprising result since pollination success intuitively would not be expected to be related to the presence of moss. The method used when calculating seed set in *F. vulgaris* may play an important role here. Since I included empty flowers as potential developers of seeds, a plant with a high proportion of empty flowers will have a low seed set. Thus, if the number of seed producing flowers depend on the habitat and high moss cover is associated with high quality meadows for *F. vulgaris* plants growing in these sites would have low numbers of non seed producing flowers and thus high seed set according to these measures. Of course it may be possible that some confounding variable influences seed sett and moss cover simultaneously but a different measure on seed set may still be suitable in this case. The number of seeds produced per *F. vulgaris* plant correlated with population density which indicates better reproductive success in high population density areas. If this is true this results opposes the view that wind pollinated species is not affected by Allee effects so easily, a view that may starts to be questioned (e.g. Davis *et al.* 2004).

Pollen limitation and population dynamics

Little is known how and when pollen limitation affects the dynamics of plant populations. It is important to note that if pollen limitation should have an effect on population dynamics, lower seed set or seed vigor must result in decreased number of offspring or poorer offspring performance. Hegland and Totland (2007) did find that pollen supplement resulted not in higher number of seeds but in seeds of higher quality in *R. acris*, which resulted in higher recruitment compared with control individuals. This may also be the case here. I could not find any differences in seed set between hand pollinated and control plants but the ones received the treatment may have superior seeds. If this is the case, individuals from high quality meadows would indeed have higher fitness than those from low quality meadows. Unfortunately it was not possible to study seed quality within the time frame of this project. Johansson *et al.* (2011) studied functional traits over a range of species and found weak positive relationships between seed mass and the likelihood to remain in abandoned semi-natural grasslands which supports the hypothesis that heavier seeds may lead to a fitness advantage. Also if plants, as seems to be the case for *R. acris*, can compensate the reduced seed set in low quality habitats by developing more flowers they may be able to persist in degraded habitats. But since many of the species in traditionally hay meadows grow short and are sensitive to shade (e.g. Ekstam *et al.* 1988) this would not likely be the case for the majority of species.

Meadows with high habitat quality are generally more species rich than meadows of low quality (Anonymous 1992). The properties of meadows with higher scores on habitat quality included many of the traits I listed as important factors for seed set in insect pollinated species (low vegetation height, low moss cover, high management intensity, low tree cover etc.). Ågren *et al.* (2008) studied the relative importance of population structure and habitat quality for spatial variations in reproductive success in a perennial insect pollinated plant. They concluded that the relative importance of local population structures and abiotic conditions may vary among years and also along the chain of events during the flowering period. Thus we may expect different limiting factors on population growth between years which make pinpointing conservation efforts harder. I had no opportunity to study among year variations but this may be important to consider when trying to reveal the fate of the traditional hay meadows since a simple snapshot from one year may hide important influencing factors. If these kinds of variations exist for insect pollinated plants in my studied system they may be more sensitive to extinction from stochastic events (Ovaskainen and Hanski 2002).

Obviously we cannot make general conclusions from a study with only two model species. To get a more community based understanding of the fate of traditionally managed hay meadows in the light of pollen limitation a wider range of species have to be included in this kind of study. This is a challenging task, if even possible with empirical studies. This is an area where theoretical models may be an excellent way to estimate the future conservation status in our meadows (e.g. Sullivan *et al.* 2011; Petit and Firbank 2006; Eriksson 2005).

Conclusions and implications

I found lower seed set in populations of the self incompatible, insect pollinated *R. acris* from low quality habitats compared with those growing in higher quality habitats. I could not conclude that the observed variation in seed set in *R. acris* was due to differences in amount of pollination services. I found no relationships between seed set and over all habitat quality in the wind pollinated *F. vulgaris*. These results may have long time consequences for the species compositions in our meadows. Wind pollinated plants may gradually replace insect pollinated ones and outcompete them in low quality habitats. Although it is too ambitious to make conclusions on their relative interspecific fitness from the data presented in this study these patterns are still worth considering. If a lack of pollination services constrain insect pollinated plants to such an extent that competition from wind pollinated plants gets deleterious it will have serious consequences for the future status of our species-rich meadows. This may be important factors explaining the dynamics leading to degraded and remnant semi-natural habitats as described in Johansson *et al.* (2011), Cousins and Eriksson (2002) and Krauss *et al.* (2010).

If plants depending on pollination show lower seed set in sites where the habitat is believed to be less attractive to pollinators we may expect pollen limitation due to insufficient pollination services. This means that conservation efforts should be concentrated to localities where sufficient pollination services can be sustained with certainty. If not, we may waste money and effort on conserving areas where species cannot persist because of insufficient reproductive success. This is much dependent on the behavior of the pollinating agent. Plants in isolated sites may survive the isolation if their pollinators are able to disperse over long distances (Jauker *et al.* 2009). These limits to where/when pollination gets insufficient are far from solved and until any consensus is reached it may be wise to place new conservation areas in connection to already existing ones and to ensure high habitat quality so that sufficient pollination services can be achieved.

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