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Interactions for pollinator visitation and their consequences for reproduction in a plant community

Stein Joar Hegland [a,b,*], Ørjan Totland [c]

[a] Faculty of Science, University College of Sogn and Fjordane, P.O. Box 133, N-5801 Sogndal, Norway
[b] Norwegian Red Deer Centre, N-6914 Svanøybukt, Norway
[c] Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway

ABSTRACT

Competition and facilitation in species interactions attract much attention in ecology, but their relative importance has seldom been evaluated in a community context. We assessed competitive and facilitative interactions for pollinator visitation among co-flowering species in a plant community, investigated the subsequent consequences for plant reproduction, and investigated whether effects could be trait-based. We removed the flowers of two species attractive to pollinators, in two separate experiments and assessed the effects on pollinator visitation rates and components of reproductive success in 11 co-flowering focal herb species. Overall, most focal species appear not to interact with the removal species with respect to pollinator visitation and subsequent reproduction (neutral interactions). Three focal species in the community had significantly higher reproductive responses (fruit production and seed weight) in the presence of the attractive removal species (facilitative interactions), but species interaction effects were less pronounced in species' flower visitation rates. A community-wide meta-analysis demonstrated that the two experiments did not have a significant effect on either facilitation or competition, and that there was no overall correlation between effect sizes for visitation and reproduction. Based on species-specific responses, it seems likely that floral traits such as similar flower colors contribute to interspecific facilitation of pollinator visitation and, in particular, that high pollinator dependence for plant reproduction, and associated pollen limitation, may contribute to subsequent interaction effects on reproduction in the focal species.

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1. Introduction

Considerable efforts have been made to investigate interspecific interactions among species (Bruno et al., 2003), including how plant species compete for pollinator visitation (e.g., Feinsinger, 1987; Palmer et al., 2003); a factor that may directly affect plant fitness (Waser, 1983; Feinsinger, 1987; Mitchell et al., 2009). Nevertheless, information about the relative importance of competitive or facilitative interactions, especially on plant pollination and reproductive success, is limited because few studies have approached interactions on a community, or multi-species, level. In experimental studies of interactions for pollination involving two to three response species, attractive plant species

appear to have both competitive and neutral effects on other species' pollinator visitation and reproductive success (Feinsinger et al., 1991; Grabas and Laverty, 1999). In contrast some observational studies focusing on pollinator visitation showed that all types of interactions, i.e., competitive, neutral and facilitative, occur within a plant community with a bias toward more facilitative rather than competitive interactions (Moragues and Traveset, 2005; Hegland et al., 2009). It has been argued that interspecific facilitation for pollinator visitation that subsequently affects plant reproduction positively seldom occurs in nature (Feldman et al., 2004; Bell et al., 2005). On the other hand, a broad range of two species interaction studies reveal that positive, negative or neutral interactions for pollinator visitation among species are roughly equally represented (Waser, 1978; Feinsinger et al., 1991; Caruso, 2002; Moeller, 2004; Ghazoul, 2006). Only a few studies have actually found that interspecific interactions for pollinators have subsequent effects on plant reproduction (Feinsinger et al., 1991; Laverty, 1992; Moeller, 2004; Ghazoul, 2006), but many previous studies did not investigate effects of co-occurring plants on both

* Corresponding author. Faculty of Science, University College of Sogn and Fjordane, P.O. Box 133, N-5801 Sogndal, Norway. Tel.: +47 41501553.
E-mail addresses: stein.joar.hegland@hisf.no (S.J. Hegland), orjan.totland@umb.no (Ø. Totland).

pollinator visitation and plant reproduction. Moreover, because few studies included more than two species, it is difficult to generalize about the relative importance of the different interactions for pollination service within a community.

Ultimately competition or facilitation for pollinators may affect the amount of pollen deposited on a plant's stigma, which subsequently affects plant reproduction and fitness. Many plant species appear to experience pollen-limited reproduction (Ashman et al., 2004) and therefore one might expect that increased or decreased pollinator visitation will cause altered reproductive output (Steffan-Dewenter and Tscharntke, 1999; Klein et al., 2003; Morris, 2003; but see e.g., Kunin, 1993). Floral characteristics and mating systems of plant species may influence how the reproductive success of species responds to variation in pollen deposition (Knight et al., 2006) and several studies have attempted to explain how floral traits contribute to the outcome of interactions (facilitation vs. competition) involving pollination services. In general, species attractive to pollinators are more likely to affect the pollinator visitation of co-flowering species (Laverty, 1992; Chittka and Schürkens, 2001). The degree of floral similarity, such as in color or shape may also explain the outcome of visitation interactions (Brown and Kodric-Brown, 1979; Bierzychudek, 1981; Schemske, 1981). Likewise, traits related to pollen placement on pollinators' bodies and breeding system have been used to understand how visitation interactions may affect the reproductive success of the interacting plant species (Waser, 1983; Feinsinger et al., 1991; Fishman and Wyatt, 1999). To date, only a handful of studies have used a community approach to connect species characteristics to the outcome of species interactions for pollinator visitation and plant reproduction (e.g. Ghazoul, 2006; Hegland and Totland, 2008; Sargent et al., 2011). Therefore, we aim to explore the relative importance of facilitation and competition for pollinator visitation and their subsequent effect on female reproductive success among several rewarding native plant species that co-occur and bloom simultaneously within a natural plant community. To achieve this end, we conducted two separate field experiments in which flowers of two frequently visited plant species were removed throughout the flowering season. The effect on pollinator visitation and plant reproduction was measured in 11 co-flowering focal plant species within a plant community. We also tested whether there was an overall effect of our two experiments in favor of any interaction type using a meta-analysis. Finally, we discuss whether species' characteristics may explain the species-specific responses.

2. Materials and methods

2.1. Study system and design

Our study site was a ca. 50 × 50 m temperate meadow on a relatively steep slope of the species-rich grassland in Rudsviki, Kaupanger, close to the shore of the Sognefjord in western Norway (61°09′27 N and 7°10′22 E). About 35 insect-pollinated flowering species occurred at the site during the study season (species names are based on Lid and Lid, 1994). The pollinator community was dominated by various species of bumblebees and dipterans in the year of study (Hegland et al., 2010).

We designed our study to determine how several plant species within a community interact for pollinator visitation and reproduction. Two experiments were conducted in which we removed the flowers of a plant species attractive to pollinators. In one experiment, we removed the flowers of *Potentilla erecta* and in the other we removed the inflorescences of *Trifolium pratense*. We used these species as "removal species" because they had a high floral abundance in the community, were frequently visited by the pollinators we focus on (flies and bumblebees, respectively), and flowered during the main part of the pollination season the year before this study (see also Hegland and Totland, 2005). These features were likely to increase the possibility that the presence or absence of these two removal species would affect pollinator visitation to "focal species" in the community, and subsequently impact their female reproductive success. In this sense the removal species could be viewed both as potential "cornucopian species" (*sensu* Mosquin, 1971) or as "magnet-species" (*sensu* Laverty, 1992). The focal species were selected on the basis of flowering overlap and visitor sharing with the removal species during the study year and the previous year (see also Table 1 in Hegland and Totland, 2005). All the removal and focal species used in the study are perennial, relatively abundant in the community, and common in the region (*personal observation*).

We used a fine-scale experimental design for this study. We subjectively placed 10 and 11 blocks in both the *Potentilla*- and *Trifolium*-removal experiment. The blocks, separated by at least 5 m, were roughly equally distributed within the study community. The selection criterion was that at least two of the focal species co-occurred in a block with the removal species, at the time of block selection. The treatment consisted of continuously removing all flowers of the removal species every day from the start of the

Table 1
Information on floral traits, plant families and reproductive units of removal (in bold) and focal plant species, used in analyses of the two pollination interaction-experiments at Rudsviki, Sognefjord, west-Norway, 2004.

Plant species	Plant family	Breeding system	Flower color	Flower shape	Reproductive unit collected
Potentilla erecta	Rosaceae	Obligatory outcross[1]	Yellow	Open	–
Carum carvi	Apiaceae	Normally outcross[2]	White	Open	infructescence
Fragaria vesca	Rosaceae	Outcross and selfing[3]	White	Open	infructescence
Plantago lanceolata	Plantaginaceae	Obligatory outcross[4]	Whitish	Open	infructescence
Ranunculus acris	Ranunculaceae	Obligatory outcross[5]	Yellow	Open	infructescence
Trifolium pratense	Fabaceae	Obligatory outcross[6]	Purple	Tubular	–
Campanula rotundifolia	Campanulaceae	Obligatory cross[7]	Blue	Open/Tubular	fruit
Centaurea jacea	Asteraceae	Normally outcross[8]	Purple	Open/Tubular	infructescence
Clinopodium vulgare	Lamiaceae	Normally outcross[9]	Purple	Tubular	infructescence
Euphrasia stricta	Scrophulariaceae	Outcross and selfing[10]	White	Tubular	fruit
Knautia arvensis	Dipsacaceae	Outcross and selfing[11]	Purple	Open/Tubular	infructescence
Prunella vulgaris	Lamiaceae	Outcross, and selfing[13]	Purple	Tubular	infructescence
Trifolium repens	Fabaceae	Normally outcross[14]	White	Tubular	infructescence

Notes: Flower color is presented according to human vision. Experimentally removed species are in bold. Classification of breeding systems are based on following references: [1] (Watson, 1969); [2] (Bouwmeester and Smid, 1995); [3] (Irkaeva and Ankudinova, 1994); [4] (van Damme, 1984), *P. lanceolata* is mainly wind-pollinated, but insect pollination may occur (Clifford, 1962); [5] (Totland, 1994); [6] (Proctor et al., 1996); [7] (Nyman, 1992); [8] (Hardy et al., 2001); [9] Based on information from the online (Ecological Database of the British, 2007) on several close relatives; [10] Based on general information on the genus *Euphrasia* (French et al., 2005); [11] (Vange, 2002); [12] (Norderhaug, 1995); [13] (Winn and Werner, 1987); [14] (Burdon, 1983). Flower color is as perceived by human eyes (see also Proctor et al., 1996). For those species where we collected a fruit instead of an infructescence we used seeds per fruit as response variable in the statistical analysis as opposed to fruits per infructescence (see Data analysis).

experiment until all flowers of the focal species had withered, after ca. four to six weeks. Within each block, we established a pair of circular 2 m diameter plots about 1 m apart. Around each plot, both removal and control plots, we created a buffer zone of 0.5 m, within which we continuously removed flowers of all species to reduce the effects of the floral neighborhood on the visitation to flowers within the plots. The size of this zone was also determined by the need to walk when sampling plots and simultaneously not disturb the vegetation of the meadow. We randomly assigned one treatment and one control plot within each block.

2.2. Data collection

We collected two different types of data that could indicate whether competitive or facilitative interactions for pollinator visitation occurred, and whether components of female reproductive success were affected. First, we observed visitation to flowers or inflorescences (depending on the species) of all focal species during 5-min periods simultaneously for both removal and control plots within the same block. Visitors were classified into main pollinator groups, namely the two dominant pollinator groups in our study community (bumblebees: *Bombus* ssp. and former *Psithyrus* ssp.; and flies: Diptera: Syrphidae and muscoid-types). Visitation observations were conducted between 0900 and 1600 h and only when there was no rain or strong wind. After each observation period, we counted the number of flowers or inflorescences of all focal species in the plots. Data were used to calculate per flower visitation rates for each species (see Data analysis). The visitation observations were made once a day per plot during six days between 31 May and 8 June in the *Potentilla*-removal experiment, and eleven times per plot during seventeen days between 23 June and 30 July in the *Trifolium*-removal experiment. The shorter flowering period of most focal species in the first experiment, and a period of cold and rainy weather early in the season caused the difference in the number of observation days between the two experiments. The total observation time is different per species and varied, in sum for both control and removal plots, between 265 and 490 min for species in the *Potentilla*-experiment and 210 and 1035 min in the Trifolium-experiment. Second, we collected fruits or infructescences to investigate how the presence vs. absence of the removal species affected components of reproductive success in the focal species. Fruits or infructescences were collected when almost mature, five to six weeks after the initiation of the experiments (depending on the species) and before the fruits opened or dehisced. We collected three infructescences per focal species per plot for the *Potentilla*-removal experiment and five infructescences per focal species per plot in the *Trifolium*-removal experiment. The fruits or infructescences were collected from ramets within the plots that appeared to occur on different individuals. First, we counted the number of seeds and fruits and then we weighed the dried seeds (dried at room temperature for six months) to obtain an average seed weight per fruit. Table 1 gives an overview of the floral traits (i.e., breeding system, flower color, flower shape) and the collected reproductive units for each focal species.

2.3. Data analysis

We used the flower visitation rate and two components of female reproductive success to assess the effects of the experiments for each focal plant species. The flower visitation rate, i.e., the "pollinator attractiveness" component in our study, was defined as the number of visits per flower or inflorescence per 5-min observation period, and we calculated separate visitation rates for *Bombus* and Diptera. The latter was split into separate variables for muscoids (mainly Muscidae) and syrphids (Syrphidae) when there

were ≥5 days of observed visitation for each group. In the flower visitation rate numbers, we included visits only when we observed the pollinators to touch the sexual organs of flowers, for example excluding flies sitting on the closed flowers of *Trifolium repens*.

Fruit production, i.e., the number of viable fruits per infructescence, was generally (see Table 1 for details per species) used as measure of the "quantity" component of reproductive success and mean seed weight was used as measure of the "quality" component. Both components can be positively affected when extra pollen is supplied (Hegland and Totland, 2007, 2008). Overall, a higher visitation rate or more reproductive success in control plots was interpreted as interspecific facilitation from the attractive removal species, whereas higher values of these variables in removal plots indicated a competitive interaction (e.g., Fishman and Wyatt, 1999).

We used the mean values (e.g., Steffan-Dewenter et al., 2002) per plot within blocks, of flower visitation rates, fruit production and seed weight as response variables in an ANCOVA-model (utilizing the univariate general linear model module in SPSS, version 12.0.1) to evaluate the relative importance of pollination interactions in the study community. Treatment (removal vs. control) was a fixed factor, block was a random factor, and the conspecific flower density (CFD) of the focal species was used as a covariate because it is likely to affect the flower visitation rate (e.g., Thomson, 1981). Before starting the model selection we tested the homogenous slopes assumption of ANCOVA (Glantz and Slinker, 2001) by running a model including the interaction between treatment and covariate (Petraitis et al., 2001). If the interaction showed a $P < 0.15$ we ran a heterogeneous slopes model that included this interaction in addition to the other factors; otherwise, we omitted the interaction from the subsequent model selection procedure (Glantz and Slinker, 2001). We removed factors or interactions sequentially if their P values were >0.25, starting this manual backward selection process with removing the variables with the highest P-value. Homoscedasity in the data was tested using the Levene's test. If variances among treatment groups were heterogeneous we used a log $(x + 1)$

Table 2
Results from ANCOVA-analysis on the effects of *Potentilla erecta*-removal on visitation rate and reproductive success variable (response) of four co-flowering herbs.

Plant species	Response variable	Additional variables in final model	Effect of treatment		
			DF	F	P
Carum carvi	Muscoids	–	1,14	0.013	0.911
	Syrphids	Block	1,5	0.004	0.954
	Fruit production		1,14	0.563	0.505
	Seed weight	–	1,14	0.469	0.505
Fragaria vesca	Dipterans	Block	1,9	3.836	0.082(*)
	Fruit production	–	1,16	0.229	0.638
	Seed weight	–	1,16	0.003	0.961
Ranunculus acris	Muscoids	–	1,14	0.155	0.700
	Syrphids	Treat × CFD	1,12	4.222	0.062(*)
	Fruit production	–	1,13	7.001	0.020*
	Seed weight	Block	1,5	0.267	0.627
Plantago lanceolata	Syrphids	Block*	1,10	0.798	0.395
	Fruit production	–	1,18	0.022	0.885
	Seed weight	Block*	1,9	1.526	0.248

Notes: A manual backward selection procedure was applied. Insect names refer to the flower visitation rates of the insect groups, fruit production and seed weights were the reproductive success variables used. Statistical analysis was based on mean values per block derived from means per plot for each variable and species. If variances among treatment groups were heterogeneous we transformed the data (log). Additional variables in the final model are Treat = treatment, Block and CFD = conspecific floral density. These variables are included in the final model if their $P = 0.10–0.25$, and if statistically significant they are indicated by (*) if $P < 0.10$ or * if $P < 0.05$ as the treatment effect. If none of the additional factors had a $P < 0.25$, this is shown by –. Denominator DF varies among species because of number of blocks species were observed in and within species dependent on the model selection.

transformation. The normality assumption was tested by using the residuals in Kolmogorov–Smirnov tests, wherein all residuals passed the tests (i.e., $P > 0.05$).

We used effect sizes for the visitation and reproduction variables of all focal species in the removal experiments to evaluate the overall interaction effects of the two experiments and to test the overall relationship between removal effects on pollinator visitation and effects on components of reproduction. Effects sizes in the meta-analysis were calculated as Hedges' d standardized effect size with MetaWin version 2 and are based on the mean values from each treatment including their standard deviations (Rosenberg et al., 2000). The mean visitation rates used here were based on the total visitation numbers of all visitor groups to obtain only one variable per species. The overall effect sizes are the weighted means and their significance is evaluated with bootstrap confidence intervals using the standard procedures in MetaWin (Rosenberg et al., 2000).

3. Results

3.1. The Potentilla-removal experiment

The flower visitation rate or reproductive success variables in two of four focal species showed marginally or statistically significant effects in response to the removal of the *Potentilla*-flowers (see

Table 2 for results of statistical analysis; Fig. 1 for visitation rates; Fig. 2 for components of reproductive success). The mean dipteran visitation rate (muscoids and syrphids pooled) to *Fragaria vesca* flowers was marginally significantly ($P = 0.082$) higher (four times) in removal plots than in control plots (Fig. 1a) indicating a potential competitive interaction with the removal species at this stage of pollination. There was no significant effect on the components of reproductive success (i.e., mean fruit production and seed weight) of *F. vesca*. The mean visitation rate of syrphids to *Ranunculus acris* also showed a marginally significant ($P = 0.062$) response to the experiment. The visitation rate to *R. acris* was more than twice as high in the control plots compared to in the removal plots, indicating a potential facilitative interaction with the removal species (Fig. 1b). The effect of the removal species on the components of reproductive success of *R. acris* was significantly positive, as seen by a 20% higher mean fruit production in control plots ($P = 0.02$, Fig. 2a). The mean visitation rate and reproductive success variables in the two other focal species (*Plantago lanceolata* and *Carum carvi*) were not significantly affected by the removal of *Potentilla*-flowers (all P's > 0.24; Tables 1 and 2).

3.2. The Trifolium-removal experiment

In three out of seven focal species, the flower visitation rate or reproductive success variables were marginally or significantly
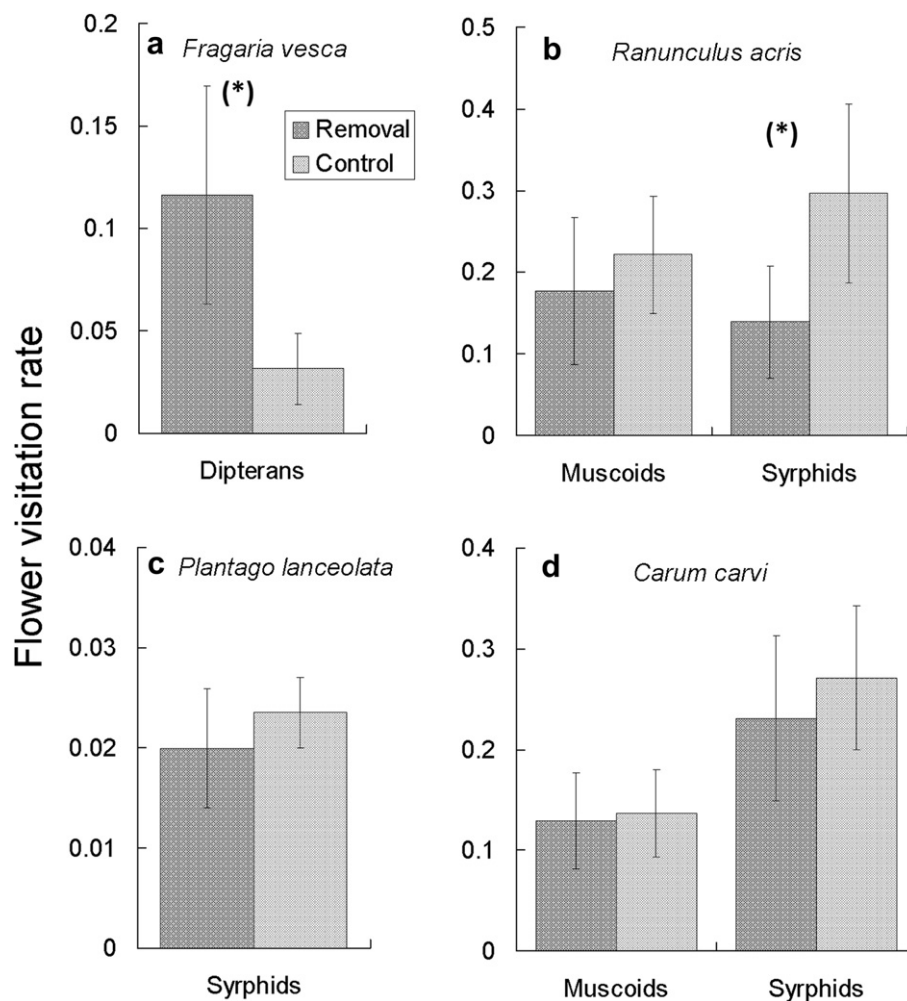

Fig. 1. The mean flower visitation rate of pollinator groups to flowers of the focal species (a–d) in control and treatment (removal of the attractive species *Potentilla erecta*) in the *Potentilla*-removal experiment in Rudsviki, Sognefjord, west-Norway, 2004. Means are per plot and treatment with ±1 SE.

altered by the experimental removal of *Trifolium* flowers (see Table 3 for results of statistical analysis; Fig. 3 for visitation rates and Fig. 4 for components of reproductive success). In *Campanula rotundifolia*, there was no significant removal effect on the flower visitation rate, but there was a significant ($P = 0.005$, Fig. 4a) treatment effect, with a nearly 100% increase in seed production in the control compared to in the removal plots. In *Centaurea jacea* there was only a marginal ($P = 0.104$) significantly higher mean visitation rate of dipterans to flowers in the control plots (152%; Fig. 3b), but the average seed weight of *C. jacea* was significantly higher (19%; $P = 0.009$, Fig. 4b). This result demonstrates a potential facilitative effect of treatment on reproduction, although fruit production was not significantly affected (Table 2). In *Knautia arvensis*, we also detected a marginally significant ($P = 0.076$) higher (127%; Fig. 3d) mean visitation rate of syrphids in the presence of the removal species. On the other hand, we found no significant effect of the experiment on the components of reproductive success in *K. arvensis*. All the visitation rate and reproductive success variables in the three other focal species (*Clinopodium vulgare*, *Euphrasia stricta*, *Prunella vulgaris* and *T. repens*) were not significantly affected by the removal of *Trifolium* flowers (all *P*'s > 0.14; Table 3).

3.3. Overall effect of the two removal experiments

We performed a meta-analysis to test whether there was an overall effect of our two experiments in the direction of either facilitation or competition for variables associated with pollination
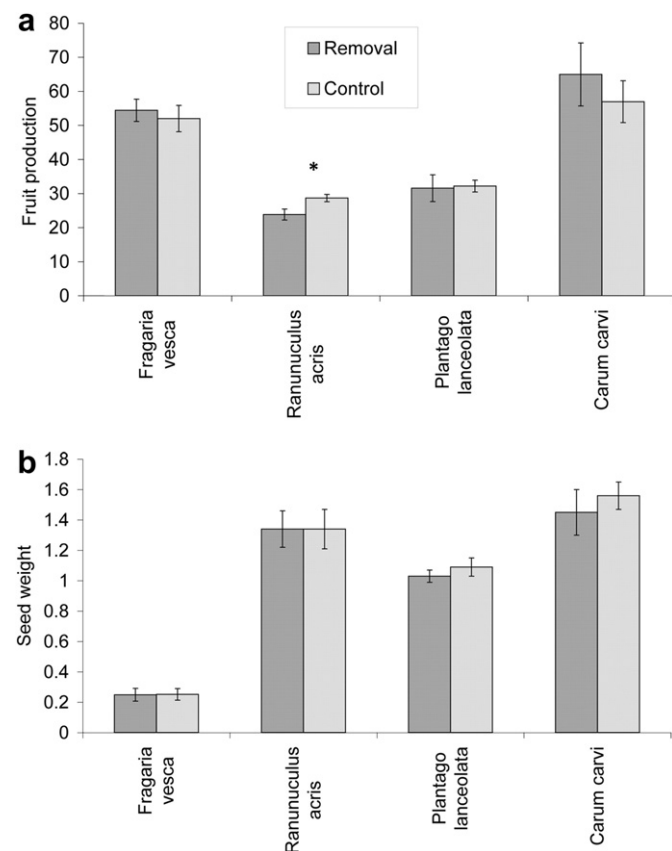

Fig. 2. The reproductive success (a) fruit production and (b) seed weight of focal plant species in control and removal plots in the *Potentilla*-removal experiment in Rudsviki, Sognefjord, west-Norway, 2004. Fruit production is mean number of viable fruits per infructescence and all seed weights are means in mg (except for *Fragaria vesca*: g). Means are per plot and treatment with ±1 SE.

Table 3
Results from ANCOVA-analysis on the effects of *Trifolium pratense*-removal on the visitation rate and reproductive success variable (response) of seven co-flowering herbs.

Plant species	Response variable	Additional variables in final model	Effect of treatment		
			DF	F	P
Campanula rotundifolia	Bumblebees	Block(*)	1,9	0.658	0.438
	Muscoids		1,19	0.103	0.752
	Seed production	Block	1,6	14.762	0.005*
	Seed weight	Block, CFD	1,6	0.504	0.504
Centaurea jacea	Dipterans (log)		1,6	3.676	0.104
	Bumblebees		1,6	0.093	0.773
	Fruit production	–	1,6	0.044	0.841
	Seed weight	Block	1,3	36.601	0.009*
Clinopodium vulgare	Bumblebees		1,11	1.461	0.252
	Fruit production	–	1,10	0.219	0.650
	Seed weight	Block	1,4	1.055	0.362
Euphrasia stricta	Muscoids		1,19	2.307	0.145
	Bumblebees	Treat × CFD, CFD*	1,17	2.355	0.143
	Seed production	Block*	1,9	0.472	0.509
	Seed weight	Block, CFD	1,8	0.089	0.774
Knautia arvensis	Muscoids		1,15	0.671	0.425
	Syrphids	Block	1,6	4.575	0.076(*)
	Bumblebees	CFD	1,14	0.442	0.517
	Fruit production	–	1,11	0.139	0.716
	Seed weight	–	1,11	0.303	0.593
Prunella vulgaris	Bumblebees	Block	1,7	1.808	0.221
	Fruit production	–	1,14	0.224	0.644
	Seed weight	Block(*), CFD	1,6	2.077	0.200
Trifolium repens	Bumblebees	–	1,18	0.417	0.527
	Fruit production	CFD(*)	1,11	1.398	0.262
	Seed weight	Block*, CFD	1,3	0.282	0.632

Notes: Significant treatment effects ($P > 0.05$) are marked with * and marginal significant effects are marked (*), see notes of Table 2 for all other notes.

success. The weighted mean effect sizes across all focal species for visitation rate, fruit production and seed weight for all species in the community were all negative (indicating facilitative effects: -0.12 for visitation rate; -0.22 for fruit production; -0.09 for seed weight), but none of these main effects were significant as bootstrap confidence intervals for all variables ranged across zero ($-0.35–0.11$ for visitation rate; $-0.55–0.07$ for fruit production; $-0.29–0.11$ for seed weight).

We also investigated the relationship between effect sizes for pollinator visitation and components of reproduction for all focal species to reveal whether there was an overall consistency for these components of pollination success for the whole community. There was no significant relationship between Hedges' d effect size for visitation rates and for fruit production ($R^2 = 0.07$, $P = 0.44$, DF = 9) nor between Hedges' d for visitation rates and Hedges' d for seed weight ($R^2 = 0.03$, $P = 0.60$, DF = 9).

4. Discussion

4.1. The relative importance of competition and facilitation

We found that most plant species in our study community did not interact strongly for pollination services (here: flower visitation rates and components of reproductive success). Still too few studies have assessed plant–plant interactions for pollination success above the single species-level to conclude whether this dominance of neutral interactions may be a general pattern in nature. Our study highlights the need to be careful in using interspecific competition as a general explanation for the observed variation in plant reproduction. The current study gives some indications that facilitative interactions for pollination-related variables may be relatively more important than competition. In particular the
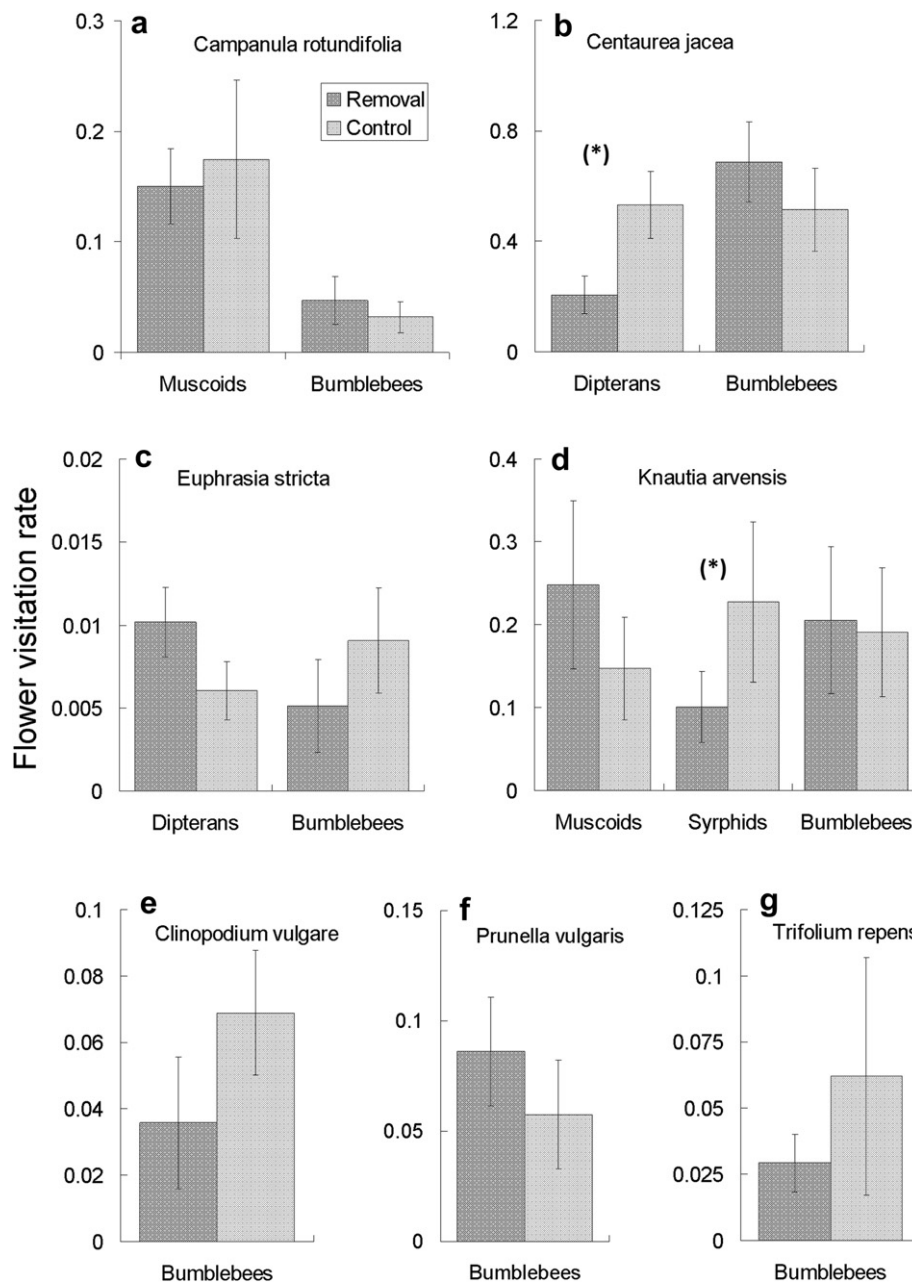
Fig. 3. The mean flower visitation rate of pollinator groups to flowers of seven focal species (a–g) in control and removal (removal of the attractive species *Trifolium pratense*) plots in the *Trifolium*-removal experiment in Rudsviki, Sognefjord, west-Norway, 2004. Means are per plot and treatment with ±1 SE.

effects on reproduction may be interpreted this way, since three focal species (*R. acris*, *C. jacea* and *C. rotundifolia*) experienced significant facilitative effects on components of their reproduction. Two of these three species also experienced marginally significant higher pollinator visitation in proximity of the attractive removal plant species. In those two focal species a more than twofold increase in flower visitation rate subsequently lead to a 20% higher fruit production in *R. acris* and 19% heavier seeds in *C. jacea* compared to plots from which the attractive species was removed.

4.2. Do characteristics of species contribute to the outcome of pollination interactions?

There are also possible biological explanations for facilitative, neutral and competitive interaction in our study. Interactions for

pollinator visitation may be modified by several traits. For example, species with similarities in flower colors may experience a collective positive density-dependence, attracting more pollinators when co-occurring (i.e., "Müllerian mimicry" Roy and Widmer, 1999). In our study, focal species showing tendencies toward facilitated pollinator visitation appeared to have similar flower colors to the removal species (The purple-flowered *C. jacea* and *K. arvensis* marginally affected by *T. pratense* and the yellow-flowered *R. acris* marginally affected by *P. erecta*), whereas the single focal species experiencing marginally competitive interaction for visitation had dissimilar flower colors (the white-flowered *F. vesca* vs. *P. erecta*). Color-stimulated facilitation for pollinator visitation may be explained by the general notion that pollinators prefer patches with high floral densities (e.g., "hot-spot-response" Thomson, 1981) and has been detected in a previous study (Hegland et al., 2009). In
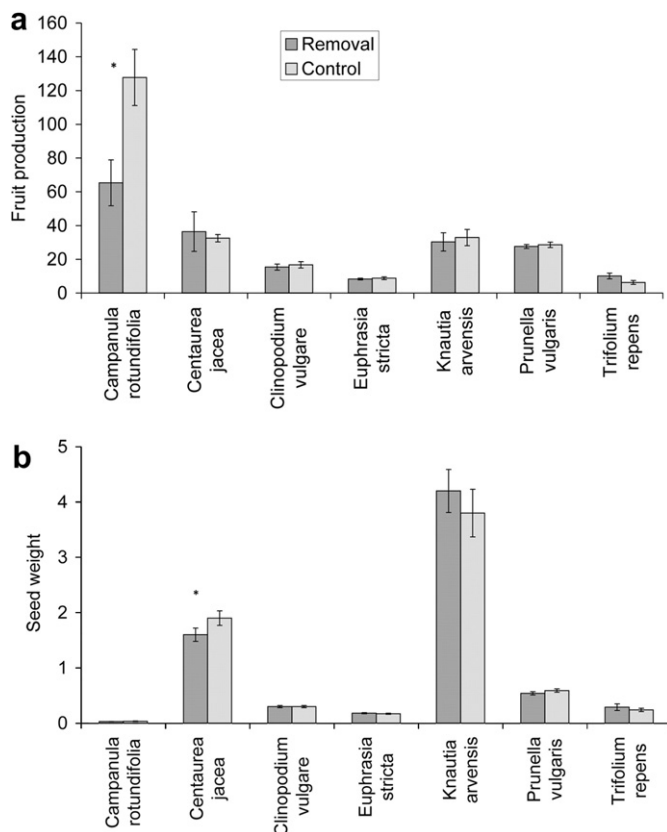
Fig. 4. The reproductive success (a) fruit production and (b) seed weight of focal plant species in control and removal plots in the *Trifolium*-removal experiment in Rudsviki, Sognefjord, west-Norway, 2004. Fruit production is mean number of viable fruits per infructescences, except values for *Campanula rotundifolia* and *Euphrasia stricta* which is mean number of viable seeds per fruit. All seed weights are means in mg. Means are per plot and treatment with ±1 SE.

addition, pollinators may not easily discriminate between flowers that resemble each other in color and shape and may therefore often switch between such species (Waser, 1986; Laverty, 1994; Chittka et al., 1997; Goulson, 2000). Consequently, species may exhibit a flower color-constancy rather than plant species-constancy (Waser, 1983; Goulson, 2000). Sargent et al. (2011) found that a focal plant species were less pollen-limited when co-occurring with close relatives, a finding that underlines the role of floral similarity in explaining facilitation for pollination services. One should bear in mind that insects may perceive floral colors in a different way than humans depending on factors such as the insects' specific color vision, the background color, the contrast with the background, and the floral color of the neighborhood (Chittka and Kevan, 2005).

In general, the treatment effects on components of reproduction showed much clearer statistical results than on pollinator visitation, probably because the temporal variation in pollinator visitation is much larger than for fruit production and to grasp the variation in pollinator visitation a larger sampling is required. To understand how interspecific interactions for pollinator visitation affect subsequent plant reproduction we may compare floral or reproductive traits of focal species showing significant effects against those showing non-significant effects on reproduction. Plants' anther position affects placement of pollen on pollinator bodies, and therefore plant species with similar stigma and anther positions have a greater probability of negatively affecting each other's reproduction, due to heterospecific pollination (e.g., Campbell and Motten, 1985; Fishman and Wyatt, 1999; Caruso and Alfaro, 2000).

The potential for such interference competition (Waser, 1983) was present within the study community as several of the focal species (*F. vesca* and *R. acris* in the *Potentilla*-removal experiment; *T. repens* in the *Trifolium*-removal experiment) had similar pollen placement on pollinator bodies as the removal species. However, in this study the mechanism did not cause competitive effects as no focal species displayed competitive effects for reproduction.

Interestingly, two of the focal species (*R. acris* and *C. jacea*) that responded reproductively to the removal experiments have also been found to respond strongly to pollen supplementation experiments within the same study community (Hegland and Totland, 2008). Some authors have suggested that altered visitation rates (either competition or facilitation) will most likely influence reproduction if species are highly dependent on outcrossing (Waser, 1983; Feinsinger et al., 1991; Fishman and Wyatt, 1999), because of self-incompatibility accompanied by pollen limitation (Knight et al., 2005). Increased visitation may be similar to the effect of higher pollen loads and pollen-limited plant species may therefore be most predisposed to experience reproductive effects of plant—plant interactions for pollinator visitation (Rathcke, 1983; Moeller, 2004). The removal experiment may thus have increased pollen limitation on reproduction in the two focal species, *R. acris* and *C. jacea*. According to such reasoning species with alternatives to outcrossing are less likely to be affected by changes in flower visitation rate. Our study supports such a view since *R. acris* and *C. jacea*, are both described as having very low selfing rates (Table 1). The two focal species that had no reproductive response after marginally significant changes in pollinator visitation (*F. vesca*, *K. arvensis*) all reproduce substantially through selfing. In addition, *C. rotundifolia*, which showed increased reproductive success without changes in visitation rates, is also reported as mainly outcrossing (references in Table 1). Thus, our results may provide additional support for the importance of breeding systems for outcomes of plant—plant interactions for pollination (Waser, 1983; Feinsinger et al., 1991; Fishman and Wyatt, 1999; Knight et al., 2005; Aguilar et al., 2006). The fact that few species in the study community appeared to be strongly pollen-limited (Hegland and Totland, 2008) may also explain why relatively few focal species respond reproductively to the manipulations in the present study.

4.3. Methodological issues in multi-species experiments

In addition to the possible overestimation on interspecific interactions and trait-based explanations (see 4.1 and 4.2, respectively), the reason why so many species did not respond significantly to the removal experiments in terms of altered visitation may relate to sampling design and inherent problems with field experiments in a multi-species setting. Methodologically, the marginally significant indications of both competition (*F. vesca*) and facilitation (*R. acris*, *C. jacea*, *K. arvensis*) for pollinator visitation, as opposed to significant results for reproduction, highlight one of the challenges in a multi-species comparison: it may be difficult to attain a large enough sample size to account for the variability of pollinators' response to variation in flower density of many species simultaneously. This may be a general problem in pollination interaction studies as it appears that several single-interaction investigations also have failed to detect changes in visitation rates when reproduction was indeed affected by removal of interacting species (e.g., McGuire, 1993; Grabas and Laverty, 1999; Bell et al., 2005). Field experiments such as ours also occur within a setting where many potentially influential factors are uncontrolled, in particular variations in floral density of other species than the removal species and variations in flower diversity (e.g., Ghazoul, 2006). Moreover, our experiments were conducted on a small spatial scale that may not affect all pollinators' behavior to a similar

extent, subsequently influencing visitation of plant species differently. In our study it appears that fly visitation may be relatively more influenced than visitation of bumblebees which concurs with such scale-related explanation.

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