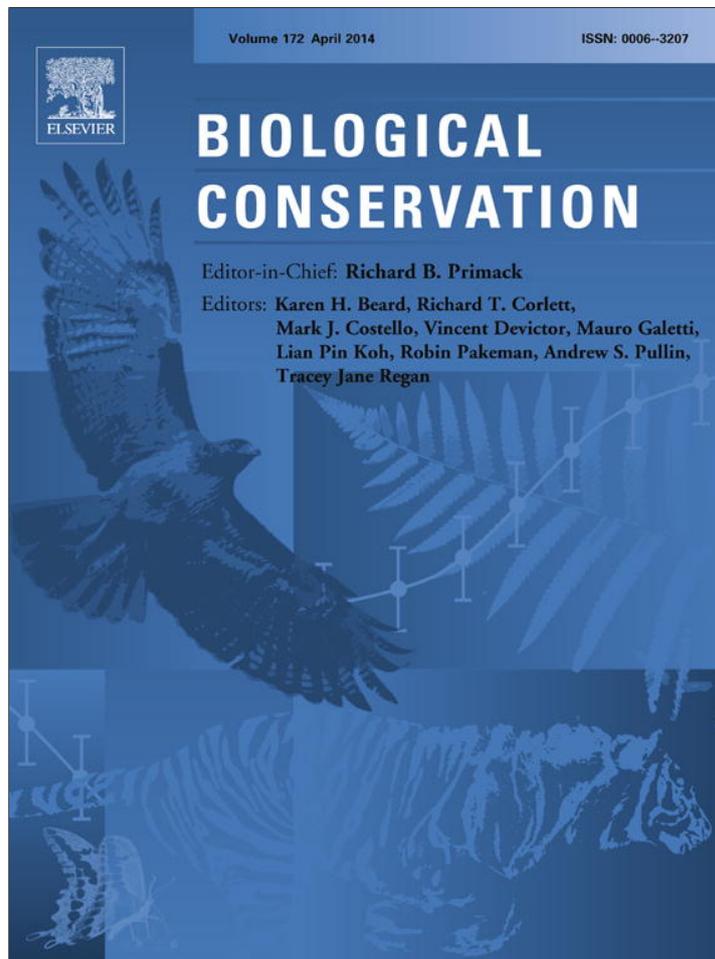


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# Late-season mass-flowering red clover increases bumble bee queen and male densities



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## ABSTRACT

Spatiotemporal resource continuity promotes persistence of mobile animal populations. Current agricultural landscapes are poor in flowers resources for bumble bees. Available forage crops are predominantly early-season mass-flowering crops (MFC). It has been suggested, but not tested, that scarcity of late-season flower resources are limiting bumble bee populations. We examined whether addition of late-season flowering red clover affected worker, queen and male bumble bee densities. Bumble bees were surveyed in flower-rich uncultivated field borders across 24 landscapes (radius 2 km) with or without a clover field in the centre, varying in semi-natural grassland (SNG) and early MFC availability. Clover fields had over ten times higher worker densities compared to field borders, suggesting red clover as favoured forage. Five times more queens and 71% more males were found in landscapes with clover fields compared to control landscapes, despite these fields constituting less than 0.2% of the landscape area. Both MFC and SNG increased the density of males, but only in the presence of clover fields. Our results suggest that late-flowering red clover positively affects bumble bee reproduction, likely by increasing temporal resource continuity. Interventions such as flower strips can thus have mitigating effects if they release population regulation by late-season resource bottle-necks.

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## 1. Introduction

Insect pollinators have been declining for decades in many agricultural areas of the world (Bartomeus et al., 2013; Biesmeijer et al., 2006; Bommarco et al., 2012; Dupont et al., 2011). The most important cause of these declines is probably large-scale agricultural intensification in agricultural landscapes. This intensification has resulted in loss of semi-natural habitat and increased use of agrochemicals, leading to loss of nesting and foraging resources for pollinators and toxic effects of insecticides (Brittain et al., 2010; Carvell et al., 2006b; Kremen et al., 2002; Rundlöf et al., 2008; Williams et al., 2010). In the northern temperate regions, bumble bees are frequent visitors of wild plants and important native pollinators of crops (Bommarco et al., 2012; Steffan-Dewenter et al., 2002; Winfree et al., 2008). Together with other wild bees, bumble bees can provide pollination

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insurance and complementation (Garibaldi et al., 2013) in times of honey bee deficit (Breeze et al., 2011) and decline (Winfree et al., 2008). We need to identify the causes of the decline of bumble bees and, based on that understanding, develop efficient interventions that enhance bumble bee densities in the landscape.

The distribution of resources over time and space is an important factor influencing the dynamics of animal populations. For a bumble bee colony, a continuous and readily available supply of food is crucial for successful establishment, growth and finally production of sexuals (males and queens) (Westphal et al., 2009; Williams et al., 2012). Bumble bees have a strongly male-biased sex allocation, partly explained by a more than three times higher cost to produce queens compared to males (Beekman and Van Stratum, 1998; Bourke, 1997). Flower availabilities during colony foundation in early spring, and colony reproduction in mid to late summer have been suggested as important resource bottle-necks in current landscapes (Fitzpatrick et al., 2007; Pelletier and McNeil, 2003). Especially the availability of early-season flower resources has often been studied in relation to bumble bee population regulation. For instance, mass-flowering oilseed rape (*Brassica napus* L.) in early spring has been shown to contribute to early colony growth and colony size in three bumble bee species, but did not

result in higher reproductive success (Carvell et al., 2011; Herrmann et al., 2007; Westphal et al., 2003, 2009; Williams et al., 2012). The promotion of populations is, however, most prominent for species that are able to exploit ephemeral and scattered but very rewarding flower resources (Walther-Hellwig and Frankl, 2000; Westphal et al., 2006). The lack of influence on reproductive success has been suggested to be caused by a deficiency of forage resources in late season (Westphal et al., 2009; Williams et al., 2012), but this hypothesis has not been tested.

Agricultural landscapes dominated by arable crops have lost many wild plants (Persson and Smith, 2013). In temperate Europe, large areas of oilseed rape can provide abundant forage resources early in the season, while fewer crops flowering in late summer are cultivated. Lack of flower resources in this period, when bumble bee colonies are large and resource-demanding, is possibly limiting bumble bee population growth.

Red clover (*Trifolium pratense* L.) is a late-flowering crop that is cultivated in monocultures for seed intended for animal fodder production in grass-clover leys. The cultivated area of red clover seed production has decreased dramatically; in Sweden by 90% during the last 70 years, down to just over 2000 ha today (Bommarco et al., 2012). The same trend has been reported from the UK (Carvell et al., 2006b), Belgium, and the Netherlands (Kleijn and Raemakers, 2008). Red clover is one of the most favoured plants for bumble bee pollen and nectar foraging in summer (Carvell et al., 2006a; Goulson et al., 2005; Kleijn and Raemakers, 2008), but the relative portion of red clover pollen collected by bumble bees is today considerably lower compared to before the 1950s (Kleijn and Raemakers, 2008). The occurrence of remaining monocultures of red clover in some landscapes allows us to test whether this resource affects bumble bee density.

In this study, we used information from whole landscape surveys of bumble bee worker, queen and male densities in flower-rich uncultivated linear habitat elements within 24 circular 2 km radius landscapes. Thirteen of the landscapes had a 4–16 ha red clover monoculture for seed production in the centre, which was also surveyed for bumble bees. This design allowed us to investigate the impact of spatiotemporal flower resource availability on the density of foraging bumble bee workers. We could also assess the densities of queens and males and use these as proxies for reproductive success within a year. We were particularly interested in assessing the potential benefit of adding a late-season mass-flowering resource to a landscape. Based on the study design, we tested the following hypotheses;

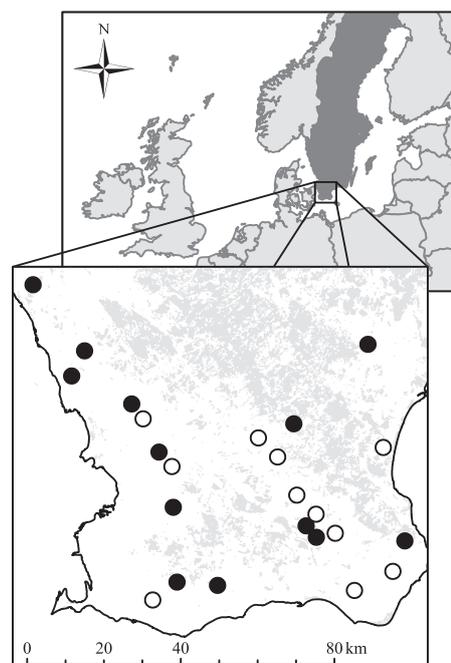
- (1) Red clover seed fields are favoured as foraging habitats over flower-rich semi-natural vegetation in linear elements. This is particularly true for workers because they forage for the entire colony and thus need high yielding resources. The attraction is also expected to be stronger for workers of species shown to be good at utilising larger areas of other mass-flowering resources.
- (2) Clover fields attract workers. Because of this the density of workers in linear elements will, during the flowering period of red clover, be lower in landscapes with, as compared to landscapes without red clover seed cultivation.
- (3) The addition of late-season flower resources promotes production of sexuals (males and queens), by increasing resource continuity in landscapes. In particular, we hypothesise that densities of queens and males in linear elements are higher in landscapes with red clover seed cultivation, compared to in landscapes without this late-season flower resource. This effect will be particularly pronounced in landscapes which also have a high availability of early-season flower resources.

## 2. Methods

### 2.1. Study design and landscape context

The study was conducted in 2008 in the province of Skåne, a region of approximately 100 km × 100 km in southernmost Sweden (Fig. 1). The province is dominated by agriculture and contains a range of landscape types; from regions of simple and intensively managed crop dominated landscapes to regions of more complex landscapes that also include extensively managed grasslands and forest (Persson et al., 2010). In the study region, we selected 24 independent ( $\geq 4$  km apart) circular landscapes, radius 2 km (Fig. 1). The 2 km landscape radius was selected because bumble bees are expected to have potential foraging ranges around this scale (Greenleaf et al., 2007) and react to the landscape context at this or even larger radii for some species (Westphal et al., 2006). Landscapes were selected along gradients of varying proportions of semi-natural grasslands (SNG) and of mass-flowering crops (MFC). Thirteen of the landscapes contained a field for red clover seed production (mean size: 8.2 ha, range: 4–16 ha). In the remaining 11 landscapes, no red clover seed production occurred within the 2 km radius.

Digital land use data from the Integrated Administration and Control System (IACS), a yearly updated database on farmland in Sweden, was used in ArcGIS 9.2 (ESRI, Redlands, CA) to characterise the landscapes. The selected landscapes were all dominated by agricultural land (55.5–94.3%) but varied in the proportions of SNG (0–16.4%) and MFC (0.9–15.5%). The SNG in the region consists of permanent grasslands traditionally used for grazing, and more rarely for making hay. These grasslands often contain a low density of flowers, but are important nesting habitat for bumble bees (Öckinger and Smith, 2007). The MFC included oilseed rape, turnip rape (*Brassica rapa* L. ssp. *oleifera*), oilseed radish



**Fig. 1.** Location of the study region in the southernmost part of Sweden and of selected landscapes with (filled circles) and without (open circles) red clover seed cultivation. Circles represent landscapes with a 2 km radius and grey areas in the lower map are forest.

(*Raphanus sativus* L. var. *oleiformis*), field bean (*Vicia faba* L.), flax (*Linum usitatissimum* L.), strawberry (*Fragaria x ananassa* (Weston) Decne & Naudin) and cultivation of other berries and fruits, but not red clover seed cultivation. Winter sown oilseed rape, flowering predominantly in May, accounted for 95.8% of the total area of MFC. The proportion of MFC in the landscape thereby represents the amount of early-season flower resources in this study. Neither arcsine-root-transformed proportions of agricultural land ( $F_{1,22} = 1.15$ ,  $P = 0.30$ ), SNG ( $F_{1,22} = 0.32$ ,  $P = 0.58$ ) nor MFC ( $F_{1,22} = 0.88$ ,  $P = 0.36$ ), differed between landscapes with and without red clover seed cultivation. The proportion of SNG and MFC were not sensitive to the spatial scale selected, because they were positively related between landscape radii 2 and 3 km (Pearson correlations; SNG  $r = 0.94$ ,  $P < 0.0010$ ; MFC  $r = 0.84$ ,  $P < 0.0010$ ;  $N = 24$ ). The proportions of SNG and MFC were negatively related when measured at a landscape radius of 2 km (for details see [Table A1 in the online Supplementary appendix](#)).

## 2.2. Bumble bee survey

Each selected landscape was divided into four equally sized sectors, representing the north-east, north-west, south-east and south-west parts of the landscape. In each sector, flower-visiting workers, queens and males of true social bumble bee species were surveyed in three 100 m<sup>2</sup> transects (1 m wide and 100 m long) of flower-rich uncultivated linear field borders (with grass and herbaceous vegetation) next to fields with non-flowering annual (predominantly cereals or sugar beets) and semi-permanent (cultivated pastures, leys and fallows) crops. Linear transects were evenly distributed across each landscape and situated 0.5–2 km from the landscape centre. Four survey transects of 50 m<sup>2</sup> (1 m wide and 50 m long) were also established in each clover seed field, parallel to a field border. Two were located 4 m from the field border and two 12 m from the same field border.

The average number of surveys per transect in linear habitats was  $1.67 \pm 0.08$  (mean  $\pm$  s.e.m.), while transects in clover field were all surveyed twice. Surveys were conducted on days with warm (>16 °C), dry and calm (<5 on the Beaufort Wind Force Scale) weather. If a linear habitat was surveyed more than once, the two surveys were separated by approximately a month. The time between surveys in clover fields was approximately 2 weeks. Transects in linear elements in the landscapes were surveyed between 26 June and 15 August and clover field transects during the flowering period of red clover between 26 June and 29 July. The survey period can be considered as late season based on the typical activity period of bumble bees in Scandinavia, with early species founding colonies in March/April and peaking in June, and late species founding in May/June and peaking in July/August (Löken, 1973). The red clover seed fields thus represent a late-season flower resource in the region.

Given the time of the surveys, the encountered bumble bee queens can be expected to be newly produced and not overwintered queens. To avoid major impacts on bumble bee populations, no queens were collected and killed. Instead, queens were determined to species in the field. Queens were separated from workers based on size, and differences in colour pattern. Bumble bee workers and males were collected, stored in individual 5 ml tubes filled with ethanol (70%), and determined to species and caste in the lab following Löken (1973), Prys-Jones and Corbet (1986) and Edwards and Jenner (2005). Because of difficulties in morphologically separating males of *B. terrestris* and *Bombus cryptarum* Fabricius, females of *Bombus lucorum* L., *Bombus magnus* Vogt and *B. cryptarum*, and males of *B. lucorum* and *B. magnus*, these were grouped based on caste and species group.

## 2.3. Flower resource survey

Local flower density was estimated by counting the number of flower units in each transect at each bumble bee survey. Flower units were individual flowers for Convolvulaceae, Papaveraceae and Rosaceae (except *Filipendula*), the number of flower clusters for Brassicaceae and *Filipendula*, the number of flower heads for Asteraceae, Dipsacaceae and Plumbaginaceae, the numbers of racemes for Fabaceae and Boraginaceae, and the number of flower stalks for Campanulaceae, Caryophyllaceae, Clusiaceae, Lamiaceae, Onagraceae, and Scrophulariaceae. The flower plant diversity was calculated as the total number of flowering plant species in the transects during the flower density surveys. The flower units differed depending on the type of flower, are not directly comparable between species and cannot directly be translated to area flower cover. The measure is, however, comparable among landscapes (Williams and Kremen, 2007). In addition, from another data set collected over 2 years in 16 semi-natural grasslands in the same region, we can confirm a positive relationship between the flower unit density and actual flower cover in our study region (Pearson correlation  $r = 0.68$ ,  $N = 32$ ; Rundlöf, Bommarco & Smith, unpublished data).

The ( $\log_e$ -transformed) density of flowers (i.e. number of flower units per 100 m<sup>2</sup>) was higher in clover field transects compared to transects in linear elements (general linear mixed model, SAS proc MIXED, with landscape identity as random factor,  $F_{1,12} = 1263.41$ ,  $P < 0.0010$ , mean  $\pm$  s.e.m.  $23,654 \pm 2107$  flower units in clover fields and  $607 \pm 72$  flower units in linear elements (based on raw data)), with an almost 40 times difference in flower density. There was no difference in flower density in linear element transects between landscapes with red clover compared to landscapes without (general linear model, SAS proc GLM,  $F_{1,22} = 0.25$ ,  $P = 0.62$ , mean  $\pm$  s.e.m.  $607 \pm 72$  flower units in linear elements in landscapes with clover and  $532 \pm 57$  flower units in linear elements in landscapes without clover). There was, however, a higher species richness of flowering plants in linear element transects in landscapes without red clover compared to landscapes with red clover ( $F_{1,22} = 11.28$ ,  $P = 0.0028$ , mean  $\pm$  s.e.m.  $22.08 \pm 1.43$  flower species in linear elements in landscapes with clover and  $29.18 \pm 1.56$  flower species in linear elements in landscapes without clover).

## 2.4. Data analysis

Bumble bee densities were calculated as the average number of individuals per 100 m<sup>2</sup> across transects and sampling rounds in a landscape, to yield comparable densities independent of the transect area surveyed in a landscape. Each combination of landscape identity, habitat and cast was thus represented by the average number of bumble bees per 100 m<sup>2</sup> of transect in a linear transect or in a clover field alike.

We performed two separate sets of analyses on effects on bumble bee densities. First, we tested for differences in habitat preference and if this depended on caste (worker, queen, or male bumble bees). We used data from clover fields and linear elements only from landscapes in which a clover field was present. Total bumble bee densities were related to habitat (clover field or linear element), caste and their interaction, both excluding and including local flower density, and the interaction between habitat and flower density as covariates. We also performed analyses of the six most abundant species separately, but here queen densities were excluded due to low numbers. For the species specific densities, workers and males were related to habitat (clover field or linear element), caste and their interaction, with local flower density as a covariate. If  $P < 0.1$  for the interaction habitat\*caste, the slice option was used to separately test differences in worker and male, and for all species also queen, densities between the two habitats.

With the slice option, tests of simple main effects can be performed in the presence of a significant interaction to reveal the effect of one factor given the different levels of the other factor (Littell et al., 2006).

Second, we tested for differences in density of bumble bees depending on whether the landscape had or did not have a red clover seed field. At the same time, we tested if the impact of landscape types (with or without clover) depended on caste and proportions of MFC and SNG in the landscape. We used data from only linear elements in landscapes with and without red clover seed cultivation. Bumble bee densities were analysed in relation to caste, landscape type (with or without red clover seed cultivation in the landscape), proportions of SNG and MFC in the landscapes, and the interactions between caste, landscape type, and proportions of SNG and MFC. Local flower density was used as a covariate to account for variation in local habitat quality. We also performed analyses for workers and males of the six most abundant species (queen densities excluded due to low numbers). For the species specific densities, bumble bee densities of workers and males were related to landscape type (with or without red clover seed cultivation in the landscape), caste and their interaction, with local flower density as a covariate. Interactions where  $P < 0.1$  were interpreted using separate models. For example, when the interaction caste\*landscape type\*SNG showed  $P < 0.1$ , we performed separate analyses for the three castes to evaluate the interaction landscape type\*SNG.

Data was analysed in SAS 9.2 for Windows (SAS Institute Inc., Cary, NC) using general linear mixed models (proc MIXED). This type of model can handle data from unbalanced designs (Littell et al., 2006), which we have with 13 landscapes with red clover seed cultivation and 11 landscapes without clover. Bumble bee and flower densities were  $\log_e$ -transformed prior to analysis, and residual plots were used to assess model assumptions of normally distributed residuals. Denominator degrees of freedom were estimated using the Kenward–Roger method (Littell et al., 2006). Landscape identity was included as a random factor, to ensure that the densities of the different castes groups were not treated as independent estimates.

The landscape proportions of SNG and MFC were negatively related, but since the Pearson correlation coefficient ( $r$ ) was  $< 0.60$  and the variance inflation factor (VIF) was  $< 2$  (Table A1, online Supplementary appendix) the two variables were included in the same model. The two proportions were not related to local flower density (Table A1).

### 3. Results

In total 5516 bumble bees (*Bombus*) of twelve species were found among workers (4266), queens (57), and males (1193) (Tables A2–3, online Supplementary appendix). The six most abundant species were; *B. terrestris* (61.8%, including also *B. cryptarum* for males), *B. lapidarius* L. (29.2%), *B. pascuorum* Scopoli (2.8%), *B. hortorum* L. (1.9%), *B. sylvarum* L. (1.4%), and *B. subterraneus* L. (1.1%). The remaining species; *B. soroeensis* Fabricius, *B. lucorum* (including *B. magnus* and *B. cryptarum* for females and *B. magnus* for males), *B. hypnorum* L., *B. pratorum* L., *B. muscorum* L., and *B. ruderarius* Müller, each constituted less than 1% of the total community.

#### 3.1. Habitat preference

The analysis of habitat preference showed that bumble bee densities differed between clover fields and linear elements, but the difference between these habitats depended on caste as seen in the interaction between habitat and caste (Table 1 and Fig. 2).

Also in the analyses of each bumble bee species separately, the densities of all six species considered were explained by an interaction between habitat and caste (Table 1).

Workers were more abundant in clover fields than in linear elements in the same landscapes (Table 1 and Fig. 2). Including flower density in the model resulted in workers still being more abundant in clover fields ( $F_{1,20} = 19.60$ ,  $P < 0.0010$ ). For all six considered species, the density of workers was higher in clover fields than in linear elements (Table 1).

Queens were, just as workers, more abundant in clover fields than in linear elements in the same landscapes (Table 1 and Fig. 2). When including flower density in the model, queen density no longer showed a difference between linear elements and clover fields ( $F_{1,20} = 0.02$ ,  $P = 0.89$ ).

Males were more abundant in linear elements than in clover fields in the same landscape (Table 1 and Fig. 2). When including flower density in the model, males were still more abundant in linear elements, but not significantly so ( $F_{1,20} = 3.04$ ,  $P = 0.097$ ). For *B. hortorum*, *B. pascuorum*, *B. subterraneus* and *B. terrestris* there were no differences in male density between habitats (Table 1). The density of *B. lapidarius* males was higher in linear elements than in clover field, and so was that of *B. sylvarum* males, but not significantly so (Table 1).

Bumble bee density was not related to local flower density ( $F_{1,19} = 1.23$ ,  $P = 0.28$ ) and there was no habitat specific impact of flower density, indicated by the interaction between flower density and habitat type ( $F_{1,15} < 0.01$ ,  $P = 0.97$ ).

#### 3.2. Impact of landscape type

When comparing bumble bee densities in similar linear habitats in landscapes with or without red clover seed cultivation, landscape type (red clover or no red clover seed cultivation) and caste (worker, queen and male) interacted both with the proportion of SNG and with the proportion of MFC in explaining the density of bumble bees in linear elements (Table 2 and Fig. 3). The densities of different bumble bee species in linear elements responded differently to presence–absence of red clover seed cultivation in the landscape and there were sometimes also differences between cast within species (Table 3). The density of *B. pascuorum* did not differ between landscapes with and without red clover seed cultivation, and there was no interaction between landscape type and caste (Table 3). The density of *B. hortorum* was higher in linear elements in landscapes without red clover seed cultivation, independently of caste (workers or males; Table 3). For the densities of *B. sylvarum*, *B. terrestris* and possibly, although not significantly so, also for *B. lapidarius* and *B. subterraneus*, there was an interaction between landscape type and caste (Table 3).

The density of *B. terrestris* was positively related to the local density of flowers, and this trend was also found for *B. sylvarum*, but not significantly so (Table 3). The densities of the other four species were not related to local flower density (Table 3).

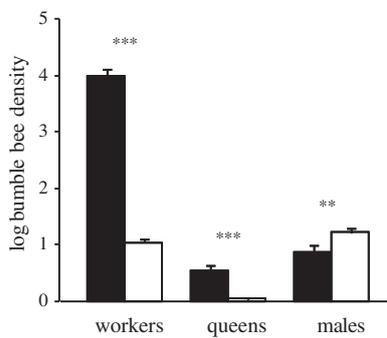
None of the predictors explained the overall density of workers (Fig. 3a and c), although there were non-significant tendencies that the worker density was higher in linear elements in landscapes without red clover seed cultivation than in landscapes with clover and that landscape type interacted with SNG in explaining worker density (Fig. 3b and Table 2). When separately testing the impact of SNG on worker densities, no relationships were found; neither for landscapes with red clover seed cultivation ( $F_{1,9} = 2.35$ ,  $P = 0.16$ ) nor landscapes without clover ( $F_{1,7} = 0.26$ ,  $P = 0.62$ ). The density of *B. sylvarum* workers was higher in landscapes without red clover seed cultivation, while there was no difference for *B. terrestris*. *B. lapidarius* or *B. subterraneus* workers (Table 3).

The density of queens was positively related to local density of flowers and showed a tendency to be higher (at level of

**Table 1**  
Density (individuals per 100 m<sup>2</sup>) of all bumble bees and of the six most abundant bumble bee species in relation to habitat (clover field or flower-rich linear habitat element), caste (males and workers, and for all species also queens) and their interaction. If  $P < 0.1$  for the interaction term, the slice option was used to separately test differences in worker and male, and for all species also queen, densities between the two habitats. Mean bumble bee densities ( $\pm$ s.e.m.) in clover fields and linear habitat elements can be found in Fig. 2 and for individual species in Table A2 in the online Supplementary appendix.

Species	All castes						Tests of effect slices					
	Habitat		Caste		Habitat*caste		Workers		Males		Queens	
	F <sub>1,12</sub>	P	F <sub>1,12</sub>	P	F <sub>1,12</sub>	P	F	P	F	P	F <sub>1,34</sub>	P
All species	158.30	<0.0010	311.81 <sub>2,24</sub>	<0.0010	249.62 <sub>2,24</sub>	<0.0010	596.16 <sub>1,34</sub>	<0.0010	8.58 <sub>1,34</sub>	0.0061	17.46	<0.0010
<i>Bombus hortorum</i> L.	12.94	0.0037	10.19	0.0077	10.36	0.0074	23.23 <sub>1,24</sub>	<0.0010	0.08 <sub>1,24</sub>	0.78		
<i>Bombus lapidarius</i> L.	56.30	<0.0010	260.08	<0.0010	184.92	<0.0010	214.19 <sub>1,24</sub>	<0.0010	11.59 <sub>1,24</sub>	0.0024		
<i>Bombus pascuorum</i> Scopoli	3.79	0.075	6.23	0.028	4.09	0.066	7.87 <sub>1,24</sub>	0.0099	0.04 <sub>1,24</sub>	0.84		
<i>Bombus subterraneus</i> L.	11.61	0.0052	7.88	0.016	15.47	0.0020	27.03 <sub>1,24</sub>	<0.0010	0.25 <sub>1,24</sub>	0.62		
<i>Bombus sylvarum</i> L.	2.05	0.18	12.23	0.0044	14.05	0.0028	14.53 <sub>1,23</sub>	<0.0010	4.03 <sub>1,23</sub>	0.057		
<i>Bombus terrestris</i> <sup>a</sup> L.	176.94	<0.0010	116.24	<0.0010	255.98	<0.0010	422.31 <sub>1,24</sub>	<0.0010	0.30 <sub>1,24</sub>	0.59		

<sup>a</sup> Includes also *B. cryptarum* for males.



**Fig. 2.** Density (log<sub>e</sub> individuals per 100 m<sup>2</sup>) of bumble bee workers, queens, and males in red clover fields used for seed production (filled bars) and in flower-rich uncultivated linear habitat elements (open bars) in the surrounding landscapes. N = 13 and error bars represent s.e.m. Significance levels \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

significance  $P = 0.055$ ) in landscapes with, compared to without, red clover seed cultivation (Fig. 3d and Table 2). Queen density was neither related to proportion SNG nor MFC (Fig. 3e–f).

The density of males increased with increasing proportion SNG, but only in landscapes with red clover seed cultivation (clover:  $F_{1,9} = 8.97$ ,  $P = 0.015$ ; no clover:  $F_{1,7} = 0.37$ ,  $P = 0.56$ , Fig. 3h). Similarly, male density increased with increasing proportion MFC in the landscape, but only in landscapes with red clover seed cultivation (clover:  $F_{1,9} = 6.24$ ,  $P = 0.034$ ; no clover:  $F_{1,7} = 0.36$ ,  $P = 0.57$ , Fig. 3i). The density of males was not related to local flower density (Fig. 3g). The density of *B. sylvarum* males did not differ between landscape types (Table 3). For *B. terrestris*, and possibly, but not

significantly so, also for *B. subterraneus* and *B. lapidarius*, the density of males was higher in landscapes with red clover seed cultivation compared to those without (Table 3).

#### 4. Discussion

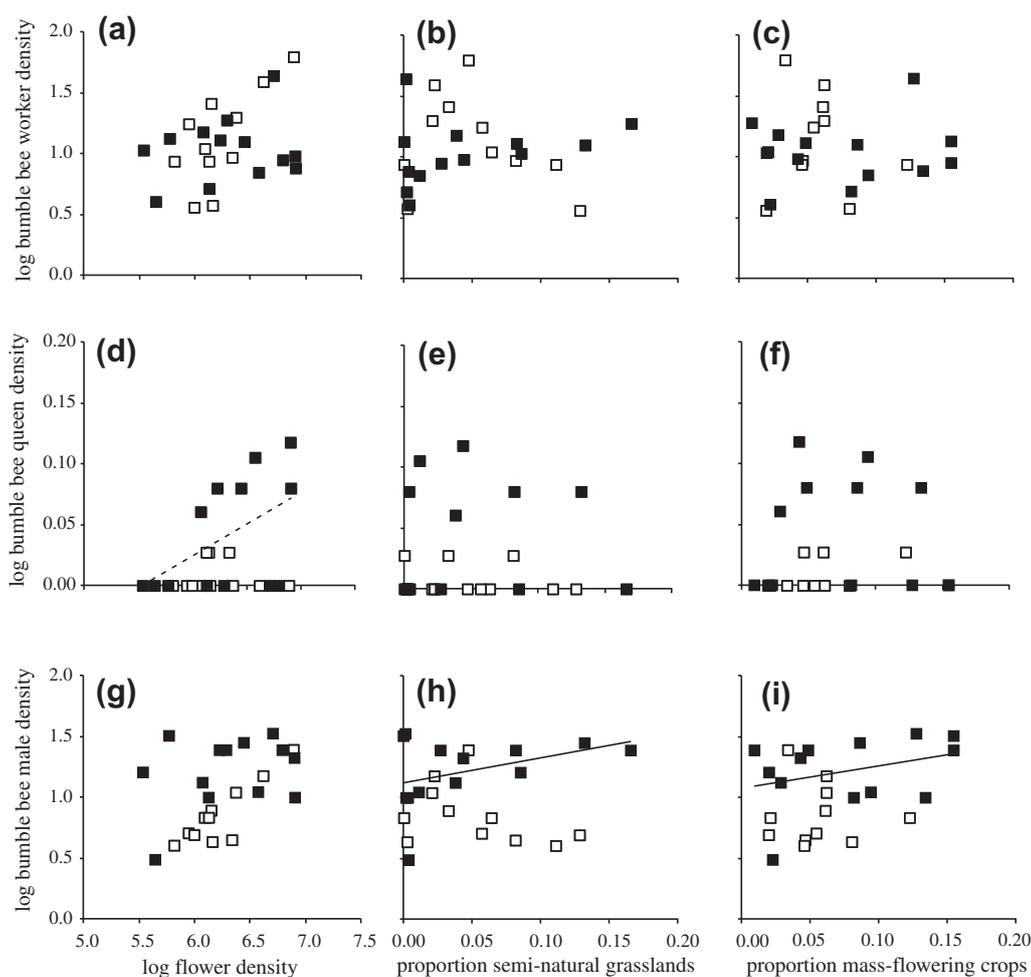
##### 4.1. Attractiveness of habitats

As predicted, we found that late-season mass-flowering red clover strongly attracts foraging bumble bees, particularly worker bees. The average density of workers was over ten times higher in red clover seed fields compared to flower-rich uncultivated field borders. Forage resource availability is limiting the size of bumble bee colonies and large colonies are more likely to achieve successful reproduction (Pelletier and McNeil, 2003; Westphal et al., 2009; Williams et al., 2012). We thus expect clover fields to both impact colony fitness and bumble bee worker distribution. Sown areas of flowers are part of European agri-environment schemes to mitigate the loss of forage resources for flower-visiting insects (Haaland et al., 2011), but such interventions are not based on a true understanding of factors limiting population persistence and growth of target organisms. An observed high density of flower visiting insects in sown patches of flowers does not tell us much about population level effects and might simply be a result of aggregation (Scheper et al., 2013).

An interesting observation in the study is the difference in habitat selection by workers and males, where worker density was higher in clover fields and male density in linear elements. Differences in habitat preferences between castes, and likely also

**Table 2**  
Density (individuals per 100 m<sup>2</sup>) of bumble bees in relation to local flower density, landscape type (red clover or no red clover seed cultivation), caste (worker, queen and male), proportion semi-natural grassland and proportion mass-flowering crops other than clover in the landscape and interaction between predictor variables. Results are presented for summed densities of all castes and for separate models for workers, queens and males. Densities in relation to predictors in Fig. 3.

	All castes		Workers		Queens		Males	
	F <sub>df</sub>	P	F <sub>1,17</sub>	P	F <sub>1,17</sub>	P	F <sub>1,17</sub>	P
Flower density (FDE)	2.06 <sub>1,17</sub>	0.17	1.47	0.24	7.78	0.013	0.98	0.34
Landscape type (LTY)	4.04 <sub>1,17</sub>	0.061	4.18	0.0057	4.25	0.055	3.16	0.093
Caste (CAS)	24.52 <sub>2,36</sub>	<0.0010						
Semi-natural grasslands (SNG)	0.39 <sub>1,17</sub>	0.54	0.91	0.35	1.32	0.27	0.04	0.84
Mass-flowering crops (MFC)	0.84 <sub>1,17</sub>	0.37	1.29	0.27	0.80	0.38	0.28	0.60
LTY* CAS	6.10 <sub>2,36</sub>	0.0052						
LTY*SNG	6.48 <sub>1,17</sub>	0.021	3.82	0.067	1.40	0.25	8.48	0.0098
LTY*MFC	3.63 <sub>1,17</sub>	0.074	2.42	0.14	2.93	0.11	4.72	0.044
SNG* CAS	1.34 <sub>2,36</sub>	0.27						
MFC* CAS	1.28 <sub>2,36</sub>	0.29						
LTY*SNG* CAS	6.63 <sub>2,36</sub>	0.0035						
LTY*MFC* CAS	4.70 <sub>2,36</sub>	0.015						



**Fig. 3.** Density (log, individuals per 100 m<sup>2</sup>) of bumble bee workers (a–c), queens (d–f), and males (g–i) in flower-rich uncultivated linear habitat elements in landscapes with red clover seed cultivation (filled squares, solid lines, N = 13) and landscapes without red clover seed cultivation (open squares, N = 11) in relation to local log<sub>10</sub> flower density (flower units per 100 m<sup>2</sup>) and proportions of semi-natural grasslands and mass-flowering crops in the landscapes. Lines indicate significant slopes (P < 0.05). Dashed line shows estimated slope for all 24 landscapes.

**Table 3**

Density (individuals per 100 m<sup>2</sup>) of the six most abundant bumble bee species in relation to local flower density, landscape type (presence or absence of red clover seed cultivation), caste (males and workers) and their interaction. If P < 0.1 for the interaction term, the slice option was used to separately test differences in worker and male densities between the two habitats. Mean bumble bee densities (±s.e.m.) in linear habitat elements in landscapes with and without red clover seed cultivation can be found in Fig. 3 and for individual species in Table A3 in the online Supplementary appendix.

Species	All castes						Tests of effect slices					
	Flowers		Landscape type		Caste		Landscape type*caste		Workers		Males	
	F <sub>1,21</sub>	P	F <sub>1,21</sub>	P	F <sub>1,22</sub>	P	F <sub>1,22</sub>	P	F	P	F	P
<i>Bombus hortorum</i> L.	0.05	0.83	15.07	0.0010	1.19	0.29	1.63	0.21				
<i>Bombus lapidarius</i> L.	1.56	0.23	0.97	0.34	10.51	0.0037	3.85	0.062	<0.01 <sub>1,31</sub>	0.99	3.13 <sub>1,31</sub>	0.086
<i>Bombus pascuorum</i> Scopoli	0.78	0.39	0.06	0.81	10.12	0.0043	0.08	0.78				
<i>Bombus subterraneus</i> L.	2.95	0.10	0.59	0.45	0.70	0.41	3.40	0.079	0.39 <sub>1,43</sub>	0.39	3.58 <sub>1,43</sub>	0.065
<i>Bombus sylvarum</i> L.	3.31	0.083	10.47	0.0040	11.51	0.0026	10.35	0.0040	20.49 <sub>1,40</sub>	<0.0010	0.37 <sub>1,40</sub>	0.55
<i>Bombus terrestris</i> <sup>a</sup> L.	12.42	0.0020	1.13	0.30	36.77	<0.0010	16.68	<0.0010	2.03 <sub>1,37</sub>	0.16	10.11 <sub>1,37</sub>	0.0030

<sup>a</sup> Includes also *B. cryptarum* for males.

between species, could partly be explained by differences in amount and type of flower resources. The larger relative attraction of workers to the clover fields is probably due to the higher density of forage resources in the red clover fields, since their main mission is to forage pollen and nectar for the colony (Goulson, 2010). Males on the other hand only forage nectar for themselves and are focused on finding and attracting mates. Males of many bumble bee species patrol a specific track, which is scent marked with

species specific pheromones, often following landmarks such as high vegetation, hedgerows, trees or fence posts (Bergman and Bergström, 1997; Goulson, 2010). If linear elements are preferred habitat for patrolling and contain guiding landmarks, it could explain the male preference for such linear elements over flower rich clover fields.

The lack of relationship between flower density and bumble bee density is somewhat surprising, since bumble bees often distribute

in relation to flower resources (e.g. Öckinger and Smith, 2007; Rundlöf et al., 2008). Our observations could be the result of averaging both flower and bumble bee densities over the season, of the flower unit measure used, or because the value of local flower resources is context dependent, i.e. that the attractiveness of local flower patches is weighed against the value of surrounding resources (Carvell et al., 2011; Scheper et al., 2013).

#### 4.2. Impact of landscape type on workers

As shown for other MFC (Carvell et al., 2011; Hanley et al., 2011; Lentitni et al., 2012; Westphal et al., 2003), large areas of attractive forage can influence the bumble bee community outside the focal habitat by drawing bumble bee workers from the surrounding landscape. However, contrary to our second prediction there was no difference in overall bumble bee worker density between landscapes with red clover seed cultivation compared to those without. The densities of *B. hortorum* and *B. sylvarum* workers were, however, lower in linear elements of landscapes with red clover seed cultivation, indicating that mass-flowering red clover acts as an attractant on these two species and therefore negatively influences densities in the surrounding landscape. Mass-flowering legumes are considered to be a particularly rewarding resource for long-tongued bumble bee species (Carvell et al., 2011). By affecting only a portion of the bumble bee community, occurrence of mass-flowering resources could result in a changed community composition of pollinators in adjacent habitats. This may in turn influence pollination of wild plants in the surrounding landscape in the presence of mass-flowering oilseed rape (Diekötter et al., 2010; Holzschuh et al., 2011). Mass-flowering can thus influence pollination of wild plants, but effects are likely to differ depending on the relationship between the flowering period of the crop and the wild plant.

#### 4.3. Impact of landscape type on queens and males

In line with our third prediction, we detected an increased density of bumble bee males, and a clear tendency also for an increase of queens, in landscapes with red clover seed cultivation compared to landscapes without. It has previously been shown that early MFC contribute to early colony growth in *B. terrestris* (Westphal et al., 2009), to colony size in *Bombus vosnesenskii* Radoszkowski (Williams et al., 2012), and possibly also to colony size in *B. pascuorum* (Herrmann et al., 2007). However, reproductive performance was only found to be enhanced for *B. vosnesenskii* males. Our results indicate that the presence of late-season flowering resources, such as red clover, promote successful bumble bee reproduction. The density of bumble bee queens was more than five times higher and that of males 71% higher, in uncultivated field borders in landscapes with compared to landscapes without red clover seed cultivation (based on raw data), even though the red clover field constituted less than 0.2% of the study landscape. The combined input of early MFC (e.g. winter oilseed rape) and/or more sparsely flowering habitats (SNG) with a small addition of late flowering MFC (e.g. red clover) appears to contribute to successful reproduction in the form of males in species such as *B. terrestris* and possibly also *B. subterraneus* and *B. lapidarius*. This is likely caused by an increased amount and temporal stability of flower resources, since bumble bees do not store large amounts of pollen and nectar and thus depend on a continuous supply of forage during the entire colony cycle (Goulson, 2010; Pelletier and McNeil, 2003). However, only more permanent habitats, such as SNG and permanent field borders, provide nesting and over-wintering sites (Goulson, 2010; Öckinger and Smith, 2007). This type of habitat cannot be replaced by neither early nor late flowering annually disturbed habitats.

From our study, we cannot conclude if the impact of red clover seed production on bumble bee male and queen densities is due to a within or between season effect of red clover seed cultivation, since the same farmer often have clover seed fields for consecutive years. Additionally, we cannot rule out attraction of queens and males at large spatial scales, because their dispersal distances have been estimated to several kilometres using molecular methods (Lepais et al., 2010; Wolf et al., 2012). However, with the relatively large landscape radius of 2 km and the large difference in densities between landscapes types, we believe that the pattern is due to effects on reproduction.

#### 4.4. Conclusions and implications

Our results indicate that red clover seed fields can have an important function for bumble bee population persistence in agricultural landscapes. Flower resources are often lacking during parts of the bumble bee colony cycle due to agricultural intensification, when uncultivated habitats are removed and agrochemicals are used to eliminate flowering weeds. It appears as if relatively small areas of red clover could mitigate the loss of pollinators by providing late-season flowering resources, which coincide with the peak of the bumble bee colony cycle and production of sexual offspring. It remains to be ascertained if this also has an impact on actual reproductive success in bumble bee colonies and queen occurrence in the following year.

Identifying limiting factors for population growth of target organisms emerges as a priority if we are to design efficient interventions to support farmland biodiversity. To our knowledge, this study is the first to demonstrate effects of added flower resources on both distribution of workers and reproductive output (queen and male densities) in natural bumble bee populations in the wider landscape outside the intervention area. Our results indicate that creation of moderately sized strips of favoured and limiting flower resources, such as red clover, could contribute to bumble bee population persistence in agricultural landscapes.

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#### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biocon.2014.02.027>.

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