Effects of floral display size and plant density on pollinator visitation rate in a natural population of *Digitalis purpurea*

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Summary

1. Pollinator visitation patterns in relation to variation in floral display size may be modified both quantitatively and qualitatively by local plant density. In this study four measures of pollinator response by *Bombus* spp. (plant visitation rate, bout length, proportion of flowers visited, flower visitation rate) were investigated under two or three different plant densities in two consecutive years in a natural population of *Digitalis purpurea* L.

2. Plant visitation rate increased with floral display size in both years, and was higher in dense patches compared with sparse ones in 1999. Bout lengths increased with display size in 1999, and bouts were longer in sparse patches. However, the actual rate of increase with display size was independent of plant density for both response measures.

3. The proportion of flowers visited decreased with floral display size in both years, and in 1999 the decline was faster in high-density patches. As a result, the proportion visited was higher in dense patches for the smallest display sizes, and higher in sparse patches for larger display sizes.

4. Flower visitation rate decreased with floral display size in both years. This is inconsistent with the idea that bees achieve an ideal free distribution across flowers. There was no significant effect of plant density.

5. These results demonstrate that local plant density variation may modify the functional relationship between floral display size and pollinator visitation rate, and potentially influence plant mating patterns.

Key-words: *Bombus*, flower visitation rate, plant density effects, plant–pollinator interactions, pollinator foraging behaviour


Introduction

Animal-pollinated plants are expected to evolve strategies that maximize the efficiency of pollen transfer to and from the plant. One such strategy may be to develop large floral displays that attract more pollinators. There is usually considerable within-population variation in the number of open flowers presented on a plant, and several studies have reported a positive relationship between floral display size and the frequency of pollinator visits at the individual plant level (i.e. plant visitation rate) (e.g. 15 out of 17 studies reviewed by Ohashi & Yahara 1999).

An increase in bout lengths on larger plants is predicted in optimality models of insect foraging (Pyke 1979). From the plant’s point of view this may not be of any selective advantage, since it will result in geitonogamous pollination (de Jong, Waser & Klinkhamer 1993) and possible inbreeding depression in self-compatible species (Darwin 1876; Charlesworth & Charlesworth 1987), or pollen discounting (Ritland 1991) and stigma clogging in self-incompatible ones (Robertson & Macnair 1995). Empirical evidence support that bout lengths usually increase with plant size, but this is often accompanied by a decrease in the proportion of open flowers probed (references in Mitchell et al. 2004). As a result, per flower visitation rate has been found to increase (Klinkhamer, de Jong & de Bruyn 1989; Andersson 1991), decrease (Andersson 1988; Klinkhamer & de Jong 1990) or show a constant relation with floral display size (e.g. Robertson & Macnair 1995; Vaughton & Ramsey 1998; Ohashi & Yahara 2002).

How pollinators respond to differences in display size may be modified by ecological factors, especially
Materials and methods

PlANT SPECIES

Foxglove, Digitalis purpurea L., is a facultative biennial herb that grows on naturally disturbed soil. The plant produces one or a few inflorescences, usually 1–2 m in height, and normally with 20–100 flowers. The self-compatible flowers (Darwin 1876) produce nectar and are mainly visited by bumble-bees (Best & Bierzychudek 1982). The basal flowers mature first (acropetal flowering). There is little spatial separation of the sexes within flowers, but temporal separation (protandry) is pronounced. The combination of acropetal flowering and protandry results in inflorescences where the sexes are spatially separated: the lowest flowers are female, usually followed by neuter flowers, male flowers and, at the top open, prefertile flowers. Nectar reward increases during flower life (Percival & Morgan 1965) and consequently induces bumble-bees to visit the lowest flowers first.

FIELD STUDY

Field work was conducted during the summers of 1998 and 1999 in a population on established pasture land in Ulvik (Hordaland County) in SW Norway (60°34′ N, 6°54′ E). An area of approximately 200 m by 100 m on the south-facing slopes towards the Hardangerfjord was used (10–50 m above sea level). The population consisted of a mosaic of areas with varying plant density, and focal plants were chosen within patches of uniform local density.

In 1998 patches of three plant density categories were used: four dense patches with plants less than 0.5 m apart, four intermediate patches with plants approximately 1 m apart, and four sparse patches with plants approximately 2 m apart. Ten neighbouring plants were haphazardly selected in each patch, yielding a total of 120 focal individuals. Approximately 900 flowering plants were present in the population. Insect activity was monitored in each patch for 10 min on each of 3 days: 11, 12 and 14 July 1998 (i.e. each patch and plant was observed for 30 min in total). The sequence of patch observations were changed at random between days to avoid observing the same patch at the same time each day. Total observation time was 6 h.

The relatively low visitation frequency observed in 1998 made us increase total observation time in the 1999 season, and the fact that fewer flowering plants were present (c. 450) made it necessary to reduce the number of patch density levels. Two density categories were used: seven dense patches with plants less than 0.5 m apart, and seven sparse patches with plants more than 1 m apart. Neighbouring focal plants were haphazardly selected in each patch according to the number available. In dense patches plant number varied from 6 to 14 with a total of 65, and in sparse patches from 8 to 11 with a total of 68. Insect visitation
was monitored for 15 min on 5 days between 29 June and 8 July 1999 (i.e. total observation time for each patch and plant was 75 min). It was not possible to monitor every patch on each day due to weather conditions: On 30 June, and 7 and 8 July, 14 patches were monitored, on 29 June and 4 July, 10 patches, and on 3 July, 8 patches. Total observation time was 17·5 h. Observations were timed to coincide with peak pollinator activity (between 10·00 hours and 17·00 hours) in both study years. Inter-patch distances varied from 5 to 20 m.

For each plant visit the species identity of the visitor and the number of flowers probed was recorded on a mini-cassette recorder. It was not always possible to determine which species of bumble-bee the visitor belonged to (the morphology of *Digitalis* flowers makes it possible to see the insect only when it enters or leaves a flower). A visit was recorded if the insect entered the corolla tube. Before each observation period the number of open flowers on the inflorescence (= floral display size) was noted for each plant in the patch. A flower was classified as ‘open’ when the corolla opening was wider than 1 cm (no bumble-bees were ever seen trying to enter a flower classified as ‘not open’). From these data, pollinator visitation rate per hour to plants and to flowers, bout lengths (number of flowers probed during a single plant visit) and proportion of flowers visited (bout length divided by floral display size) were determined.

In the 1999 season several plants were infested by larvae of the species *Eupithecia pulchellata* Stephens (Lepidoptera: Geometridae). Oviposition takes place on immature buds and the presence of the larva inhibits flower opening, although the ‘bud’ grows to a size well beyond the point where it would normally open (J. M. Grindeland, unpublished observation). Infested flowers situated among normal open flowers were considered to be part of the floral display and consequently treated as ‘open’. When movements within an inflorescence were considered only truly open flowers were included.

### DATA ANALYSIS

Because of the slightly different methods (i.e. length of the observation periods and different categorization of plant density), we analysed the data from the two years separately. Plant visitation rate and bout length (relatively small integer counts) were analysed by log-linear models, i.e. Poisson response distribution and log-link function (Crawley 1993; Agresti 1996). Proportion of flowers visited was analysed by logit models, i.e. binomial response distribution and logit link function (Agresti 1996). Flower visitation rate met assumptions of normal distribution and constant variance and was analysed by linear models (Sokal & Rohlf 1995). In all models, plant density and observation date were included as fixed categorical factors and floral display size as a continuous covariate. In all analyses backward selection and Type III tests (retain criterion $P < 0·05$) were used to find the most appropriate model starting with all pairwise and three-way interactions. The goodness of fit of logit and log-linear models were assessed by Pearson chi-square statistics and always found appropriate. This validates and confirms the implicit assumption of our modelling strategy, that random patch and plant effects were relatively unimportant and consequently not needed in the models. The linear models were performed with SPSS 11·0 (SPSS Inc. 2001), the logit and log-linear models with the Insight module in SAS 8·02 (SAS Institute 2001).

### Results

**VISITORS**

All visitors to *D. purpurea* observed were bumble-bees (*Bombus* spp.) except one visit by a honey-bee (*Apis mellifera* L.) in 1998. Bumble-bees were mainly foraging for nectar, with a handful of observations of pollen collecting (i.e. turning upside down once inside the corolla tube). In total for both years four species of bumble-bees were observed (Table 1): *Bombus hortorum* (L.), *B. pascuorum* (Scop.), *B. lucorum* (L.) and *B. hypnorum* (L.). The most frequent visitor by far was *B. hortorum*, which accounted for more than half of all visits in both years (Table 1). The other species varied in frequency both within year and between years. *B. lucorum* was a relatively frequent visitor in 1998, but nearly absent the second year. For *B. pascuorum* the opposite was the case. The fourth species, *B. hypnorum*, was observed in 1998 only and then at fairly low frequency (Table 1). The frequencies are conservative estimates as not all visitors were identified to species (*Bombus* sp. 12%...
In 1998 the average floral display size was substantially higher than in 1999, 9.4 ± 4.1 (SD) as compared with 5.5 ± 2.9 (SD). This difference was partly due to the presence of parasitized flowers in 1999, but also the fact that inflorescences on average were 20 cm shorter in 1999. The range in floral display size was also somewhat higher in 1998. Since in 1998 there were twice as many flowering plants each on average with nearly twice as many open flowers, a crude estimate gives a four times as many simultaneously open flowers in the population this year.

PLANT VISITATION RATE

In 1998 a total of 108 plant visits were recorded, while the total for 1999 was 953 (Table 1). The mean frequency of visits varied more than threefold between years: 1.8 visits per plant per hour in 1998 and 5.9 in 1999. The range in number of visits was nearly twice as high in 1999; 0–32 visits per hour compared with 0–18 visits per hour in 1998. The proportion of plants left unvisited was also lower.

Table 2. Generalized linear model analyses of the effects of floral display size, density and date on plant visitation rate and bout length (Poisson errors, log link), and on the proportion of flowers visited (binomial errors, logit link). Only significant interactions are included.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Plant visitation rate</th>
<th>Bout length</th>
<th>Proportion visited</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>χ²</td>
<td>P</td>
</tr>
<tr>
<td>1998</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Display size</td>
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<td>Density</td>
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<td>0.307</td>
</tr>
<tr>
<td>Date</td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Display size</td>
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<td>54.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density</td>
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<td>0.012</td>
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<tr>
<td>Date</td>
<td>5</td>
<td>174.27</td>
<td>&lt;0.001</td>
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<tr>
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<td>Display * Density</td>
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<tr>
<td>Display * Density * Date</td>
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<td>12.99</td>
<td>0.023</td>
</tr>
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</table>

In 1998 only floral display size affected plant visitation rate significantly (Table 2). The visitation rate increased moderately with number of open flowers (Fig. 1a). In 1999, floral display size, density, date and the plant density–date interaction affected plant visitation rate significantly (Table 2). Plant visitation rate showed an accelerating increase with floral display size, and it was higher in dense patches than in sparse patches (Fig. 1b). Mean visitation rate increased fourfold from the first to the last observation date, and it also differed increasingly between the two density levels at the later observation dates.

BOUT LENGTHS

The average bout length per plant was slightly higher in 1998 than in 1999 (2.1 ± 1.3 (SD) vs 1.8 ± 1.2 (SD)), and the maximum bout length was five flowers longer. On average the most frequent visitor, *B. hortorum*, had the longest bout lengths in both years. In 1998 59% of all visits was to one flower only, in 1999 43%. In 1998 neither floral display size, plant density nor date affected bout lengths significantly (Table 2, Fig. 2a). In 1999 there was a highly significant main effect of

Fig. 1. The observed (mean ± SE) and predicted relationship between floral display size and plant visitation rate in (a) 1998 and (b) 1999.
floral display size, with bout lengths increasing with the number of open flowers (Fig. 2b). Bouts were also longer in sparse than in dense patches (Fig. 2b), and there was a significant effect of date (Table 2). No interactions were significant.

**PROPORTION OF FLOWERS VISITED**

The average proportion of flowers visited was higher in 1999 than in 1998 (0.42 ± 0.26 (SD) vs 0.20 ± 0.15 (SD)). It declined with increasing floral display size in both years (Fig. 3a, b). In 1998 only floral display size affected the proportion of flowers visited significantly (Table 2). In 1999 there was a significant three-way interaction between floral display size, plant density and date, and the main effects of display size and plant density were highly significant (Table 2). The proportion visited declined at a faster rate in high density patches (Fig. 3b), and this relationship was strongest at the earlier observation dates.

**FLOWER VISITATION RATE**

Mean flower visitation rate was considerably higher in 1999 than in 1998: 3.1 visits per flower per hour in 1999 and 1.3 in 1998. It decreased with increasing floral display size in both years (Fig. 4a, b). In 1998 there was a significant main effect of floral display size and no significant interactions (Table 3). The effect of density was marginally non-significant, with higher flower visitation rates in low density patches. In 1999 the main effects of floral display size and observation date were highly significant (Table 3), mean flower visitation rate almost doubled from the first to the last observation date. There was no effect of plant density, and neither of the interactions were significant (Table 3).

**Discussion**

This study demonstrates that plant density-variation at the patch level may alter pollinator response to variation in floral display size. The proportion of flowers visited on equally sized plants of *Digitalis purpurea* declined at a faster rate in dense patches compared with sparse ones. This density-dependent difference in foraging behaviour may affect plant mating patterns at a local scale, in particular in systems where geitonogamy is common.

**PLANT VISITATION RATES**

Plant visitation rates were substantially higher in the 1999 season compared with 1998, probably because of...
the combined effect of a higher number of pollinators foraging in the population and fewer plants available to them. *Bombus hortorum* was probably the most important pollinator both years, since visitation rates from this species were high and fairly constant throughout the observation periods. Plant visitation rate increased with floral display size in both years in our study population. The relationship was slightly accelerating, whereas most earlier studies have found this relation to be decelerating (reviewed by Iwasa, de Jong & Klinkhamer 1995; Ohashi & Yahara 2001).

In 1998 plant visitation rate was independent of density, while in 1999 pollinators visited plants in dense patches more frequently (on average 6.6 times per hour vs 5.3 times per hour in sparse patches). The lack of any significant effect of density in 1998 could be due to weaker competition for nectar this year, as bumble-bees should be less selective in their visitation pattern when reward levels are high. There was a general increase in pollinator activity during the 1999 season, with much higher overall visitation rates at the later observation dates, and this was accompanied by increasing differences in visitation rate between the two patch density levels. This suggests that the standing crop of nectar was increasingly depleted, and low reward levels induced bumble-bees to keep interplant movements at low cost, and consequently choose dense patches at later dates. However, the bumble-bees’ actual response curve to floral display size was independent of density: dense patches attracted more visitors, but once in the patch the bumble-bee would discriminate between displays of different size in the same manner as in sparse patches.

**BOUT LENGTHS AND PROPORTIONS**

In 1998 bout lengths of foraging bumble-bees were unaffected by the number of open flowers of *D. purpurea*, while in the 1999 season there was a marked increase in bout lengths with floral display size, and bouts were also longer in sparse patches. We expected lower rates of increase in dense patches, as the difference in flight costs between inter- and intraplant movements should be smaller here. Although such a trend was present in 1999, no significant effect of density was found. As insect activity increased during the 1999 season we expected bouts to be shorter at later dates, but though date was significant in the analyses, bout length showed no systematic variation with date.

Bouts increased less than proportionally with the number of open flowers presented on a plant, and the resulting proportion of flowers visited declined with floral display size in both years. The proportion probed was expected to be lower in dense patches, but the effect was absent in 1998 and proved to be dependent on display size in 1999. At small display sizes the proportion in fact was higher in high-density patches, but as the relationship also declined at a faster rate, the resulting response curves crossed at a display size of approximately four flowers, which was a little below mean display size (5.5 this year). This means that on equally sized plants below this size, pollinators would show greater tenacity in dense patches, whereas above this size tenacity would be highest in sparse patches. A similar pattern was found by Ohashi & Yahara (2002) in their study of *Cirsium purpureatum*, but they compared bumble-bees foraging in either high or low-density areas. A bumble-bee foraging in a population of *D. purpurea* will frequently move between...
areas of differing local density, and our study indicates that pollinators may adjust their behaviour according to density-variation at this small, within-population scale.

**FLOWER VISITATION RATES**

The fact that pollinators tend to visit large displays more often, but leave a greater proportion of the flowers behind has repeatedly been observed (Ohashi & Yahara 2001). These countervailing factors have in most studies resulted in a constant relationship between display size and flower visitation rate (e.g. Robertson & Macnair 1995; Goulson *et al.* 1998; Ohashi & Yahara 2002; Mitchell *et al.* 2004), whereas in *D. purpurea* flower visitation rate declined with increasing floral display size in both study years. Unless nectar production rate (NPR) per flower is negatively correlated with display size, this contradicts the idea that pollinators achieve an ideal free distribution (IFD) across flowers (Fretwell & Lucas 1970; Dreisig 1995). Since we lack data on NPR from this population, we cannot completely dismiss the possibility that bumble-bees approximated an IFD. However, very few studies have found a negative relation between NPR per flower and display size (references in Harder & Cruzan 1990), and in a common garden experiment on *D. purpurea* there was no significant correlation between plant size and NPR (J. M. Grindeland & N. Sletvold, unpublished observation).

The decrease in flower visitation rate with display size indicates that average reward levels were higher on large plants. In view of this it is puzzling that bout lengths tended to be so short on large displays. One possible explanation could be that the unpredictable variation in per flower reward level increases with display size (Biernaskie & Cartar 2004). *D. purpurea* has a highly organized, vertical inflorescence, but as flower number increases, more flowers will be situated at approximately identical height. The likelihood that some flowers will be missed during a foraging bout is thus higher on large displays (J. M. Grindeland, unpublished observation; Best & Bierzychudek 1982).

If pollinators use reward levels gained at each flower to decide whether to leave a plant (Hodges 1985), a larger within-variability in reward level on large plants could result in early departures (Pappers & Lucas 1970; Dreisig 1995). Since we lack data on NPR from this population, we cannot completely dismiss the possibility that bumble-bees approximated an IFD. However, very few studies have found a negative relation between NPR per flower and display size (references in Harder & Cruzan 1990), and in a common garden experiment on *D. purpurea* there was no significant correlation between plant size and NPR (J. M. Grindeland & N. Sletvold, unpublished observation).

We found no effects of density on flower visitation rate. The higher plant visitation rate observed in dense patches was counterbalanced by the higher decline in proportion of flowers visited. This is similar to the results of Ohashi & Yahara (2002), where average visitation rate per head in *Cirsium purpuratum* was independent of plant density.

**Conclusion**

In view of the results of our study, we hypothesize that bee behaviour on *D. purpurea* inflorescences is governed by correlated changes in reward level and reward variability. The higher profitability of large plants attracts more visitors, but the occurrence of newly emptied flowers within the display may cause the bee to leave after just a few flowers have been probed. Detailed measurements on the amount of nectar gained at the flower level will be necessary to test whether this really is the case. Our results also suggest that plant density variation at a within-population scale may influence pollinator foraging behaviour, and consequently size-dependent plant mating patterns (i.e. selfing rates). Further work is required to determine if this is common in other systems, and the inclusion of plant density in studies on pollination ecology is recommended.

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**References**


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