Pollination processes and the Allee effect in highly fragmented populations: consequences for the mating system in urban environments

Pierre-Olivier Cheptou and Lyz G. Avendaño V
UMR 5175 CEFE–Centre d’Ecologie Fonctionnelle et Evolutive (CNRS), 1919 Route de Mende, F-34293 Montpellier Cedex 05, France

Summary

• The urban environment was used to study the plant reproductive system in small fragmented populations as well as the potential adaptations of plants to urban conditions. We examined the effect of density on the pollination process and on reproduction in urban populations of the allogamous species Crepis sancta. The habitat is composed of small uncultivated square patches (c. 2 m²) regularly spaced along the pavement in streets of the city of Montpellier, France.
• Pollinator behaviour (the presence of pollinators, the number of flowers visited and the duration of each visit) and seed set as a function of the number of plants in patches and selfing rates, determined using progeny array analysis, were studied. The propensity for the urban populations to produce seeds by self-fertilization in insect-proof glasshouse was also analysed.
• We found strong evidence of reduced pollinator activities at low densities, resulting in reduced pollination and a reduction in seed set from 80 to 20% of ovules fertilized (the Allee effect).
• Progeny array analysis revealed a slight increase (marginally significant) in selfing rates in urban populations compared with large populations. In spite of lower pollinator activity, urban populations did not show a greater ability to self-fertilize compared with rural populations from the nearby countryside.

Key words: Allee effect, fragmentation, metapopulation, pollination, self-fertilization, urban environment.

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Introduction

Fragmentation in ecological systems has been a subject of interest for the last 20 years. This interest has arisen from concerns about the recent consequences of human activities such as deforestation or changes in agricultural practices, as well as the recognition that natural systems are structured in space and time. Urban environments represent newly fragmented habitats, and thus provide the opportunity to study the impact of fragmentation in ecological systems. They may also be of particular interest for analysing recent evolutionary processes, as shown in recent studies (Shocat et al., 2006).

Compared with a single large population, fragmented populations exhibit reduced population size and reduced gene flow among remnant demes. One of the main changes accompanying the reduction in population size is the increased importance of stochastic processes (Saunder et al., 1991; Aizen & Feinsinger, 1994; Young et al., 1996) resulting in a higher probability of a local population becoming extinct (Shaffer, 1981; Ellstrand & Elam, 1993).

Low population densities are also expected to threaten population demography in a deterministic way. At low density, the difficulties of gametes in encountering one another may cause a decrease in the per capita growth rate, a phenomenon
named the ‘Allee effect’ (Allee et al., 1949). This is defined as a positive relationship between density or population size and any components of fitness (Stephens, 1999) sometimes referred to as ‘inverse density dependence’ (Courchamp et al., 1999). Low population sizes and/or low population density (the number of individuals per unit area) can result in an Allee effect, but the two factors often interact in empirical studies (Ghazoul, 2005). Depending on the strength of the Allee effect, population dynamics can be disrupted and populations may eventually go to extinction deterministically, as shown in theoretical (Schreiber, 2003) and empirical studies (Groom, 1998).

Because plants are sessile, sexual reproduction depends on pollen vectors and low densities lead to a lower probability of pollen transfer, resulting in a lower seed set (Ashman et al., 2004). Moreover, in animal-pollinated plants, a small population size may decrease the attractiveness to pollinators (e.g. insects), resulting in a smaller quantity of pollen available for outcrossing. Also, pollen quality may decrease at low density because of higher relatedness among individuals or because of similar self-incompatibility alleles shared by pollen donors and pollen recipients in the case of self-incompatible species (Kearns & Inouye, 1997). Pollinator behaviour (foraging) can change too. By disrupting plant pollinator interactions, low densities resulting from fragmentation could therefore create conditions necessary for the Allee effect to occur.

Plants have evolved mating strategies to cope with pollen limitation and particularly with the Allee effect. When faced with recurrent low population sizes such as expected in fragmented systems, populations may evolve towards selfing in order to ensure seed production (Darwin, 1876; Baker, 1955). Reproductive assurance has been considered to be one of the major forces for the frequent transition from outcrossing to selfing in hermaphrodites (Holsinger, 2000). Thus, analysing the Allee effect in fragmented populations provides a relevant ecological context for analysing selection pressures on mating systems in hermaphroditic plants (Cheptou, 2004; Moeller & Geber, 2005).

In this study, we used the urban environment to investigate the pollination process and reproduction in fragmented populations in the mostly outcrossing species *Crepis sancta* (Asteraceae). Urban environments consist of a matrix that is mostly unsuitable for plants because of constructions, asphalt and buildings. Within this mainly unsuitable area, small and sparse suitable patches constitute highly fragmented habitats where plants can persist (mostly ruderal species). Interestingly, ecological studies on urban systems have sometimes reported higher variety diversity than in surrounding agricultural landscapes (Wania et al., 2006). Because of their artificiality, urban environments provide a useful system for investigating population dynamics by taking advantage of the regular geometry of habitats and their well-known history. The study species *C. sancta* is widespread in the city of Montpellier (France) where it occurs in very small and isolated populations (from 1 to 40 plants maximum in our study system). Interestingly, this species has been intensively studied in the near countryside where it occurs in very large populations, sometimes more than a hundred thousand individuals per hectare (Cheptou et al., 2002). This study aimed to investigate the impact of population density as a consequence of fragmentation on the pollination process and its demographic consequences (the Allee effect). Specifically, we addressed the following questions:

- Is pollinator activity positively related to density in urban populations?
- Is seed set positively related to density (the Allee effect) in urban populations?
- Do urban populations exhibit higher selfing rates than rural populations from the nearby countryside?
- Is there evidence for a greater ability to self-fertilize in urban populations?

### Materials and Methods

#### Study system

The study system consisted of small habitats around trees regularly spaced along the pavement (hereafter called ‘patches’) in streets of the city of Montpellier (France). These patches are widely distributed (several thousands) and are mostly colonized by plants that persist in patches because of the absence of management by public authorities. Patches are small areas of c. 1–2 m² suitable for plants in a matrix of unsuitable habitat (streets and concrete matrix). In the spring of 2005, more than 95 species were identified in the patches in Montpellier, mostly annual species (F. Beilhe, personal communication). The most frequent species were *Stellaria media* (L.), *Crepis sancta* (L.) Bornm, *Sonchus oleraceus* (L.), *Poa annua* (L.) and *Senecio vulgaris* (L.) (F. Beilhe, unpublished). Each patch constitutes a deme with small population size (from 0 to 40 plants for the species studied here). These habitats are, however, frequently disturbed, and are thus maintained at a young successional stage. Because of its simplicity, this system allows suitable and unsuitable habitats to be defined easily and thus constitutes a metapopulation (Hanski & Gilpin., 1997). Patches are regularly distributed within streets (each patch is equally isolated), which facilitates experimental investigations. Moreover, because patches have equal areas (within each street), the number of plants per patch is equivalent to its density.

The plant studied, *C. sancta*, is an annual Mediterranean Asteraceae found in the south of France in ephemeral habitats (wasteland) or in the first stages of the successional process. *C. sancta* flowers for 6–8 wk in early spring. It produces two types of achenes: a few peripheral achenes (3–10 per capitulum) without a pappus and numerous central achenes (70–100 per capitulum) with a pappus (Imbert et al., 1996). Pollination is strictly entomophilous. Honeybees are the most frequent pollinator both in the countryside and in the urban environments,
although other hymenopterans can be found. During our pollination survey in 2004, only _Apis mellifera_ (L.) was found in both rural and urban environments. _C. sancta_ is a highly outcrossing species and possesses a self-incompatibility system, although low levels of self-compatible variations have been reported in natural populations (Cheptou et al., 2002).

In order to analyse reproduction, three distinct alignments of patches were selected in Montpellier, each c. 1 km from one another. We studied patches along three streets. In Henri Marès Street (HM), the patches had a constant area of 2.25 m$^2$ and were 4.6 m apart. In La Roqueturière Street (Roque), the patches had a constant area of 2.3 m$^2$ and were 7 m apart. In Auguste Broussonnet Street (AB), the patches had a constant area of 1 m$^2$ and were 3.6 m apart. The number of plants varied from 0 to 20 flowering plants per patch and 0–70 capitula (synchronously flowered) per patch in 2004 when the pollination experiment was performed. Given the small distance between patches, patches are likely to be connected by pollen dispersal by bees, and possibly by seed dispersal.

We checked that the patches studied were not close to large populations of _C. sancta_ that could disturb our pollination observations. Specifically, no populations were found within 800 m of the studied streets. This did not ensure that our populations were genetically isolated, but at least pollination processes could be expected to be little influenced by large populations.

Pollination surveys in large populations were conducted in order to compare pollination processes and reproductive success in _C. sancta_ between populations in the city and nonfragmented populations. We considered a population of 25 m$^2$ with c. 750 plants in Chemin de Barques (CB) in Montpellier and an abandoned vineyard at Claret (30 km from Montpellier) with c. 200 000 plants per hectare.

Pollination and reproductive success in patches

We carried out 15-min floral visitor watches in arbitrarily selected patches in each street. We stood 2 m away from the patch and recorded pollinator presence and visiting time per patch. The watch duration was chosen on the basis of the probability of observing at least one insect (see also Steffan-Dewenter & Tscharntke, 1999). We observed 64 patches (over the three streets). The experiment was run over a short period during the day (from 11:00 to 15:00 h) from 4 March to 20 April 2004. The 40 h of observation were obtained when sunny, clear and calm conditions favoured pollinator activity. During a 15-min floral visitor watch, three pollination measurements were made. First, we recorded the presence or absence of pollinators. Secondly, when pollinators were present, we counted the number of visits. A visit is defined as a period during which the pollinator stays on the flower and pollinates (Dafni, 1992). The visits during the 15-min period may be by the same insect or by different insects. Thirdly, we measured the visit duration. Finally, we counted the number of flowering plants per patch and the number of flowering capitula per patch. Using a linear regression, we checked that the number of capitula per plant did not vary with the number of plants per patch ($F_{1,137} = 0.69, P = 0.4$). This indicates that density does not affect the resources per plant.

After the peak of flowering, we estimated the reproductive success of plants using the seed:ovule ratio, i.e. the proportion of ovules fertilized. Because the number of plants per patch is low, a direct measure of the ratio by harvesting capitula would disturb the demography of the patch, which would disturb the patch dynamics in subsequent years. To avoid this, we used an indirect method. After fructification, the capitulum is cone-shaped. Because seed maturation enlarges the width of the capitulum, we estimated the seed:ovule ratio indirectly by measuring the capitulum width. A large capitulum width is expected to be correlated with a high seed:ovule ratio, whereas a small width is expected to be correlated with a low seed:ovule ratio. A preliminary study using a small number of capitula randomly chosen in the three streets was performed to estimate the relationship between the seed:ovule ratio and capitulum width. In order to calibrate the relationship between capitulum width and seed:ovule ratios, 108 capitula were measured in the three streets. When seeds were mature but before the capitula were open (i.e. before seed dispersal), we measured the capitulum width and height, and wrapped the capitulum with adhesive tape to avoid seed dispersal. A week later, when the fruits were mature, we counted the number of viable achenes and the total number of florets per capitulum with a binocular microscope. Viable seeds are plump and dark and can easily be distinguished from unfertilized ovules. We found a strong and significant relationship between seed:ovule ratio and capitulum width ($P < 0.001$) but not height ($P > 0.05$). We proceeded to measure capitulum width for 595 heads, for which the total number of flowering plants (and the total number of flowering heads) per patch was recorded at the time at which the head measured was receptive.

In order to obtain comparative values from nonfragmented areas, we carried out 15-min floral visitor watches at Claret and CB by choosing 1 m$^2$ at random (1 m$^2$ represents the lower limit of patch size in the city). We also measured the seed:ovule ratios directly in these two populations.

Progeny array analysis

We used allozymic markers for the progeny array analyses. In 2003, when the seeds were mature, open pollinated progenies were collected from nine patches at HM and 10 patches at Roque. One capitulum per plant chosen at random was used for the analysis. In autumn, the seeds were sown in the greenhouse. Because germination rates were high (>$90\%$) and inbreeding depression is low at this stage (< 5%; Cheptou et al., 2000) potential outcrossing estimate bias because of early deaths of inbred genotypes is expected to be low (< 2%).
Table 1  Number of flowering plants per patch and density (number of individuals/m²) for sampled progenies of *Crepis sancta* in Henri Marès Street (HM) and La Roqueturière Street (Roque) in 2003

<table>
<thead>
<tr>
<th>Family</th>
<th>Number of individuals</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM35</td>
<td>1</td>
<td>0.44</td>
</tr>
<tr>
<td>HM28</td>
<td>1</td>
<td>0.44</td>
</tr>
<tr>
<td>HM03</td>
<td>2</td>
<td>0.89</td>
</tr>
<tr>
<td>HM17</td>
<td>2</td>
<td>0.89</td>
</tr>
<tr>
<td>HM08</td>
<td>3</td>
<td>1.33</td>
</tr>
<tr>
<td>HM06</td>
<td>4</td>
<td>1.78</td>
</tr>
<tr>
<td>HM14</td>
<td>4</td>
<td>1.78</td>
</tr>
<tr>
<td>HM15</td>
<td>6</td>
<td>2.67</td>
</tr>
<tr>
<td>RoqueA4.1</td>
<td>1</td>
<td>0.42</td>
</tr>
<tr>
<td>RoqueB40</td>
<td>1</td>
<td>0.42</td>
</tr>
<tr>
<td>RoqueA17</td>
<td>2</td>
<td>0.84</td>
</tr>
<tr>
<td>RoqueA7.4</td>
<td>4</td>
<td>1.68</td>
</tr>
<tr>
<td>RoqueB12</td>
<td>12</td>
<td>5.04</td>
</tr>
<tr>
<td>RoqueA40</td>
<td>15</td>
<td>6.30</td>
</tr>
<tr>
<td>RoqueB14</td>
<td>15</td>
<td>6.30</td>
</tr>
</tbody>
</table>

Patch size in 2.25 m² for HM and 2.38 m² for Roque.

We used 83 individuals from HM (eight families) and 79 individuals from Roque (seven families), with 6–12 individuals per family, as suggested by Ritland (1986). Each family analysed came from a different patch. The density per patch in the patches from which families were sampled is shown in Table 1. When seedlings measured an average of 7–8 cm in diameter, enzymes were extracted from fresh leaves with sand using 0.05 M Tris HCl buffer (pH 7.5), 5% sucrose (weight/weight) and 0.6% mercaptoethanol. Extracts were conserved at −70°C. We used five highly polymorphic enzyme systems (Cheptou et al., 2002). Gels with 12% starch were used for electrophoresis. Enzyme systems PGI-1 (E.C. 5.3.1.9) and PGD (E.C. 1.1.1.44) were resolved on histidine gels at pH 6.5 (Soltis & Soltis, 1989). Enzyme systems PGI-2, PGM (E.C. 5.4.2.2) and ADH (E.C. 1.1.1.1) were resolved on lithium borate gels at pH 8.3 (Soltis & Soltis, 1989). Enzyme stain recipes were those described by Soltis & Soltis (1989).

Self-incompatibility in the glasshouse

Plants from HM and Roque were cultivated in an insect-proof glasshouse in order to measure their ability to self-pollinate in a controlled environment. To this end, 23 plants from 13 patches in HM were harvested in December 2003 at the seedling stage (two to four leaves). Rosettes were transplanted to an insect-proof glasshouse at the CEFE–Centre d’Ecologie Fonctionnelle et Evolutive (CEFE-CNRS) experimental garden in Montpellier. Twenty-one seedlings from Roque (chosen at random) harvested from 10 patches in spring 2003 were transplanted. Twenty-two seedlings from Claret (a large population in a vineyard) were harvested in December 2003 in order to compare self-compatibility in the urban environment with self-compatibility in the countryside. All the seedlings were transplanted in January in 1-l pots and maintained in the same glasshouse until the flowering season (March–April 2004).

Pollinations were performed by the same person (Lyz G. Avendaño V) when all the florets from the same capitulum were receptive (50–100 florets) as described in Cheptou et al. (2000). Each plant was subjected to three treatments: (1) no pollination (autonomous self-fertilization), (2) self-fertilization by hand pollination and (3) outcross pollination by hand pollination. Hand pollinations were carried out with a paintbrush. Outcross pollinations were carried out by collecting pollen from a minimum of five randomly chosen plant donors (Kearns & Inouye, 1993; see Cheptou et al., 2002). Each cross was repeated on two capitula. After fertilization, the number of viable seeds and the total number of florets per head were measured with a binocular microscope using the method previously described. We calculated the seed:ovule ratio by directly measuring the number of viable seeds and the total number of florets (one floret produces either one seed or none).

**Data analysis**

**Pollination and reproductive success**  Because the number of plants per patch and the number of capitula per patch were highly correlated (Pearson correlation coefficient 0.73, $P < 0.0001$), statistical analyses of these variables produced the same results. We therefore used the number of plants as the predictive variable in linear models. Data were analysed using a general linear model (SAS, 1999). The presence or absence of pollinators during 15 min was analysed in order to estimate the probability of pollinators occurring in patches. A logistic regression (logit link and binomial error) was performed using the *GENMOD* procedure (SAS, 1999). The factors considered were number of plants present at the time of observation, streets (or population) and interactions. The ‘street’ factor included various sources of variation among streets, in particular heterogeneity in patch areas and distances between patches. Nonsignificant factors were removed using a backward elimination of nonsignificant factors.

The number of visits was analysed with the *GENMOD* procedure using a log link and a Poisson distribution error. Because the number of observations was low, the ‘street’ factor was not included in the analysis. Because the visits were more frequent at high density, we used a single measurement per 15-min watch (the mean of visit durations) in order not to over-represent high-density measurements. The mean visit duration was regressed against the number of plants.

In order to infer the seed:ovule ratio from capitulum width, the ratio was regressed against capitulum width using a two-order polynomial function (*GLM* procedure; SAS, 1999). We checked the absence of a street effect by comparing the slopes between streets. The ratio was transformed using angular transformation, arcsin ($\sqrt{(x)}$), to satisfy the normality of residuals (Zar, 1996).

Capitulum width was regressed against the number of plants per patch. Because two capitula within a patch were not
statistically independent (because of pollinator presence), we used the mean capitulum width per patch. The street factor was included. A simple observation of the data showed that the relationship saturated as the number of plants increased. We then analysed the capitulum width as a linear function of ln (number of plants) (see also Ward & Johnson, 2005).

**Progeny array analysis** Genetic data were analysed using the MLTR program (available from K. Ritland, University of British Columbia, Vancouver, BC, Canada). In this analysis, we used the genotypes of progenies to estimate the mating system parameters of the maternal parent in the field using maximum likelihood estimates (Ritland, 1990). Outcrossing rate estimates were calculated using multilocus and average single locus estimates (respectively $t_m$ and $t$) based on Ritland’s mixed-mating model (Ritland & Jain, 1981). The comparison between the two outcrossing estimates ($t_m - t$) provides an estimation of biparental inbreeding, i.e. inbreeding as a result of crossing with related individuals (Ritland, 1986). In this analysis, $t_m$ is considered to be the nonbiased outcrossing rate estimate. MLTR also estimates the correlation of outcrossed paternity ($r_p$) within progeny arrays (Ritland, 1989). The correlation of outcrossed paternity can be viewed as the proportion of full-sibs among outcrossed sibs (Ritland, 1989), and $1/r_p$ gives an estimation of the number of different paternal parents within progeny (Sun & Ritland, 1998). The MLTR program can estimate allelic frequencies in the pollen pool and in the ovule pool separately. As pollen and ovule allele frequencies did not differ significantly, we constrained the equality of frequencies to increase the statistical power for other parameters. Standard errors on estimates were calculated using 500 bootstraps, the unit of resampling being the progeny array. Thus, $t_m$, $t$ and $r_p$ were estimated for each population (HM and Roque). Given the large number of progeny needed to infer individual selfing rates, we were not able to estimate selfing rates for individual patches. Data were therefore used to infer mating system parameters for each street separately. For each population, we tested whether the multilocus outcrossing rate, $t_m$, was significantly lower than the mean rural population outcrossing rates estimated on nine populations from the countryside ($r = 0.91$; Cheptou et al., 2002) using the Z1-tail test. We combined probabilities of the tests from the two urban populations using Fisher’s method (Sokal & Rohlf, 1995, p. 794) in which the quantity $-2 \sum \log p$ is distributed as $\chi^2$, where the number of degrees of freedom is twice the number of independent tests.

**Self-incompatibility variation** Data from experimental crosses were analysed using the general linear model in Proc GLM (SAS, 1999). The response variable was the seed:ovule ratio. Two capitula per cross and per individual were analysed. We performed a mixed-model analysis of variance (ANOVA) with cross treatment (three levels) and population (three levels: two streets and Claret) as fixed effects. The genotype effect (nested in population) was specified as a random effect. The denominator degrees of freedom were calculated using Satterthwaite’s approximation. Type III sums of squares were used to calculate F-ratios. The seed:ovule ratio was transformed using angular transformation in order to satisfy model assumptions (Sokal & Rohlf, 1995).

**Results**

During our pollination survey, the only active pollinator found on the species *C. sancta* was *Apis mellifera*.

**Pollination and reproductive success**

We observed pollinators to be present on 25 occasions in 137 observation sequences. The probability of presence was found to be positively related to the number of plants ($\chi^2_{(1)} = 6.04$, $P < 0.05$; Fig. 1). Neither street ($\chi^2_{(1)} = 1.5$, $P > 0.05$) nor its interaction with number of plants ($\chi^2_{(2)} = 1.71$, $P > 0.05$) was significant. The residual $\chi^2$ was not significant, which ensures the validity of the model (absence of overdispersion). The number of visits was also found to be positively correlated to the number of plants ($\chi^2_{(2)} = 55.41$, $P < 0.001$; Fig. 2). In contrast, the mean duration of visits was not significantly influenced by the number of plants ($P > 0.05$; Fig. 3).

In dense populations, based on five observations, we recorded 100% presence and 13 pollinator visits per 15-min watch in Claret. The mean visit duration [and standard error (SE)] was 5.12 (0.37) s. Based on four 15-min floral visitor watches in CB, we observed 100% pollinator presence and 19.5 visits per 15 min. The mean visit duration was 9.05 (0.71) s.

The indirect estimation of the seed:ovule ratio was described by a two-order polynomial function. The street factor was found to be nonsignificant ($F_{2,103} = 2.44$, $P > 0.05$) but the...
linear coefficient was found to be significant \( (F_{1,103} = 45.5, P < 0.001) \) as was the quadratic term \( (F_{1,103} = 33.09, P < 0.001). \) We therefore obtained the following equation:

\[
\text{Mean capitulum width} = -3.83 + 1.63 \text{ width} - 0.13 \text{ width}^2,
\]

\( R^2 = 0.47 \)

The mean capitulum width was found to be positively correlated to \( \ln(\text{number of plants per patch}) \) \( (F_{1,69} = 17.21, P < 0.001; \) Fig. 4). The effect of street factor was also significant \( (F_{2,69} = 9.92, P < 0.001). \) We compared slopes of regression, which were found not to differ between streets \( (F_{2,67} = 0.05, P > 0.05). \) The variance explained by the model is \( R^2 = 0.40. \) This analysis indicates a positive relationship between individual reproductive success and density. The estimated ratios (estimated from width) varied from 0.3 to 0.9 over the density range.

By comparison, the mean ratios estimated from capitula chosen at random by direct counting in nonfragmented areas were high. For CB, the mean ratio was 0.80 \( (SE = 0.04, n = 35 \text{ capitula}). \) For Claret, the mean ratio was 0.85 \( (SE = 0.02, n = 61 \text{ capitula}). \)

**Mating system analysis**

The polymorphism of the five enzymatic loci used here was equivalent in the two streets. In particular, \( PGI-1 \) (E.C. 5.3.1.9) possessed five alleles. \( ADH \) (E.C. 1.1.1.1) and \( PGM-2 \) (E.C. 5.4.2.2) were dimorphic and \( PGD-2 \) (E.C. 1.1.1.44) possessed three alleles. \( PGD-1 \) possessed three alleles for HM but only two for Roque.

As the parental inbreeding coefficient \( (F) \) was never significantly different from zero, it was constrained to zero to increase the statistical power on other parameters (K. Ritland, personal communication). HM showed a moderate outcrossing rate \( (t_m = 0.65) \) whereas Roque showed a higher outcrossing rate \( (t_m = 0.87; \) Table 2). As a comparison, the outcrossing rates obtained in Cheptou et al. (2002) are presented in Table 2b. Neither the HM outcrossing rate nor the Roque outcrossing rate was found to differ from the mean rural outcrossing rates \( (t_m = 0.91) \) but the combination of probabilities using Fisher’s method revealed that urban populations tended to have a higher rate than rural populations \( [\chi^2_{4} = 8.37, P = 0.07]. \)

In both streets, the correlation of outcrossed paternity \( (r_p) \) was very high and the paternal pool \( (1/r_p) \) was close to 1, which means that only one pollen donor contributed to the outcrossed fraction within the capitula.
Self-incompatibility variation

A total of 410 capitula were analysed. Like previous self-incompatibility measurements on *C. sancta*, seed:ovule ratios for autonomous or hand self-pollination were low but varied continuously. Thus, self-incompatibility is not expressed as a binary response but in a quantitative way (Cheptou et al., 2002). The effect of crossing was highly significant (*P* < 0.001; Table 3, Fig. 5). Mean seed sets were highest for outcrossed pollinations and lowest for autonomous selfing. The genotype factor within populations was also highly significant (*P* < 0.001; Table 3). This finding was also obtained by Cheptou *et al.* (2002) and can be interpreted as polymorphism for reproductive traits within populations. The population effect was not significant (*P* > 0.5; Table 3, Fig. 5). However, the interaction between population and treatment was significant (*P* < 0.001; Table 3). In particular, autonomous selfing and selfing by hand pollination were similar in Roque but differed in HM and Claret, thus creating a significant interaction (Fig. 5). The proportion of autonomous selfing at Claret was higher than that in the two urban populations.

**Discussion**

Our aim was to study the species *C. sancta* in an urban environment in order to investigate the effect of small population densities as a consequence of fragmentation on pollination processes and mating systems. To this end, we used complementary approaches based on field study, the use of genetic markers and cultivation of plants from different origins in the same environment. Overall, our study showed that pollination and reproduction were strongly affected by density in the urban environment, which indicates that the range of population sizes in patches consistently affected pollination and reproduction. However, no evidence for greater selfing ability was detected in urban populations.

**Pollination services in urban fragmented populations**

We found that pollinator behaviour was significantly affected by flowering plant density in fragmented populations. The probability of pollinators being present during a 15-min
period significantly increased with the density of flowered plants, from c. 10% at low density to c. 80% at high density. This result is consistent with the higher attractiveness of dense patches relative to sparse ones. The same result has been reported in other studies (see Ghazoul, 2005 for a review). Waites & Agren (2004), studying the species *Lythrum salicaria* on small islands, found a positive relationship between visits and population sizes but for a larger range of population sizes.

We found that the number of flowers visited increased with density, a result similar to those obtained by Klinkhamer & De Jong (1990) in *Echium vulgare* and Mustajärvi et al. (2001) in *Lychnis visciaria*. However, the visit duration was not significantly related to density. The mean visit duration in the urban environment was 9.32 s. Interestingly, the mean time per visit in urban patches was about twice the mean time in the large and dense Claret population (5.12 s, estimated from 65 visits). Although using our protocol we could not exclude the effects of factors other than fragmentation in the urban environment, the pattern observed is consistent with the optimal foraging theory (Charnov, 1976). Indeed, bees tend to minimize energy lost by flying when foraging. Therefore, when resources are patchy as in urban populations, we expect bees to spend more time on a flower than in dense populations where distances between different sources are low. A similar result has been found on the species *Echium vulgare* (Klinkhamer & De Jong, 1990). The visit duration for the urban population CB with several hundred individuals (8.75 s) was similar to that found in urban patches, although the population size was higher. This suggests that pollinators may adapt their behaviour to the urban environment (mainly patchy) as a whole and may not adapt their foraging behaviour to the local density of plants within the city. Moreover, progeny array analyses in urban populations revealed that the number of fathers contributing to the outcrossed fraction within a capitulum was close to 1, which is consistent with the long pollination time and the low visitation rate. In contrast, Cheptou et al. (2002) reported that the number of fathers varied from 1.6 to 6.25 in large populations of *C. sancta* from the countryside, consistent with the short pollination times and high visitation rate reported here.

Reproductive output in the allogamous species *C. sancta* demonstrates an Allee effect in urban populations

Using the seed:ovule ratio as an individual measure of fitness, we found a positive relationship between fitness and density, which demonstrates an Allee effect in urban populations. Because the number of capitula per plant did not vary with patch density, individual status (e.g. resource limitation attributable to density) can be dismissed as a factor accounting for the pattern observed. The predicted proportion of ovules fertilized varied from 20% at low density to 80% at high density. There is no clear reason why the Roque population exhibited a higher rate of ovule fertilization. In our self-incompatibility study, Roque had a higher propensity to produce seeds autonomously without pollinators, which may explain the higher seed production whatever the density.

There are two possible reasons for reduced seed set in small populations (Wilcock & Neiland, 2002). First, pollination by genetically related plants or geitonogamy may cause a decrease in seed set because of inbreeding depression or because of similar self-incompatibility alleles shared by the pollen donor and pollen recipient. Secondly, the greater pollinator attraction of dense populations may result in higher individual pollination and therefore higher seed set (Klinkhamer & De Jong, 1993). This hypothesis requires that the pollination service for individual plants must increase with density. We have to keep in mind that pollination services were measured on the whole patch. In order to infer pollination services for individual plants, our estimation must be scaled by the number of plants. From our experimental data, the product of the fitted probability of presence and the fitted number of visits (Herrera, 1987, 1989; Kunin & Isawa, 1996) scaled by the number of plants per patch tells us if pollination at the individual level changes with density (given that mean pollination duration does not change with density). The scaled product increases with density: from 0.2 (at low density) to more than 2.2 (at high density). Although this product must be considered with caution because of the statistical uncertainties on each curve, the pollination pattern is consistent with an Allee effect and is likely to explain the increase of individual seed production with density. In contrast, we have little evidence for the reduced seed set being caused by related pollen. Indeed, progeny arrays within the capitula analysis did not demonstrate significant biparental inbreeding (\(t_m - t_s\)). This means that crosses among related individuals were not likely. Moreover, it is unlikely that mating among sibs caused a reduction in seed set because previous studies have failed to detect any reduction for crosses among full-sibs (Cheptou et al., 2000). Overall, urban environments have lower seed:ovule ratios (which vary from 20 to 80%) compared with large populations (mean seed:ovule ratios higher than 80%). Interestingly, the seed:ovule ratio at the upper limit of patch sizes is around 80%, which emphasizes the importance of density as a factor limiting seed production in urban populations. Groom (1998) found that the effect of density in *Clarkia concinna* was pronounced in small populations but no clear relation was found when population size exceeded 50 individuals. Our results show that the range of size at which an Allee effect occurs is of the same order of magnitude.

No evidence for a greater ability to self-pollinate in the urban environment in spite of higher realized selfing rates

The estimation of selfing rates inferred from the multilocus estimation in HM and Roque by means of progeny array
analysis revealed significant selfing, i.e. 35% for HM and 13% for Roque. These values are at the upper limit of the range of values previously found in large populations of *C. sancta* near Montpellier (Cheptou *et al.*, 2002). The combination of tests revealed that the urban populations tended to exhibit a higher selfing rate than populations from the countryside (marginally significant). We were not able to detect biparental inbreeding (see, however, Griffin & Eckert, 2003 for a critical view of the method). Interestingly, all the progeny of a higher selfing rate than populations from the countryside (considered as full-sibs. In contrast, there was a tendency for higher selfing in Claret as revealed by higher seed set for spontaneous selfing and self-fertilization by hand pollination. Cheptou *et al.* (2002) reported between-population variation for self-incompatibility in the countryside, some populations being more self-incompatible than that at Claret. The pattern observed may result from a historical artefact and we have no evidence of selection acting on the mating system in urban populations. The slight increase in the realized selfing rate in HM and Roque is likely to be caused by poor pollination conditions, especially at low density, which makes outcrossing rates lower in urban populations. This phenomenon has been reported in experimental studies (Van Treuren *et al.*, 1994) but the number of family samples used in this study did not allow us to correlate the selfing rates to density. Specifically, the finding that the measured selfing rate was higher than the spontaneous selfing rate measured in controlled conditions may have been a result of the fact that the fraction of outcrossing ovules was low (Allee effect), which artificially increased the ratio of selfed ovules.

Our study has revealed a lower pollination rate in urban patches compared with rural populations. This may be a consequence of the lower attractiveness of small patches (as a result of fragmentation) or other factors linked to urbanization (e.g. few pollinators independent of plant density). Pollen limitation is an important selection pressure towards self-pollination (Holsinger, 2000; see, however, Cheptou, 2004). But why has self-pollination not evolved in urban populations? First, it is also feasible that urban populations have not adapted to urban conditions yet because of their recent foundation, even if the ecological conditions for selfing to evolve do exist in our system. We know that the patches used in this study have existed for more than 20 years, but populations may actually be more recent as a result of extinctions. Secondly, it is also possible that gene flow from large populations close to the city of Montpellier where pollen limitation does not occur is substantial, preventing urban populations from adapting their mating strategy (source-sink dynamics).

In this study, we have shown that patchy urban populations provide a useful framework for the analysis of pollination in fragmented environments. Plant–pollinator interactions have been found to be modified by the urban environment, and plant density appears to be a key factor in this system. As a result, low densities are expected to endanger population demography (the Allee effect), potentially affecting patch extinction rates (see Lennartsson, 2002). Thus, the Allee effect in local populations demonstrated here may affect metapopulation dynamics. However, we found no evidence of mating system adaptation in urban populations. The analysis of gene flow between patches and large populations will be crucial in investigating the possibility of genetic differentiation in the urban environment.

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