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A population study of
***Hyacinthoides non-scripta*:**
Density dependence, Phenology and Environment

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Master of Science by Research

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A Population Study of *Hyacinthoides non-scripta*: Density dependence, Phenology and Environment

Natalie Allum

Abstract

This study investigated different factors affecting seed production in the English bluebell, *Hyacinthoides non-scripta*, and the role of density and phenology during their reproductive stage. The study sought to understand processes such as pollination facilitation, i.e. positive density dependence, and the complex interaction of density acting on different stages, and even parts, of the plant. A simulation model was developed to investigate population growth and spread under varying degrees of density dependence. Data were collected during the flowering season in 2015, from woodland surrounding Durham University (North-East England, UK), to investigate the impacts of conspecific density, flowering phenology and environmental factors on seed production (used here as a proxy for plant fitness).

The unspecialized manner of bluebell dispersal leads to extremely slow spread and influences the spatial structure of the population. Population simulation using baseline parameters (excluding density dependence except for on adult survival) predicted the population to take more than 100 years to reach plant densities and population sizes seen in the field, indicating that new populations of bluebells may take many years to establish and expand. The model also highlighted the importance of seedling survival and fertilisation for population growth and spread, and the necessity for high adult survival for population existence.

Data from the field suggest that flowering date is an important plant trait that is likely to be subject to strong selective pressure; as plants starting their flowering in the first two weeks of the flowering season produced more than double the number of seeds produced by later plants. Aspect was the most important environmental factor. Data from several years are needed to verify the model further, and determine if the trends seen in the data are common for the English bluebell in the North-East of England, or are the result of a non-optimal growth season.

[Word count: 300]

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Chapter 1: Introduction

In biology populations are often described by their size, structure and density. Generally, population density is the number of individuals per patch or area, but as argued by Kunin (1997) this concept of density is only clear when applied to uniform populations, i.e. populations where the individuals are equally distributed across space. As populations are normally patchier and more varied in nature, other approaches have been used in studies on effects of population density; density of smaller patches (local density), number of individuals in a patch (patch size) and distance to neighbours (neighbourhood density, ecological neighbourhood) (Kunin 1997; Silvertown & Charlesworth 2001; Peters 2003; Ghazoul 2005). In animal systems the density of the population can only be measured indirectly, and in such highly mobile systems it may only represent a snapshot of the population at the time it was measured. However, in largely sessile systems such as plants the effect of density can be studied more easily (Kunin 1997).

In population ecology, density dependence is often of interest because of the effect it has on population growth. Processes are density dependent if they are regulated by the density of the population, e.g. density dependent mortality in the form of increased susceptibility to pathogens at high densities (Bell et al. 2006). Plant-plant interactions, both intra- and interspecific, can also be dependent on the density of plants around them. These interactions can range from facilitative to competitive depending on factors such as timing and habitat (often related to level of stress on the plants) in addition to spacing and abundance of surrounding individuals (Brooker & Callaghan 1998; Peters 2003; Johnson et al. 2012; García-Cervigón et al. 2013). Positive density dependent interactions were largely neglected in earlier studies of population dynamics and growth, as the effects of competitive interactions were thought to be bigger and thus be more important. However, since the mid-1980s, positive interactions have received more attention (Brooker & Callaghan 1998) and the

question of competition versus facilitation is now being more intensely studied (Feldman & Morris 2011; Johnson et al. 2012).

Phenology, i.e. the timing of life cycle events, is also of interest when studying the ecology of a species. Specifically, when studying plants, events such as the timing of flowering, seed dispersal and seed germination are of interest. These events are timed by cues, abiotic or biotic, that are often species specific (Rathcke & Lacey 1985; Klimas et al. 2012). Because of climate change, many species' phenology is shifting, e.g. flowering occurring earlier (Fitter & Fitter 2002). This is problematic when different species are phenologically synchronised and dependent on each other, e.g. plants relying on pollinators for fertilisation, and one species shifts out of synchrony with the other (Kudo & Ida 2013; Rafferty et al. 2015). Such phenological mismatch can be detrimental to either or both species, especially if the species involved are highly specialised, i.e. a pollinator foraging on only one species, or a plant depending on a single species of pollinator (Kudo & Ida 2013).

The following paragraphs review the effects of density on aspects of reproduction in plants, focusing on the effects of conspecific density, but interspecific interactions will also be briefly considered. In addition, the potential effects of phenology and temporal density on reproductive success will be discussed.

Positive density dependence, Allee effects and facilitation

Interspecific facilitative interactions are typical of alpine communities, where cushion plants have been shown to have positive effects on the plants around them and on biodiversity, potentially at their own expense (Schöb et al. 2014). Interspecific facilitation has also been found in forest communities where having heterospecific neighbours shelters the focal individual from conspecific competition and acts as pest/pathogen protection (Peters 2003). Ghazoul (2006) found that co-flowering heterospecific neighbours increased the pollinator attraction to the patch and the fertilisation of the focal species. However, there was a limit to this benefit, as above a threshold density the interaction turned to competition (see below). Ghazoul (2006) hypothesised that this facilitation is only possible if pollinators do not discriminate

between florally distinct species or if pollinators seek different rewards from different species, e.g. nectar or pollen.

A similar type of pollination facilitation can also occur between conspecifics, as has been shown for several flowering herbs (Kunin 1997; Groom 1998; Knight 2003). Plants at lower densities are expected to be less visible and/or attractive to their animal pollinators. This can be highly species specific and depend upon factors such as how generalist their pollinators are (Duffy & Johnson 2011; Duffy et al. 2013; Lundgren et al. 2013). Wind-pollinated plants are not expected to show density dependent fecundity, given that they are not completely isolated and self-incompatible, as they rely on an abiotic factor for pollination. Nottebrock et al. (2013) found this to be true for the wind-pollinated *Leucadendron rubrum*. They also showed that the fecundity of the animal-pollinated *Protea repens* was indeed density dependent.

Reduced visitation is expected to lower the amount of pollen transferred between flowers and plants (Groom 1998; Young et al. 2012). This could mean reduced conspecific (compatible) pollen receipt, increased heterospecific (incompatible) pollen receipt if other species that are favoured by the pollinators are present, or both. The response is also highly species-specific, and depends on the level of self-incompatibility in the species, pollinator dependency and the level of visitation the species is adapted to (Lázaro et al. 2014). Lázaro et al. (2014) suggest that if there is a sudden decline in pollinator abundance, species that under normal circumstances receive frequent pollinator visits will suffer more than species accustomed to lower visitation rates. This arises since the latter will often have a breeding system that either does not require outcross pollen, or only requires a small amount of pollen, for seed production.

As the density of the plant patch increases, the attractiveness, and so the pollinator abundance, is expected to increase with it and therefore reduce pollen-limitation (Ashman et al. 2004). This is known as positive density dependence, or the Allee effect, where an increase in density benefits the species. The Allee effect can have different impacts on different life stages or growth forms, and is not limited to plant-pollinator interactions but rather any positive effect of increasing density on a component of individual fitness or population growth rate (Stephens et al. 1999; Courchamp et al. 1999; Keitt et al. 2001).

Interestingly, pollinators have been found to “linger” in lower density patches, i.e. the visitation rate per flower is increased (Ghazoul 2005). This can lead to increased selfing and may not increase fecundity in a self-incompatible species, especially if it exhibits late-stage self-incompatibility (Duffy & Johnson 2011), in which case the plant is effectively wasting ovules on its own pollen. A self-compatible species, however, may benefit from the increased overall pollen receipt, regardless of the outcross pollen to self-pollen ratio.

Negative density dependence and competition

While higher densities of plants might lead to an increase in pollinator abundance, the level of competition is bound to increase between conspecifics and between heterospecifics that require the same resources. Density dependent competition occurs when one or more resources required fall below the level of combined demands by the individual plants (Clements et al. 1929, quoted in Ford & Diggle 1981). Habitats are not equal in their level of resource availability, for instance some habitats are more nutrient rich than others, meaning that the level of competition is dependent upon the environment and the context of the interaction (García-Cervigón et al. 2013; He et al. 2013; Schöb et al. 2014).

In sessile systems such as plants, individuals normally only compete directly with their neighbours or over limited spatial scales (Kunin 1997; Nottebrock et al. 2013). The effective density and its impact upon an individual are therefore determined by the immediate neighbours of that individual (Kenkel 1988). Plants compete mainly for light and space; due to their limited mobility a common way to compete for light is to grow taller, which depends on having the space to do so (Silvertown & Charlesworth 2001). Competition for other resources such as water and nutrients also depends upon the availability of space, as the plant’s main way of obtaining such resources is by growing roots. The intensity of competition is therefore expected to increase with density (Feldman & Morris 2011), and there may be differences in intensity between root and shoot competition. Weiner (1990) separated root and shoot competition for *Ipomoea tricolor*, and showed that root competition had a significant effect on mean plant size,

but did not increase the size inequality between the individuals significantly, while the opposite was true for shoot competition.

There are different types of competitive interactions, such as interspecific and intraspecific competition. The former can often determine community-level structure and the distribution of different species within a habitat, while the latter influences population structure. In addition there is interference competition, where individuals compete directly via aggression (mainly in mobile systems) or by physically limiting the opponent, and exploitation competition, where individuals compete via an intermediate, often a limiting, resource (Goldberg et al. 2001). The latter is often seen in plants, although Goldberg et al. (2001) found interference competition to be the defining factor at the emergence stage of seeds. Density dependent mortality has been shown to be especially strong for seeds and seedlings of many species (Ris Lambers et al. 2002; Peters 2003; Ghazoul 2005; Bell et al. 2006), and Peters (2003) found that density could also influence the survival of even quite large trees. Competition between heterospecifics can have negative impacts on both species, if the competition is symmetrical, i.e. they have equal effects on each other. If the competition is asymmetrical, one species will have a bigger effect on the other, a greater effect than would be expected from mere size differences between the individuals. The latter scenario can lead to competitive exclusion, where one species “wins” and the other is excluded from the habitat (Weiner 1990; Honnay et al. 1999; Ghazoul 2006). This is often seen in grasslands where vigorous, dominant graminoids exclude flowering herbs (Segre et al. 2014).

Competition between conspecifics can lead to reduced growth rate and survival of individuals in the patch or population (García-Cervigón et al. 2013). Grabham and Packham (1983) found that English bluebells (*Hyacinthoides non-scripta*) growing at high densities had smaller bulbs and smaller inflorescences than plants at low densities, which is consistent with the idea that exploitation competition increases with density and reduces growth (Goldberg et al. 2001). Reduction in growth with increased density cannot go on forever, as plants cannot become infinitely small and still survive; the number of plants in the population will therefore decrease with crowding. This type of density dependent mortality is known as self-thinning (Kenkel 1988; Silvertown & Charlesworth 2001). Swamy et al. (2011) found that the probability

of a seedling growing to become a sapling (seed efficiency) was strongly correlated with the distance from large conspecifics; the further away the higher the chance of survival. The effect of large trees was highly species-specific; whilst conspecifics exerted a strong negative effect on seed efficiency, heterospecific trees had minimal influence.

Asymmetric competition between conspecifics, and crowding, can have big impacts on plant populations, because increased size inequality within populations leads to inequality in survival and fecundity (Weiner 1990; Silvertown & Charlesworth 2001). Inequality in fecundity reduces the effective population size, especially in plants that need to be of a certain size to produce seeds, and scenarios where a small fraction of the population contributes disproportionately to future generations can occur, affecting the genetic diversity of the population (Silvertown & Charlesworth 2001).

As mentioned above, at high densities plants may not only compete for resources, but also for pollinator attention. This may be particularly true if the pollinator-to-flower ratio is low, as pollinators may then afford to be pickier about which plants they choose to visit, which increases the competition between flowers (Lázaro et al. 2013).

Pollinators should, according to the theory of ideal free distribution (IDF), distribute themselves on a variable resource so that individual food intake rate is the same at all local areas or patches (Dreisig 1995). A smaller patch of plants should therefore have fewer foragers visiting than a larger patch of plants. Garbuzov et al. (2015) did indeed find this; for both plant species included in their study (*Borago officinalis* and *Lavandula x intermedia*) the number of foraging insects per patch was positively linearly related to patch area, which was highly correlated with the number of flowers in that patch. In a study of *Cirsium purpuratum* Ohashi & Yahara (1998) found that the number of flowering heads visited on a plant increased linearly with display size, but the visitation rate of bumble bees (*Bombus* spp.) per plant was a decelerating function of floral display. Grindeland et al. (2005) studied *Digitalis purpurea* and found that while plant visitation rate increased with floral display size, the proportion of flowers visited decreased which is inconsistent with IDF across flowers. Goulson (2000) found evidence of decelerating rates of inflorescence visitation with increased floral display size in larger patches of *Trifolium repens*, and suggests pollinators, specifically bumble

bees, are less likely to search for the remaining un-visited flowers in larger patches than smaller patches. Using a large pan-European, multi species dataset, Dauber et al. (2010) found increases in visitation rates in small patches and decreases in visitation rates when the patches were large. This may be due to saturation of the pollinator population due to an abundance of flowers available (Dauber et al. 2010). Grindeland et al. (2005) also found some evidence of density dependent differences in bumble bee foraging behaviour; plant visitation rate was higher in dense patches compared with sparse patches, and the proportion of flowers visited on equally sized plants declined at a faster rate in dense patches. Furthermore, Totland (2001) studied alpine populations of *Ranunculus acris* and found that visitation rate was higher in warmer compared to colder habitats. IDF is therefore not always achieved and it is likely dependent on both species and community context (Grindeland et al. 2005; Dauber et al. 2010; Lázaro et al. 2013).

Facilitation versus competition

Competition and facilitation are two major forces driving community structure in a habitat (García-Cervigón et al. 2013) and thus also of individual populations. According to these authors the plant-plant interactions are highly environmentally-dependent, and the change between competition and facilitation determined by abiotic stress. At high levels of stress intraspecific facilitation is the leading factor influencing the population, while competition becomes increasingly important as stress decreases.

Low density patches, whilst often suffering from pollen limitation as discussed above, may have an increased survival rate (Bell et al. 2006; Feldman & Morris 2011). As the number of conspecifics decreases the remaining individuals benefit from decreased competition for resources such as nutrients. This release from negative density dependence means the plants are free to grow and their chance of survival increases. Thus a demographic Allee effect may not be observable in such patches as the component Allee effect on fecundity is masked by the increased survival (Feldman & Morris 2011).

A study by Grabham and Packham (1983) also found a reduction in seed output in patches with higher densities of *H. non-scripta*, indicating resource limitation in seed development as well as in growth, but they did not look into fertilisation or pollinator visitation. Mass-flowering is expected to lead to competition between conspecifics and to pollen limitation (Johnson et al. 2012). Using a Consumer-Resource model approach, Holland and DeAngelis (2010) argue that plants cannot overexploit pollinators, as the pollinators are providing a function and not a limited resource, but as discussed above, visitation rates might decline at higher densities or larger patches of plants due to pollinator saturation. Additionally, pollen limitation occurs quite commonly in nature regardless of patch size and density (Ashman et al. 2004; Ghazoul 2006), which could be due to low pollinator abundance in general. We might expect the fertilisation-density relationship to have a hump-shaped curve, where fertilisation increases with density at first, decelerates and then drops at high densities. The nature and magnitude of facilitation, competition, or both, is dependent on the focal species, its pollinator(s) and habitat, and the wider community; either process might be dominant at different times in the lifecycle of the plant or affect different growth factors of the focal species. Each process is also likely to affect different flowers, and plants, differently within a flowering season (Casper & Niesenbaum 1993).

Phenology and effects of temporal density

Phenology is defined as the study of the seasonal timing of life cycle events (Rathcke & Lacey 1985; Fenner 1998). In plant systems this includes events such as the opening of flowers and germination of seeds. These events can be triggered by both biotic and abiotic cues, and can vary from year to year (Brody 1997). Climate change has in recent decades been shown to drive changes in the timing of these events (Campbell & Powers 2015), especially in high-altitude and high-latitude systems, and in spring (Kudo & Ida 2013). The changes are commonly species-specific in magnitude and direction (Rafferty et al. 2015).

Phenological modifications within and between communities should influence the biological interactions between species, which include both antagonistic and mutualistic relationships (Kudo & Ida 2013). A mutualistic relationship that has often

been studied in relation to phenological changes is that of pollinator and plant.

Pollinators and plants might use different cues when timing events such as emergence and flowering. If the timing of these cues changes asynchronously this may result in a phenological shift or phenological mismatch, which could reduce recruitment to either population. Kudo and Ida (2013) found that when spring came early, the flowering of the spring ephemeral *Corydalis ambigua* tended to be ahead of pollinator emergence, which resulted in low pollination service and thus low seed production. The onset and duration of flowering is therefore of high importance as to maximise fertilization the plant must match flowering to the presence of pollinators (Fenner 1998).

Within a population the phenology of flowering may vary as plants, while responding to the same cues, might grow at different rates depending on resource availability, different genotypes or phenotypic plasticity (Rathcke & Lacey 1985). Larger plants may, for instance, be better at storing and allocating resources and thus be able to produce more flowers and/or flower earlier (Khanduri 2012). This variation in flowering phenology within a population strongly influences the reproductive success of a plant. Slight asynchrony is often beneficial because it promotes outcrossing and reduces competition for pollination (Rathcke & Lacey 1985). Asynchronous flowering also changes the effective population size (Rathcke & Lacey 1985), especially if the species has separate male and female phases, in which case the number of males and females in the population will vary throughout the flowering season and directly impact pollen availability and fertilisation success (Bartkowska & Johnston 2014). While some synchrony is obviously necessary for outcrossing to occur, increased synchrony may be either advantageous (by increasing the attractiveness of floral displays) or disadvantageous (by satiating pollinators). The effect of synchrony is likely dependent upon species, population size and density (Rathcke & Lacey 1985).

Because of the likelihood of slight asynchrony in flowering, the component density (e.g. the density of open flowers in a population) will not be constant throughout the flowering season. In theory, the beginning of the flowering season would be expected to have low flower density and so the population would be less attractive to pollinators, and the same can occur at the end of the season. Some species, however, have been reported to have skewed flowering, with abrupt synchronous flowering at the beginning of the season and then tailing off as fewer and fewer flowers are open

(Rathcke & Lacey 1985). At the peak of the flowering density the pollinator attraction might be high, but competition for pollinators might occur, potentially limiting pollination (see above). While Thomson (2010) did find that pollen limitation was most intense for the intermediate cohorts (i.e. peak flower abundance) of the lily *Erythronium grandiflorum*, the pollen limitation of later cohorts was ameliorated by the continued emergence of bumblebees, *Bombus* spp., the main pollinator. Galen and Stanton (1991) found that late flowering individuals of an alpine buttercup (*Ranunculus adoneus*) population growing in a snow bowl suffered reduced seed set in one of their two study years. However, in the second year a different, continuous sampling method showed that while late flowering did have a negative effect on seed set, the spatial aspect of the snow bowl balanced the seed set along the exterior-interior gradient. The interior of the snow bowl provided shelter, meaning snow melt occurred later, but the habitat was more favourable once the snow did melt. Thus the seed set is dependent on both the presence of pollinators and a favourable habitat, which may lead to great variation from year to year. Galen and Stanton (1991) also found a reduction in seed size with delayed flowering due to reduced time for seed growth, which could have severe impacts on seedling survival as larger seeds generally are more successful.

Variation in reproductive phenology can potentially account for differences in pollination success between populations and species, influence gene flow between and within populations and affect seed size, timing of seed dispersal and risk of seed predation within populations (Galen & Stanton 1991), but these effects can seemingly be negated or increased depending on the environment.

Aims and project outline

This study investigates different factors affecting seed production in the English bluebell, *Hyacinthoides non-scripta* (L.) Chourde ex Rothm, and the role of density and phenology during their reproductive stage. The study seeks to understand processes such as fertilisation facilitation and the complex interaction of density acting on different stages, and even parts, of the plant. Negative density dependence is expected to decrease the population growth and the reproductive output of the plants, whilst

positive density dependence is expected to increase these factors. The following chapters will focus on a computational simulation model that was developed to investigate population growth and spread under varying degrees of density dependence, and analysis of data collected from woodland surrounding Durham University, in North-East England, United Kingdom. The model allows for investigation of population growth over large timespans, and can in future be used to predict the spread of *H. non-scripta*. The field data were collected to investigate changes in the reproductive output over a shorter time span, i.e. one flowering season.

The study species, *H. non-scripta* (Asparagaceae), is a spring-flowering, geophytic herb native to North-West Europe (Grundmann et al. 2010), although it has been introduced to the US and Canada. Between 25% and 50% of its global population can be found in the UK, where it has been protected from commercial overexploitation under the Wildlife and Countryside Act 1981, since 1998. The plant is in some areas suffering from hybridisation with, and in some cases competitive exclusion by, invasive cultivars of *H. hispanica* (Spanish bluebell) or the resulting hybrid between *H. non-scripta* and *H. hispanica*; *H. x massatiana*. The Spanish bluebell was introduced as a garden plant more than 300 years ago, but it took another 200 years before it was present in the wild. The increasing distribution of *H. hispanica* and the hybrid bluebell was recognised in the late 1980s and the English bluebell has received significant attention more recently from conservation groups (Kohn et al. 2009). The English bluebell can be distinguished from its competitors by its more tubularly shaped flowers; the way the raceme nods when the plant is flowering; and its pollen, which is cream-coloured as opposed to the blue pollen of the Spanish bluebell. Identifying hybrids can be extremely difficult, as they are highly morphologically variable; some with traits similar to *H. non-scripta* and some looking more like *H. hispanica* (Grabham & Packham 1983; Kohn et al. 2009; Grundmann et al. 2010). A key to distinguish the different *Hyacinthoides* spp. can be found in Grundmann et al. (2010).

The bluebell represents a good study system because the fruit are easily collected and the size of the ovules makes it possible to observe whether they have been fertilised or not, and as mentioned above, plant systems are better suited for density studies than animal systems, as the limited mobility of plants makes density effects easier to observe, although the density dependent interactions themselves remain highly

complex (Knight 2003; Bell et al. 2006; Nottebrock et al. 2013). Previous studies on bluebells that have included density effects and phenology have been based on comparing habitats (Grabham & Packham 1983; Gonzales Sierra et al. 1996). The present study investigates populations in very similar habitats making any potential effects more obvious.

Chapter 2: The role of density dependence on population dynamics of *Hyacinthoides non-scripta*: a comparison of predictions from a simulation model and field data

Introduction

The age-structure of a population can greatly influence its growth and dispersal (Silvertown & Charlesworth 2001). A population consisting of only immature plants will not increase in number nor claim new habitat until the plants become mature and are able to produce seeds, unless a means of vegetative reproduction is possible before sexually reproductive age is reached. The length of this immature stage differs between annuals and perennials, and between species. The stage is typically long in shade-tolerant forest herbs such as *Teucrium scorodonia*, *Allium victorialis* and *Hexastylis arifolia* (Bierzychudek 1982), and the English bluebell *Hyacinthoides non-scripta* (Van der Veken et al. 2007). *H. non-scripta* normally takes five years to reach the mature stage (Merryweather & Fitter 1995a; Van der Veken et al. 2007) and is likely due to plants taking time to accumulate enough resources to develop a bulb before allocating resources to flower production. Rix (2004) reports that *H. non-scripta* typically only flowers after four years of age, indicating that flowering is dependent on resource accumulation rather than some internal mechanism that takes exactly five seasons of growth. Merryweather and Fitter (1995a) studied bulb growth of *H. non-scripta* in the lab and developed a method of calculating age based on bulb size and the depth at which they grow. When applied to their field data they found four classes of immature bluebells which might represent four annual cohorts; “seedling”, “setaceous leaf”, “flat leaf”, and “two leaves”.

Once the plants are reproducing, growth and spread are possible. Density dependence, as discussed in Chapter 1, may play a role on dispersal and in shaping the spatial

structure of the population. Seedling survival is typically density dependent (Chapter 1) and seedlings might therefore have a greater chance of establishing themselves around the edge of the population, rather than in the middle (López-Barrera et al. 2006). This potential for edge effect coupled with a long immature stage may lead to a 'lag' effect at the edge and slow dispersal; dispersal being dependent on seedling establishment around the edge and survival to reproductive age. This lag may be especially prominent in species with poor dispersal ability, such as *H. non-scripta*. Van der Veken et al. (2007) found that transplanted populations of *H. non-scripta* had very limited patch expansion after 45 years and estimated their spread to be between 0.006 and 0.06 metres per year. Van der Veken et al. (2007) studied *H. non-scripta* in woodland similar to that of this study, i.e. dominated by *Quercus* and *Fagus* spp. (See Methods and Materials: Field observations), which are normally quite stable habitats and this likely contributed to the slow spread rates observed in their study, and is also likely to influence patch expansion of the bluebells in Durham. Evidently, this species must have experienced some sort of long distance dispersal since the last glaciation in order to achieve its current range across the British Isles, but little evidence of exactly how this happened can be found in the literature.

Dispersal and spread are of particular importance when looking at invasive species/populations and their native or non-invasive counterparts. Burns et al. (2013) found that dispersal ability, coupled with potential for high fecundity, was higher in species that had previously been classified as invasive. If the introduced species has higher fecundity, greater plasticity in fecundity in a way that benefits them, or greater dispersal ability than the native plant, then it is likely to have the competitive advantage over the native species and become invasive (Burns et al. 2013). In the case of the English bluebell, the Spanish bluebell is generally considered as an invasive competitor (Kohn et al. 2009). There is a dearth of information to be found in the literature, however, about the fecundity and dispersal of either species. Van der Veken et al (2007) reported very slow spread for the English bluebell, and due to their similar dispersal method, this is likely also true for the Spanish bluebell. Kohn et al. (2009) suggest that, if the spread is indeed equally slow, then hybridisation is likely the bigger threat to the native bluebell.

This chapter explores the spread, population growth and proportion of flowering plants in a population of *H. non-scripta* using a simulation model. Population models allow us to test hypotheses and carry out virtual experiments that would take years under field conditions, e.g. models and their outcomes can be used to infer which strategy is the best to adopt in forest management or agriculture settings (Fourcaud et al. 2008). The model utilised here considers how different population parameters and the different life stages of bluebells may be affected by density dependence, and allows us to investigate how changes in density dependent parameters changes the long-term trajectory of the population's spread and growth. For instance, if competition for resources affects the number of flowers a plant can produce, how will this affect population growth over a period of 150 years? The lack of information about bluebell population growth and spread is likely due to such studies requiring many years of data collection, especially for a slow growing perennial such as this, but a model might provide some insight into these issues. In addition to growth and spread, the model is used to compare pollination facilitation and pollen competition, by changing the relationship between flower density and pollination rate. For instance, if the relationship is positive, i.e. facilitation, we would expect the population to increase, whilst the opposite would be expected if flowers compete for pollinator visitation.

To test the consistency of the model's output with field observations, two data sets were collected from field populations of *H. non-scripta*; transects were run through *H. non-scripta* patches to quantify the relation between density of plants and the proportion of flowering plants. Bulbs were also collected to investigate size at flowering and to look for evidence of cohorts predicted by the model.

Materials and Methods

Simulation model

For this study a simulation model using R (The R Project, <http://www.R-project.org/>) was developed and used to investigate the role of density dependence in regulating a plant population and its spread. The model also investigated the impact of positive and

negative density dependence on different stages of a plant's life cycle (e.g. pollination rate and adult survival) and how it may affect population growth.

The model assumes a linear arrangement of l patches (see Table 2.1 for all parameters and descriptions). Each patch may be occupied by one or more bluebell plants. Plants are censused at the start of the flowering season and each plant may be in one of five age-classes. Let $N_{i,j}^t$ denote the density of bluebell plants in patch i that are in age-class j at time t . The subscript $j = 1 \dots 5$ denotes the age of the plants, i.e. one year olds, two year olds, three year olds, four year olds and five+ year olds (plants that are five years or greater in age are referred to as mature plants). Only plants in the 5+ age-class can flower, because evidence suggests that bluebells flower around their fifth year (Blackman & Rutter 1954; Van der Veken et al. 2007).

Each year the mature plants ($j = 5$) flower and disperse seeds as follows. Let N_i^t denote the total number of plants in patch i at the start of year t .

$$N_i^t = \sum_{j=1}^5 N_{i,j}^t \quad (2.1)$$

The number of flowers produced by each mature plant, f , is assumed to be negative density dependent with respect to the number of plants in the patch:

$$f(N) = f_a e^{-f_b N} \quad (2.2)$$

where N is the total density of plants in the patch and f_a and f_b are non-negative constants. The total number of flowers produced in patch i during year t is:

$$F_i^t = f(N_i^t) N_{i,5}^t \quad (2.3)$$

Each flower has a certain number of ovules, O . The probability of an ovule being fertilised can be made to be negatively or positively density dependent (p , table 1) with respect to the number of flowers in a patch. If the probability of fertilisation is assumed to be positive density dependent and the density of flowers in a patch is F , then the probability ovules are fertilised, denoted p , is given by:

$$p(F) = p_a + (1 - p_a)(1 - e^{-p_b F}) \quad (2.4)$$

where p_a and p_b are non-negative constants. p_b is the probability of fertilisation when flower production is minimal, and fertilisation success increases to 1 as flower production increases. The probability of fertilised ovules surviving inbreeding depression is denoted g . The maximum proportion of seeds a plant can develop, d , is negatively density dependent:

$$d(N) = d_a e^{-d_b N} \quad (2.5)$$

where d_a and d_b are non-negative constants. The number of seeds produced in patch i during year t is:

$$S_i^t = \min\{d(N_i^t), gp(F_i^t)\}OF_i^t N_{i,5}^t \quad (2.6)$$

This model assumes that surviving inbreeding depression is not density dependent, i.e. it assumes that the proportion of selfing to outcross remains constant.

Suppose that fraction q of the seeds are randomly dispersed to either of the two neighbouring patches. This model ignores rare long-distance dispersal, i.e. dispersal to patches other than the immediate neighbours. Also, suppose that seeds survive the winter to become one year old plants with probability y , and that this probability is negative density dependent with respect to the total number of plants in the patch during year t , after dispersal has taken place. Specifically, if N is the total number of plants in the patch then seeds survive with probability:

$$y(N) = y_a e^{-y_b N} \quad (2.7)$$

where y_a and y_b are non-negative constants. All plants survive winter in a density dependent manner with probability, w , given by:

$$w(N) = w_a e^{-w_b N} \quad (2.8)$$

where w_a and w_b are non-negative constants.

The state of the population next year across patches can be updated as follows. First the number of one year old plants is given by:

$$N_{i,1}^{t+1} = y(N_i^t) \left[(1 - q)S_i^t + \frac{q}{2}(S_{i-1}^t + S_{i+1}^t) \right] \quad (2.9)$$

The model assumes that seeds dispersed outside the linear arrangement are lost. Next, the number of older plants is updated using:

$$N_{i,j+1}^{t+1} = w(N_i^t)N_{i,j}^t \quad (2.10)$$

for $j = 1 \dots 3$, and:

$$N_{i,5}^{t+1} = w(N_i^t)(N_{i,4}^t + N_{i,5}^t) \quad (2.11)$$

for $j = 4$ and 5 .

For simplicity, the model assumes an initial condition of a single one year old plant in the central patch, $i = 26$, in year $t = 1$; that is $N_{i,j}^1 = 0$, except $N_{26,1}^1 = 1$. The model also assumes symmetrical growth and spread.

Table 2.1: Baseline parameters used in the model, with descriptions. The model run with baseline parameters only factors in density dependence on plant survival, to avoid exponential population growth.

| Parameter name | Parameter value | Parameter description |
|----------------|-----------------|--|
| f_a | 20 | Maximum flowers per plant (Corbet, 1998) |
| f_b | 0.00 | Density dependence (effect of plant density on flower production) |
| p_a | 0.045 | Lowest fertilisation probability (Estimated from Corbet (1998)) |
| p_b | 0.00 | Density dependence (effect of flower density on visitation/fertilisation) |
| d_a | 1.0 | Highest seed development probability, resource limitation |
| d_b | 0.00 | Density dependence (effect of plant density on seed development) |
| y_a | 0.01 | Maximum seed survival, proportion of seeds surviving |
| y_b | 0.00 | Density dependence (effect of plant density on seed survival) |
| w_a | 0.95 | Maximum annual plant survival of adults (5+), proportion of adults surviving |
| w_b | 0.001 | Density dependence (effect of plant density on adult survival) |
| O | 30 | Ovules per flower, averaged 10 ovules per locule (personal observation) |
| I | 51 | Number of patches |
| G | 0.67 | Probability a fertilised ovule does not get aborted (Estimated from Corbet (1998)) |
| q | 0.3 | Proportion of seeds dispersed |

The model's 14 parameters, denoted $\Theta = \{f_a, f_b, p_a, p_b, d_a, d_b, y_a, y_b, w_a, w_b, O, q, g, l\}$ (Table 2.1) are either derived from the literature, personal observations from the field, or estimated to get realistic model outputs (i.e. outputs that are consistent with field observations), and it is worth noting that the certainty may therefore vary for each parameter. Maximum flowers per plant (f_a), lowest fertilisation probability (p_a) and the probability a fertilised ovule does not get aborted (G) are all derived from Corbet (1998), and the number of ovules per flower (O) is an average based on personal observations; these are all parameters that have a relatively high certainty compared to highest seed development probability (d_a), maximum seed- and adult survival (y_a and w_a respectively), and the proportion of seeds dispersed (q), which are all estimates and therefore less certain. The parameters can be manipulated to reflect different environments, e.g. from high or low resource availability or pollen limitation. The relative strength of density dependence can also be manipulated (by varying β in the equations seen in Figure 2.5) to explore how the population responds to rapid changes in for instance competition for resources.

Field observations

Study area and focal species

Data were collected from the woodland surrounding the School of Biological and Biomedical Sciences (SBBS), specifically Great High Wood and Little High Wood (Figure 2.1), at Durham University in the North-East of England, United Kingdom (LatLong: 54.764783, -1.572332). In Britain, the English bluebell is found from the very south of England to the North coast of Scotland (Preston et al. 2002, Figure 2.2), so the study area is situated well within the range of the species. The woodland consists mainly of beech (*Fagus sylvatica*) and oak (*Quercus robur* and *Q. petraea*) but other tree species also feature, such as species of *Sorbus* and *Betula*. As well as *H. non-scripta*, the understorey consists of mainly *Anemone nemorosa*, *Luzula sylvatica* and *Rubus* spp. The site is well drained, there being several small streams present and the soil is mostly a rich loam, although some sandy, drier areas are also present. Great High Wood is largely situated on South- and East-facing slopes, but small valleys within the forest result in a wide variety of aspects. Little High Wood is largely situated on North-

facing slopes, but again with some variety due to uneven topography. The data were collected during the flowering season in May and June, 2015.

The focal species, *H. non-scripta* (Asparagaceae), is a perennial herb, but the bulb and root system are renewed annually, and the roots are quickly colonised by mycorrhizae (Daft et al. 1980; Grabham & Packham 1983; Merryweather & Fitter 1995a; Kohn et al. 2009). The bulb renewal can sometimes lead to the bulb splitting in two, i.e. clonal reproduction (Wilson 1959; Grabham & Packham 1983; Merryweather & Fitter 1995a), although seed dispersal seems to be the main recruitment method (Corbet 1998; Van der Veken et al. 2007). Seed dispersal is achieved when the raceme and fruit dry and the plants collapse or are knocked to the ground by wind action or by animals, i.e. barochory (Honnay et al. 1999).

Transect survey

Ten small transect surveys were undertaken in Little High Wood, on patches that looked like they were still expanding, i.e. patches that were not bordered by footpaths or otherwise unable to spread. Transects were drawn from the edge of a patch to the relative middle of the patch. The transects were divided into 50cm by 50cm quadrats, and for each quadrat the number of flowering plants and total plants were counted and recorded. The data were analysed using General Linear Mixed Effect Models (GLMM) in R.

Bulb collection

50 plants were randomly sampled from a range of patches at different locations in Great High Wood and their bulbs were carefully excavated. The width and length of the bulbs and their leaves, as well as bulb circumference, were determined. For each bulb the number of leaves and, if present, the number of flowers were recorded. Additionally, the mean distance to the bulb's conspecific neighbours was measured and recorded.

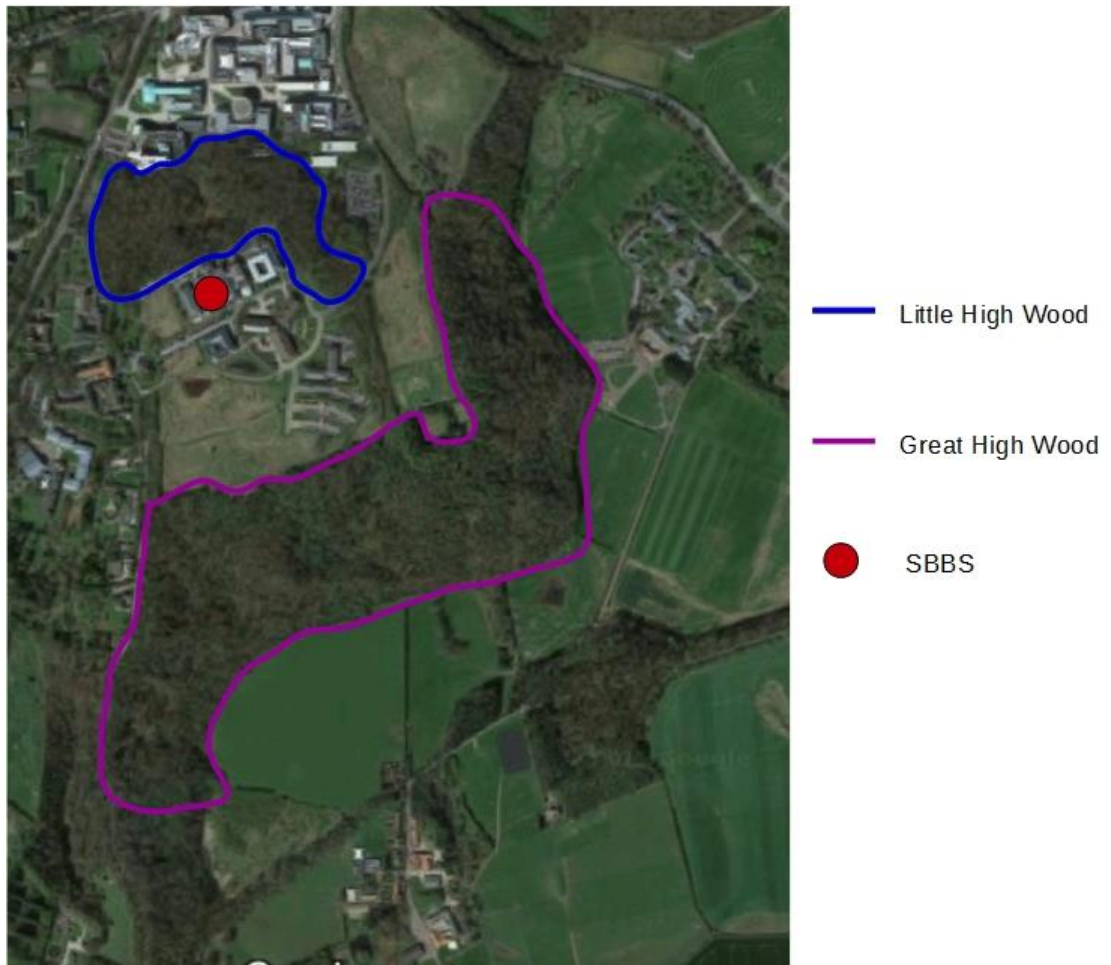


Figure 2.1: Map showing the location of the study areas, Little High Wood and Great High Wood, in relation to the School of Biological and Biomedical Sciences, Durham University.

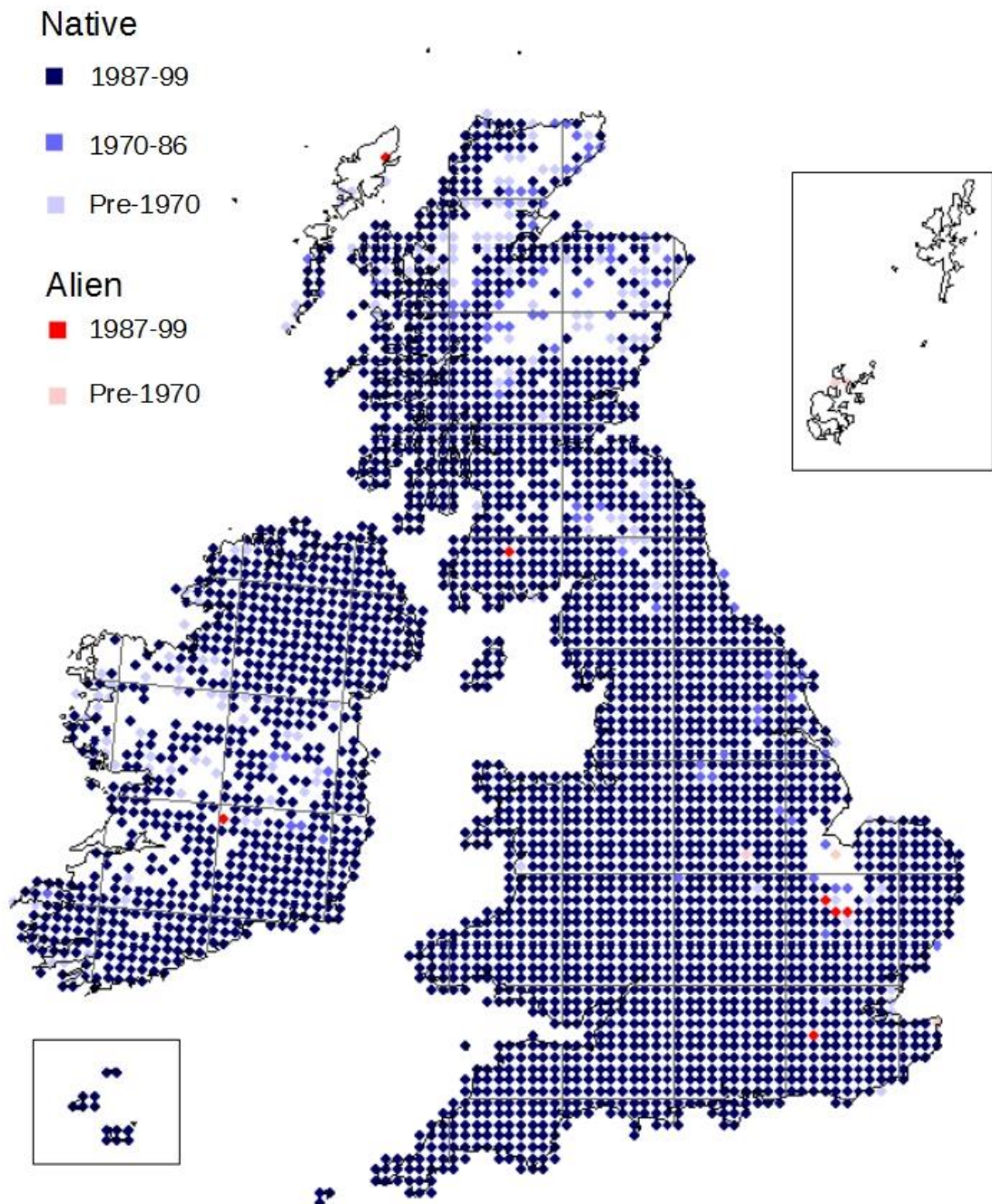


Figure 2.2: The distribution of *H. non-scripta* across the British Isles. The grid is divided into 10 km by 10 km cells. Blue indicates that the bluebell is native in that area, whilst red indicates that it has been introduced. The different shades refer to when the species was last recorded in that area, with dark being the most recent. Figure from Preston *et al.* (2002).

Results

Simulation model

Edge effects and spread

After 150 years of growth and expansion, the population had only spread to six patches on either side (Figure 2.3). Under the baseline conditions (Table 2.1), the carrying capacity of the patches was slightly less than 70 plants in each, or around 280 plants per m². Of these plants, just over 60% were flowering plants. The number of plants in each patch decreased towards the edge of the population. The proportion of mature plants remained relatively stable as number of plants decreased, with a slight decrease at the edges (Figure 2.3). The average bluebell can grow 50 cm tall (Rix 2004), so each patch is imagined to be 50 cm by 50 cm. The population expanded six patches to either side after 150 years, which would translate to a total of 3 metres, or 0.02 metres per year. This is within the range of the annual spread of 0.06 to 0.006 metres reported by Van der Veken et al. (2007).

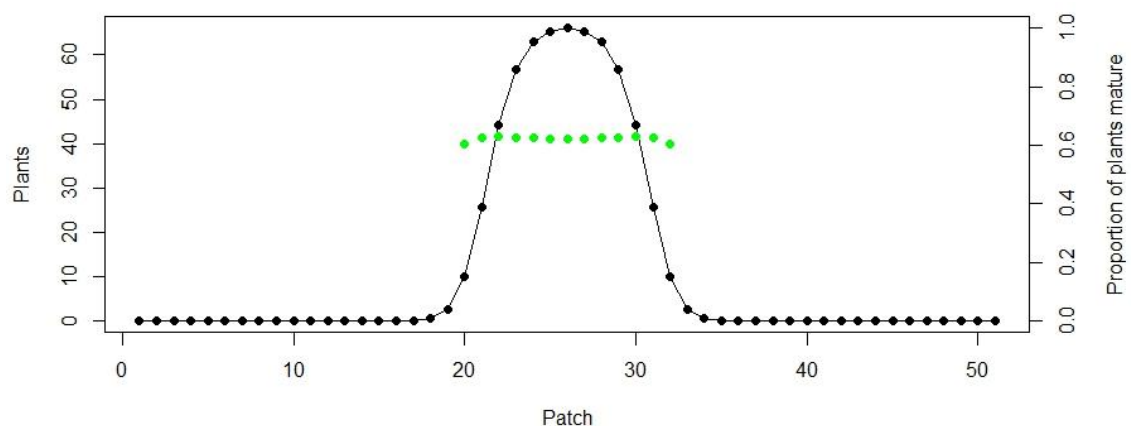


Figure 2.3: Simulation showing population growth and expansion of total plants (black) and flowering plants (proportion of flowering plants: green) after 150 years of growth under baseline conditions (Table 2.1).

Important parameters for population growth and expansion

Three parameters (seed dispersal q , seedling survival y_a and fertilisation success p_a) that were expected to influence the population's growth and expansion (namely population density, proportion of flowering plants, and spread) were changed, one by

one (Figure 2.4). The remaining parameters were kept at their baseline value (Table 2.1) and no density dependence was included, except for on adult survival.

The proportion of seeds dispersed (q) had a slight negative effect on the population's density, but not on the proportion of the plants in the population that can flower. Seed dispersal had a positive effect on the spread of the population, as is expected (Figure 2.4a, d, g).

Unsurprisingly, population density and population spread were greatly increased when seedling survival (y_a) was high. High seedling survival lead to a decrease in proportion of flowering plants in the population due to the increased number of younger plants (Figure 2.4b, e, h).

Similarly, the probability of ovules being successfully fertilised (p_a) was important for the population density and dispersal (Figure 2.4c, f, g), and had bigger impact upon the population than did seedling survival. Higher chance of fertilisation lead to more seedlings and thus a lower proportion of flowering plants, in a similar fashion to increased seedling survival. Population density and spread increased as the probability of fertilisation increased, but the proportion of flowering plants decreased. Increasing the probability of fertilisation allowed the population to spread 1250cm to either side, the furthest allowed by the model, i.e. all 51 patches were occupied by bluebells.

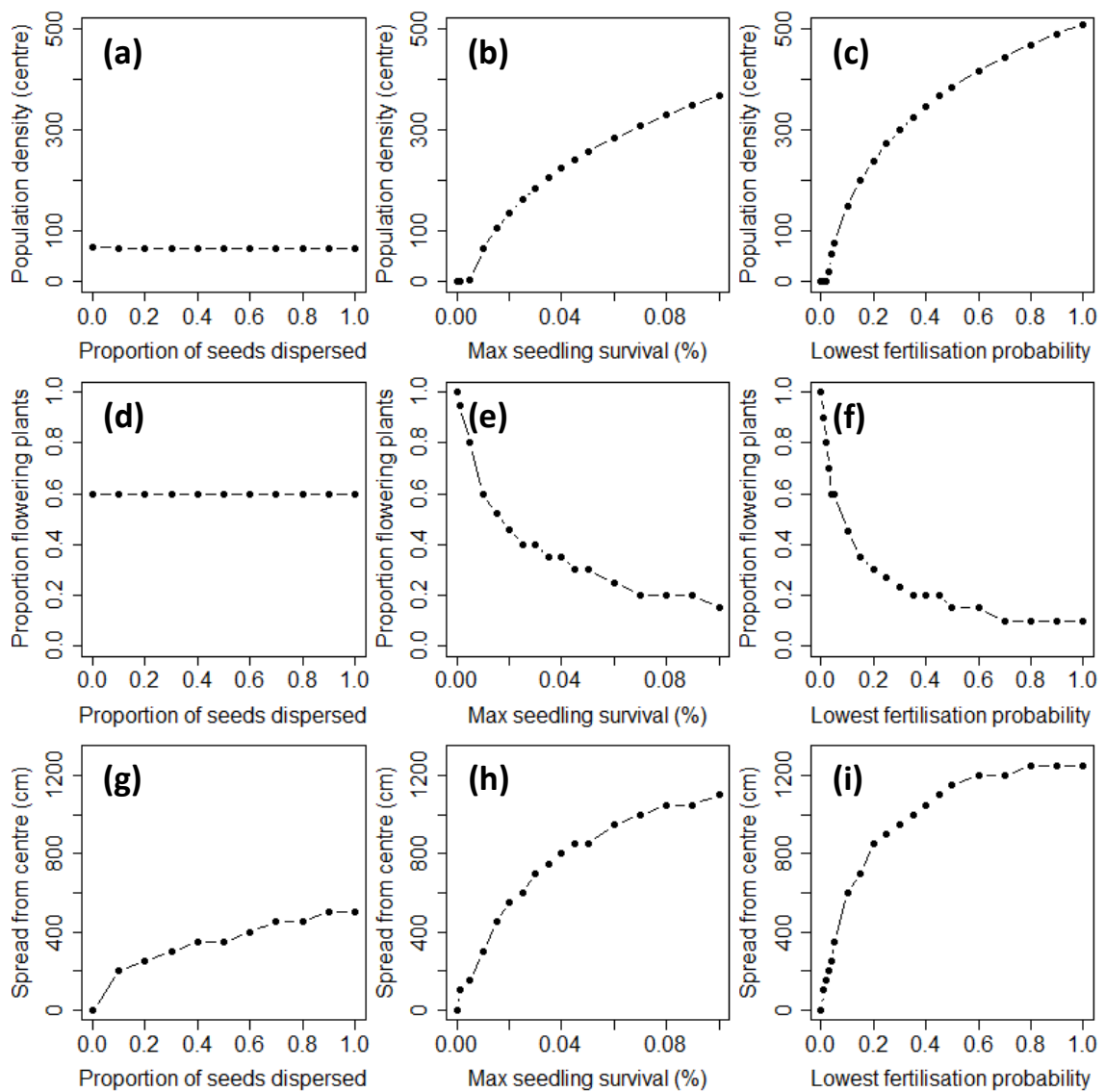


Figure 2.4: Effects of seed dispersal (q) (shown in a, d, g), seedling survival (y_a) (shown in b, e, h) and fertilisation (p_a) (shown in c, f, i) on population spread, plant density, and proportion flowering plants. Spread from centre is calculated based on each patch being 50cm by 50cm. 1250cm was the maximum spread allowed by the model. Simulations ran for 150 years.

The effect of density dependence on population growth and expansion

The model allows for variation in the density dependence of different factors affecting the population growth and spread, such as adult- or seedling survival or the probability of ovules getting fertilised. For instance, “mildly” density dependent pollination would mean that plants, and thus ovules, are less sensitive to the presence of conspecifics than if pollination is “strongly” density dependent. If the relationship is positive, i.e.

higher flower densities facilitate pollination, then “strong” density dependence would result in a larger fraction of ovules getting pollinated (and fertilised) at a lower flower density than would “mild” density dependent pollination; i.e. facilitation occurs at a lower flower density when density dependence is “strong” (Figure 2.5).

As is expected, negative density dependence (black) on flower production (f_b), seedling survival (y_b), adult survival (w_b) and seed development (d_b) all resulted in reduced population density (Figure 2.6a, b, c, d). The effect of negative density dependence on adult survival had the greatest effect; even small changes in the strength of density dependence on this factor were enough to significantly reduce the density of the population. Negatively density dependent flower production and seedling survival also had severe effects on the final density of the population; density quickly dropped when density dependence was introduced. Seed development showed the weakest effect on the population density when the factor was negatively density dependent. The lessened effect was due to the low fertilisation rate of the baseline population; very few seeds were getting fertilised, thus density dependent seed development only affected the population when it got severe enough to affect production of even low numbers of seeds. Adult survival, seedling survival, seed development and flower production were all dependent on the density of plants in the patch (N).

Fertilisation success (ρ_b) was dependent on the total number of flowers in the patch (F), and could be positively– or negatively density dependent (Figure 2.6e). The effect of negative density dependence on fertilisation success had similar effects on population density compared to Figure 2.6a and c, i.e. density decreased, but it was more sensitive to density (because the number of flowers in the population is a factor of mature plants). Positive density dependence (green) had, as expected, the opposite effect, increasing the density of the population until the carrying capacity for the patch was reached. The increase was extremely rapid once positive density dependence had been introduced, which again highlights the low probability of fertilisation in the baseline population.

Negative density dependence, with respect to both total number of plants and total number of flowers, increased the proportion of flowering plants in all cases but one

(Figure 2.6f, g, h, i, j); negative density dependence on adult survival resulted in a very slight initial decrease in proportion of flowering plants before stabilising. Negative density dependence on flower production and seedling survival lead to more rapid increases in proportion of flowering plants than did positive density dependence on seed development and adult survival, but all lead to an eventual 80 % flowering plants in the centre of the patch. Again, positive density dependence on fertilisation has the opposite effect, and the proportion of flowering plants decreased as higher fertilisation lead to an increased number of seedlings and immature plants.

In all cases the spread increased, if only slightly in most cases (Figure 2.6k, l, m, n, o). The biggest increase occurred when density dependence positively influenced fertilisation, in which case the population spread much further, as is expected due to the increased number of seeds.

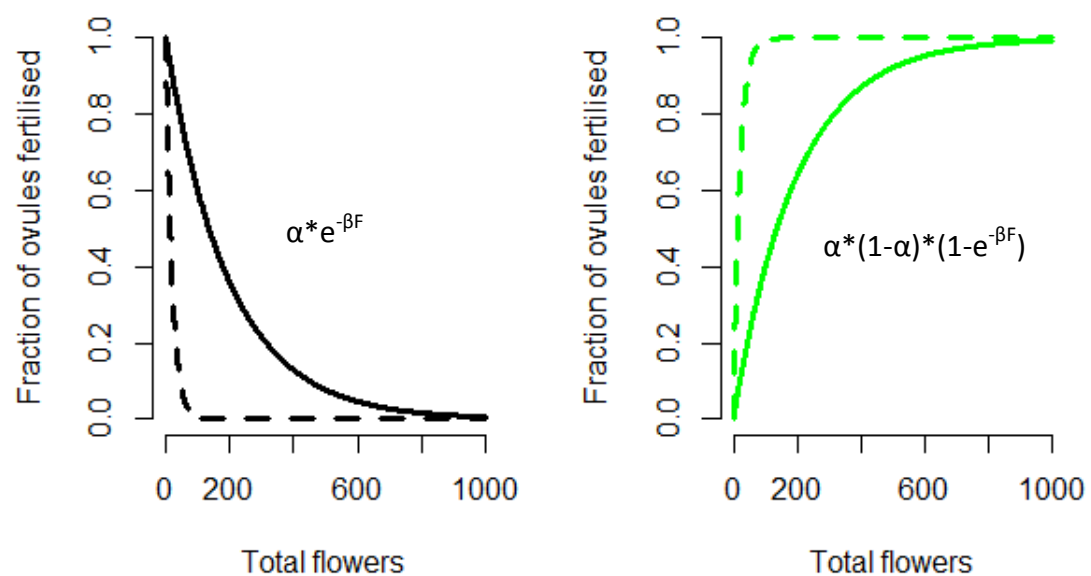


Figure 2.5: Hypothesised negative and positive effects of flower density on fertilisation success demonstrating the effect of changing the “strength” of density dependence. Black is negative density dependence, i.e. competition, green is positive density dependence, i.e. facilitation. “Strong” density dependence; $\beta = 0.05$ (dashed), and “mild” density dependence; $\beta = 0.005$ (solid). The equations used to generate the curves are presented.

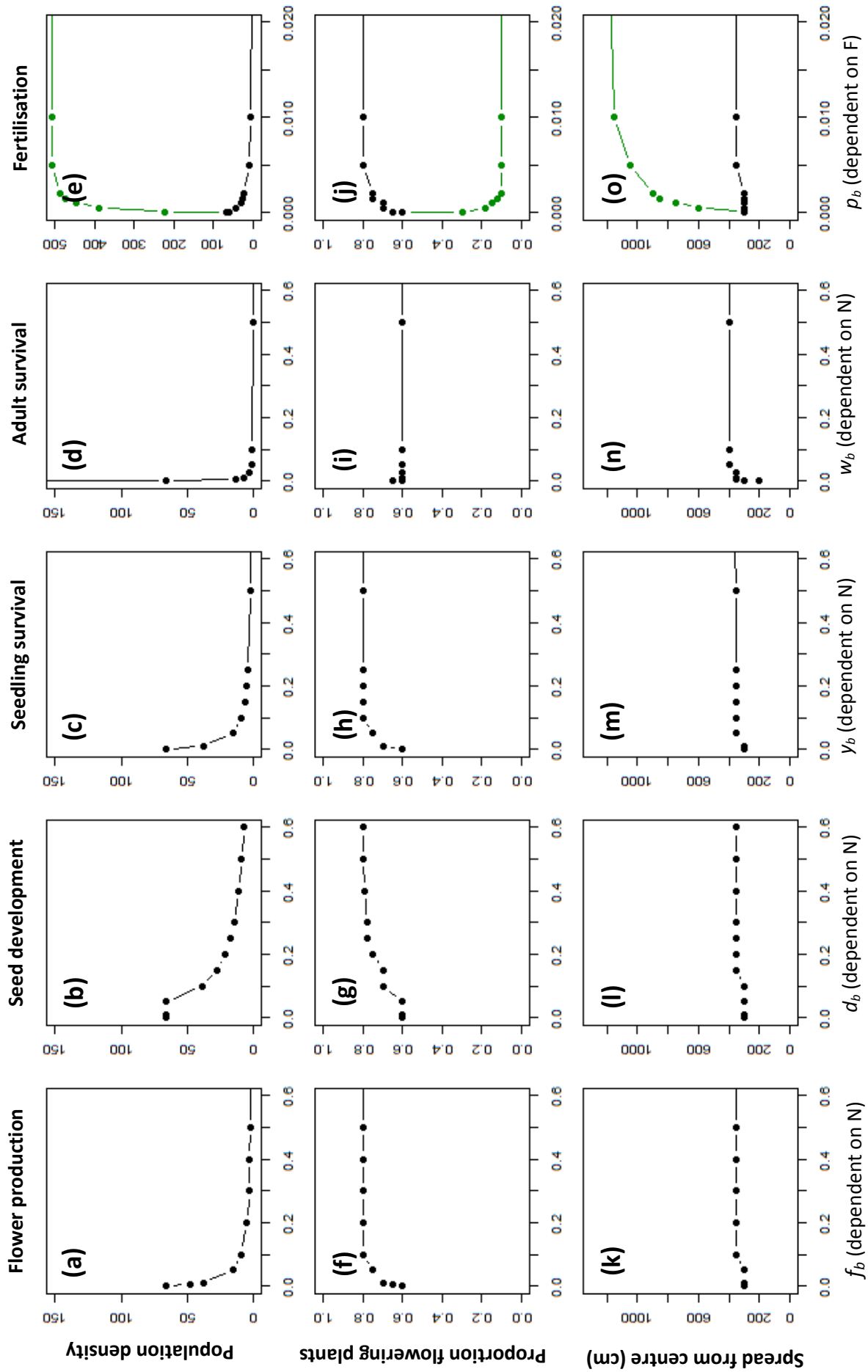


Figure 2.6: The effects of increasing the impact of density dependence on the different processes affecting the population. All graphs show negative density dependence (Black). The last column also shows positive density dependence (Green). Note different axes on e, j and o, as they depend on F rather than N. Population density and Proportion of flowering plants are measured in the centre of the population. Simulations ran for 150 years.

Field observations

On average, plant density increased steadily with distance from patch edge (Table 2.2, Figure 2.7). Similarly, the proportion of flowering plants increased with distance from patch edge (Table 2.2, Figure 2.8). Only one transect covered more than three metres, so these predictions are applicable for up to three metres. This pattern is consistent with the model's predictions (Figure 2.3).

Table 2.2: Summary of the two GLMMs performed. For both models, the covariate investigated is the distance from the edge of the patch. Plant density was assumed to exhibit Poisson variation and the proportion of flowering plants was assumed to exhibit binomial variation.

| Model | G | df | P-value | Effect size |
|-----------------------------|--------|----|---------|-------------|
| Plant density | 189.5 | 1 | <0.001 | 0.303 |
| Proportion flowering plants | 86.146 | 1 | <0.001 | 0.566 |

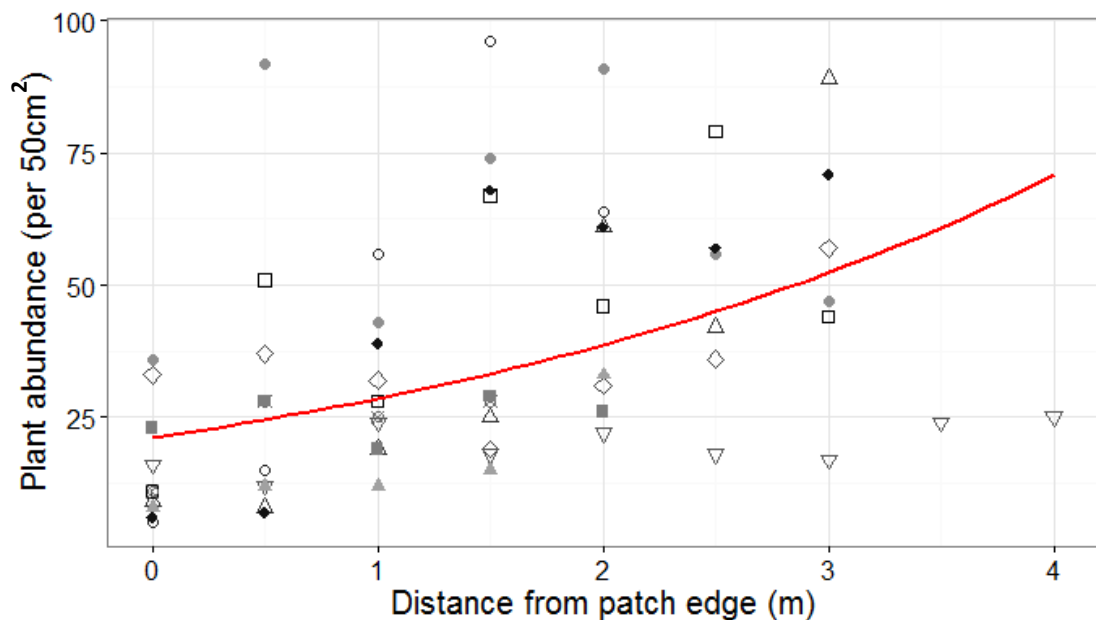


Figure 2.7: Changes in plant density along each of the 10 transects and the best-fit model prediction. Each set of markers represent a different transect.

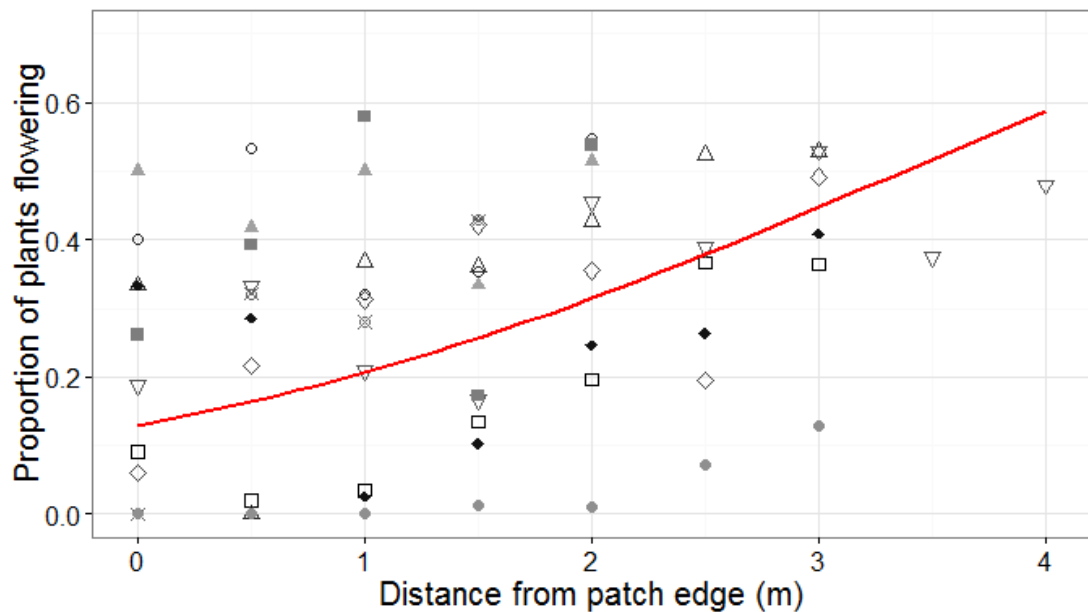


Figure 2.8: Changes in proportion of flowering plants along each of the 10 transects and the best-fit model prediction. Each set of markers represent a different transect.

Bulbs

The smallest flowering bulb had an estimated volume of 1.43 cm^3 (bulb circumference was 4.4 cm and bulb length was 1.5 cm) and the largest non-flowering bulb had an estimated volume of 1.7 cm^3 (bulb circumference was 5.4 cm and bulb length was 1.7 cm) (Figure 2.9). Mature, flowering plants had significantly larger bulbs than immature plants (Mann Whitney $U = 38$, $p < 0.001$, Figure 2.9) and as expected the number of leaves and, eventually, flowers increased with bulb size (Figure 2.10 and 2.11). The classes of juveniles reported by Merryweather and Fitter (1995a) are inconsistent with the findings in this study. Specifically, Merryweather and Fitter (1995a) describe bulbs with two leaves as being four years of age or older. However, several of the non-flowering bulbs found in this study had three to four leaves, sometimes as many as six, meaning they were either more than five years old, but not flowering, or young, abnormally large clones. Other evidence of 'cohorts', such as groups of similar masses or a clear correlation between number of leaves and bulb volume were not found.

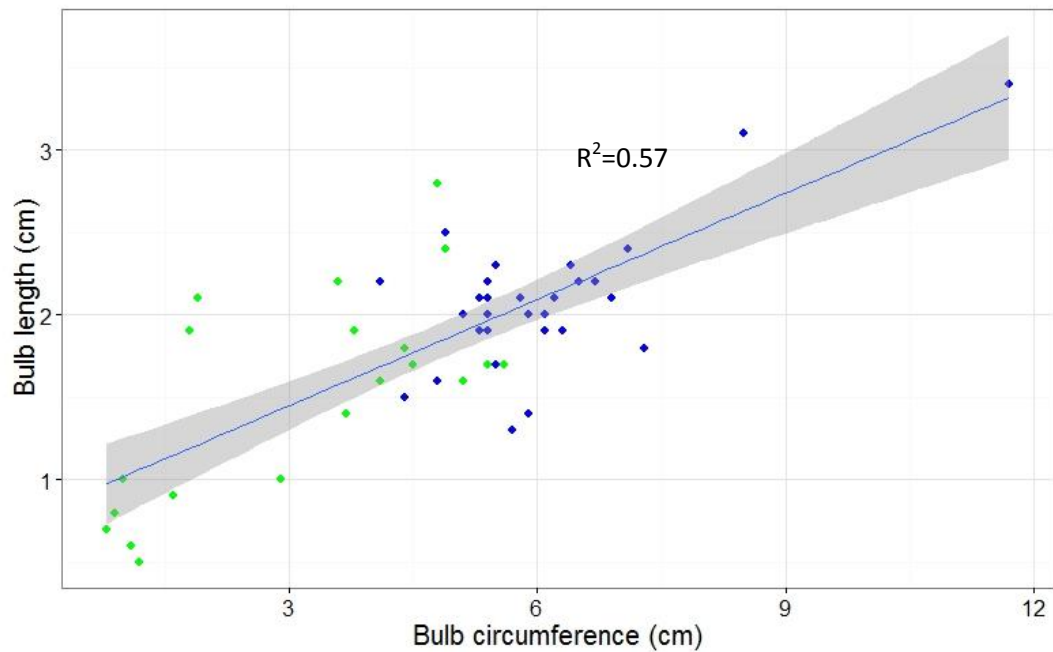


Figure 2.9: Size of collected bulbs. Blue represents flowering plants, green represents non-flowering plants. The grey shaded area around the trend line indicates the 95% confidence band based on Standard Error (estimated using R).

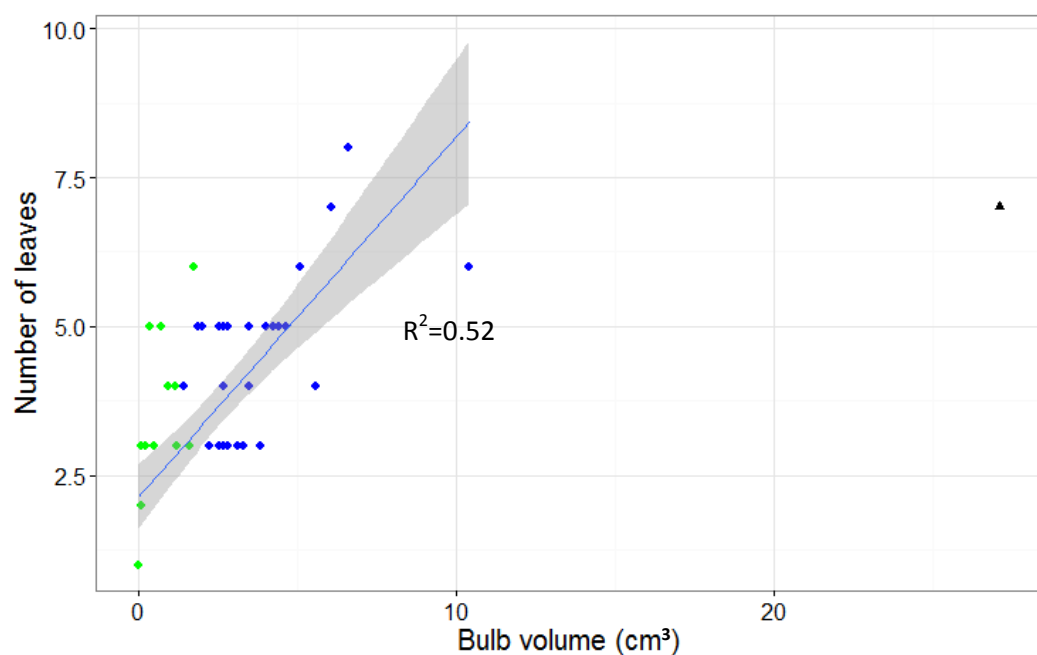


Figure 2.10: Number of leaves increased with bulb volume. Blue represents flowering plants, green represents non-flowering plants. The grey shaded area around the trend line indicates the 95% confidence band based on SE (estimated using R). The largest bulb (black triangle) is considered an outlier and excluded from the trend line. Model fit including outlier: $R^2=0.33$.

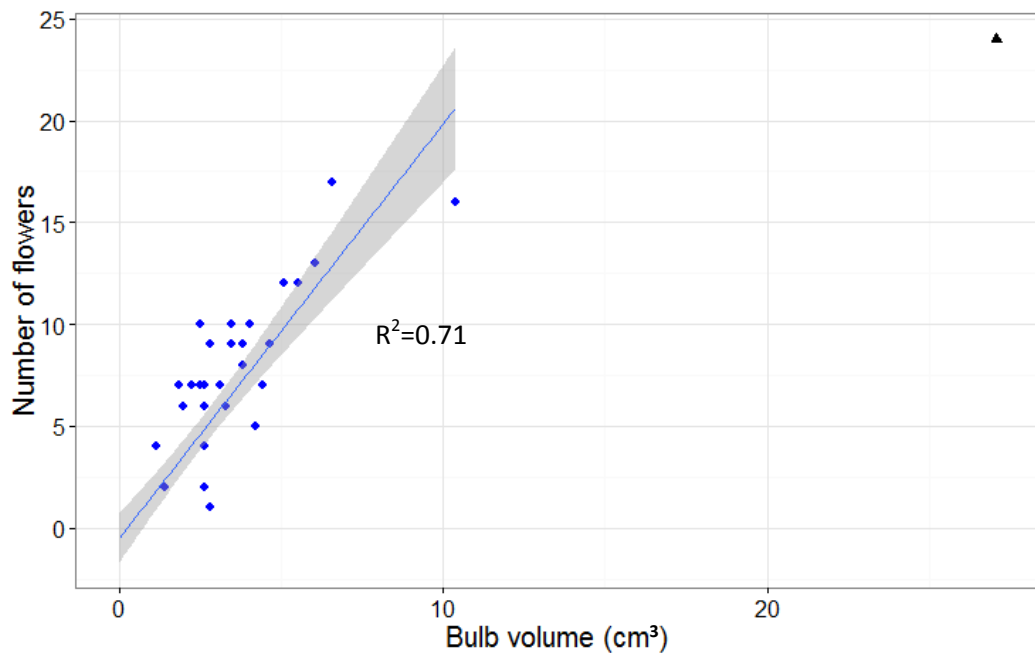


Figure 2.11: The number of flowers increased with bulb volume. Only flowering plants were included. The grey shaded area around the trend line indicates the 95% confidence band based on SE (estimated using R). The largest bulb (black triangle) is considered an outlier and excluded from the trend line. Model fit including outlier: $R^2=0.63$.

Discussion

The model was manipulated to investigate both the role of competition and facilitation in shaping a *H. non-scripta* population. These predictions were then compared with empirical data to determine, if possible, which form of density dependence may be dominating (See Chapter 4). The model has made some important assumptions; that the number of flowers a plant can produce depends on the level of competition from surrounding plants (N) and that the number of ovules fertilised depends on the number of surrounding flowers (F). Additional assumptions, based upon data presented literature, have also been included; the probability a fertilised ovule failing to develop due to inbreeding (calculated from Corbet (1998), and plants requiring five years to flower (Blackman & Rutter 1954; Van der Veken et al. 2007).

The model predicted very slow spread, which is consistent with the findings of Van der Veken et al. (2007). The treatment of space by the model means that the only plants contributing to the spread of the population are the ones in the outermost patches. It is possible that some of the seeds are shaken out by either wind or animals before the raceme breaks, but as the seeds are relatively heavy they are not likely to get very far (Van der Veken et al. 2007). Because seed dispersal is inefficient, seeds from the central plants are unlikely to reach the leading front of the population, but there is a possibility that seeds from the second outermost patches might infrequently disperse to the front. In order to keep the model simple, however, all kinds of long distance dispersal have been ignored, including dispersal from the relative edge to the leading edge. In addition, the model ignores clones, which may contribute to population density and so might indirectly affect spread, but which is unlikely to have a big effect on spread in itself.

The model predicts a decrease in plant density towards the edge of the population, which is consistent with the transect data. The lower density around the edge of the population is expected to be more favourable for seedling survival. The model assumes no other species are present, which would not normally be the case in a field setting, however, the area in which the transects were investigated (beech woodland) had an understorey consisting mostly of bluebells and greater wood-rush (*Luzula sylvatica*). The patches were clearly defined and rarely consisted of any species other than *H. non-scripta*, so in this case the model assumption seems reasonable.

The model failed to predict the decrease in proportion of flowering plants and the lag-effect at the leading edge that could be expected due to the poor dispersal and long immature stage of *H. non-scripta*; while the number of mature, flowering plants did decrease towards the edge, the proportions remained very similar. However, results from the transects showed that the proportion of flowering did decrease with distance from the middle of the patch, meaning the edge consisted mostly of immature, non-flowering plants. The model predicts about 60% flowering plants in the population, which is comparable to the centre of the patches in the transect study. It is unclear which assumptions and/or parameters of the model need to be changed to produce the observed edge effect in the model output, thus making future predictions more accurate. Where patches were bordering footpaths, there was no obvious decline in

flowering plants, indicating that the patch was expansion limited, likely by the increased mortality risk of trampling (personal observation, Littlemore & Barker 2001). The data from the transect study support the hypothesised 'lag' effect, for the population to advance the leading edge will first have to reach maturity.

The three different seed-related parameters investigated (seed dispersal q , seedling survival y_a and fertilisation p_a) had a pronounced effect on the three chosen aspects of the population (density, proportion flowering plants and spread from centre) when ignoring density dependence (Figure 2.4). The proportion of seeds dispersed had a slight negative effect on population density, but the main impact of this factor was, as is expected, on population spread. The model assumes seeds can only disperse to neighbouring patches (i.e. long-distance dispersal is not included) and all plants disperse an equal number of seeds. In the field, patches are less homogenous, both in terms of plant density and plant reproductive output, so it is unlikely that seed dispersal will be the same across the population. Hampe (2011) argues that long-distance dispersal is critical for rapid dispersal and range expansion in plants. As discussed above, long-distance dispersal is unlikely for *H. non-scripta*, but Van der Veken et al. (2007) suggest that it does happen, albeit infrequently, and that these long-distance dispersers form little satellite populations that are later joined with the expanding front of the main population. Although the model ignored long-distance dispersal it was able to predict rates of spread consistent with those previously observed in the field by Van der Veken et al. (2007), suggesting that long-distance dispersal may not contribute significantly to measureable patch expansion. However, long distance dispersal must have happened in the past in order for the species to attain its current distribution.

Population density and rate of spread from the population centre both rapidly increase as seedling survival increases, before decelerating. This pattern is expected because increased survival of seedlings means increased seedling establishment, which is crucial for both growth and expansion. At extremely low seedling survival the population density is very low, but a slight increase in survival causes the population density to 'boom' before steadily decelerating, though still increasing. The spread, while also increasing rapidly at first, shows a more pronounced deceleration (Figure 2.4). Increase in seedling survival can have a negative effect on the proportion of

flowering plants at the leading edge if the increase in immature plants increases plant competition. Such density dependent seedling mortality has been observed in other herbaceous perennials such as violets, *Viola* spp. (Schellner et al. 1982) and also in tropical trees (see Peters 2003, and references therein). Schellner et al. (1982) found *Viola* seedling survival to be negatively affected by density, while adult ramets exhibited density independent mortality. Peters (2003) found over 80% of the species in their study of tropical trees to exhibit density dependent mortality, which was prevalent for trees up to 10cm diameter at breast height (the largest trees included in the study).

The probability of pollination had the greatest influence on the three aspects of the population (plant density, proportion of flowering plants and spread from centre), and had largely the same effects on the population as seedling survival. Due to the increase in seed production with increased pollination, there was an increase in the number of juvenile plants, which had a positive effect on growth and spread, but lowered the proportion of flowering plants. The baseline population was likely very pollen limited, which is why this parameter had such a great impact.

The effects of competition and facilitation on the population

As discussed in Chapter 1, density dependence can have both positive and negative influence on the growth of a population. The simulation model assumes that density affects flower production, seed development, seedling survival, and adult survival negatively, because these are all factors that can be linked to competition (Weiner 1990, Chapter 1). Fertilisation is assumed to be influenced either positively or negatively, as evidence for both competition and facilitation can be found in the literature (Johnson et al. 2012; Bartkowska & Johnston 2014; Chapter 1). Negative density dependence on all five investigated population aspects (flower production, seed development, seedling survival, adult survival, and fertilisation) lowered the population density as expected, because increased competition leads to higher mortality rates and less reproductive output (Peters 2003; Ghazoul 2006). The effect was especially dramatic on adult survival, which at no density dependence resulted in very high population density, but decreased rapidly with small increases in competition

levels. This suggests that adult survival is very high in bluebells, as low adult survival lead to predictions of unrealistic population densities. Whilst seedlings of *H. non-scripta* often germinate in the uppermost leaf litter of the soil, bulbs descend deeper into the soil every year by means of contractile roots. Older bulbs are therefore protected against weather fluctuations and frost, which reduces their mortality (Merryweather & Fitter 1995a). Additionally, Bierzychudek (1982) suggests that ageing virtually does not happen in renewing geophytes, as no tissue ever reaches senescence stage. This could mean that, once established, bluebells are not only extremely long living, but adult plants may not be experiencing high levels of competition for resources. Schellner et al. (1982) found that larger ramets of three *Viola* spp. were more sensitive to environmental factors rather than density, and exhibited density independent mortality. The situation might be similar for *H. non-scripta*.

Negative density dependence on four of the five aspects caused the proportion of flowering plants to increase, because it lowered the number of juvenile plants. Density dependent adult survival did not have an effect on the proportion of flowering plants, apart from a small drop when density dependence was introduced. Spread was weakly positively influenced by negative density dependence as well as by positive, although the latter had a much bigger effect. Negatively density dependent adult survival had the biggest influence on spread, in which case the population spread 400cm from the centre (after 150 years). Negative density dependence on flower production and fertilisation had the least impact on spread. The overall trend of increased spread with increased competition is non-intuitive.

Positive density dependence had opposite effects from negative density dependence, except for on spread (Figure 2.6). Positive density dependence on fertilisation, i.e facilitation, increased population density greatly, even when density dependence was “mild”. Increased fertilisation is likely to lead to increased seed output, which in turn leads to more juvenile plants, which is why facilitation had a negative impact on the proportion of flowering plants. Positive density dependence on fertilisation greatly increased the spread; the model predicted 1250 cm dispersal from the population centre when fertilisation was “strongly” positively density dependent, which is the furthest spread allowed by the model. This increase in dispersal is likely due to the increased output of seeds.

Bulbs

English bluebells are usually expected to flower in their fourth or fifth year (Blackman & Rutter 1954; Rix 2004). The bulbs were collected to look for any evidence of annual cohorts, but no such pattern could be found. According to the classes developed by Merryweather and Fitter (1995a), the non-flowering bulbs used in this study are quite old, even the very small ones. This could mean that it takes *H. non-scripta* more than five years to flower, or that these bulbs are clones which have recently split off from the parent bulb. Bluebells develop a new bulb within the old at the end of the flowering season, and at this point the initials for leaves and shoots for the coming year are starting to develop. During the bulb renewal, bulb splitting may also occur, creating one or several clonal bulbs (Blackman & Rutter 1954; Daft et al. 1980). A clonal bulb would therefore not be able to flower before its second year. There is some overlap between the non-flowering and the flowering plants in terms of bulb size which could be explained by the larger non-flowering bulbs being young clones. However, only four of the twenty non-flowering bulbs studied were found within a short enough distance to suspect bulb splitting. The bulb data were collected during the flowering season, i.e. when the new bulb is undergoing development. Later in the season, when the leaves and racemes dry, the initials for next year's leaves and flowers are being laid down (Blackman & Rutter 1954). It is possible that the larger non-flowering individuals were in their last juvenile year and that the smaller flowering plants had not fully developed their bulbs yet, but more empirical data and a different experimental setup would be needed to confirm this. Ideally, bluebells would be grown from seeds and monitored every year to calculate rate of resource allocation and determine age at flowering. Merryweather and Fitter (1995a) also found that bulb age and depth at which it grew were correlated, and that this depth was a more reliable indicator of age in the field. Depth at which the bulbs were found was not recorded, but this could be considered in future studies.

Conclusion

One of the main findings of the model, one that is in agreement with the long term dispersal study by Van der Veken et al. (2007), is that spread is slow for *H. non-scripta*. New populations of bluebells are expected to take many years to establish and spread;

the baseline simulation predicted the population to take more than 100 years to reach densities seen in the field. In terms of constant population growth parameters, seedling survival was by far the most important. High seedling survival leads to increases in both population density and spread. Both the literature and field data indicates that seedling survival is extremely low. The model predicted unrealistic rates of spread when seedling survival was higher than 8%. Similarly, adult survival had great impact on population spread; some competition was needed to produce realistic plant densities, but adult survival still had to be high to reflect the densities seen in the field.

Chapter 3: Does timing of flowering and the local flower display affect fitness in *Hyacinthoides non-scripta*?

Introduction

Chapter 1 discussed phenology, the timing of life cycle events, in the context of plants. For plants, these events may be germination, flowering, or fruiting. The timing of these events may have a substantial impact upon plant fitness (i.e. seed production), especially if the cues the events depend upon change and phenological shifts occur (Kudo & Ida 2013; Campbell & Powers 2015; Chapter 1). These cues can be biotic or abiotic. For instance, flowering can be triggered by temperature, rainfall and humidity (Khanduri 2012) or by photoperiod (Elzinga et al. 2007).

The time of flowering is an important determinant of plant fitness; if a plant flowers too early, it might not have accumulated sufficient resource capital to produce maximal number of seeds, whereas if it flowers too late it might have an increased capacity for seed production, but limited time for pollinator visitation or for seed development (Elzinga et al. 2007). Flowering earlier, or later, may reduce risk of flower-, fruit- and seed-predation, if herbivores are attracted to the high densities present at peak times (Elzinga et al. 2007). A plant that flowers early might also miss the peak foraging time of its pollinators (Schmitt 1983a). Climate change has been linked to phenological shifts in flowering and mismatches with pollinators (Chapter 1). Bartomeus et al. (2011) suggest that most generalist bees are quite good at keeping up with their plant food sources, however, many studies still find that early plants produce less seeds. Thomson (2010) found that when fruit set was not limited by frost, pollination limitation was especially frequent in early cohorts of *Erythronium grandiflorum*. Schemske (1977) reports reduced seed set in early- and late-flowering cohorts (compared to intermediate cohorts) of the spring plant *Claytonia virginica*, and suggests that this is caused by reduced pollinator visits in the former and reduced photosynthetic capabilities in the latter, due to the development of the tree canopy.

Increased seed set in intermediate cohorts is also reported by Schmitt (1983b) for the grassland annual *Linanthus androsaceus*. Gross and Werner (1983) found that intermediate- or late-flowering phenological groups had increased seed set in three of their four focal *Solidago* species. *Solidago juncea*, however, showed improved seed set when flowering early (Gross & Werner 1983). In a study of *Lupinus lepidus*, Bishop and Schemske (1998) found that an initial selection pressure for later flowering was negated by greater fruit predation on late-blooming flowers in one of their study years, showing the complexity of studying phenology in the field. Overall, many factors determine the optimal flowering time for a species.

In addition to matching the peak abundance of pollinators and having the resources available for seed production, plants also need to attract pollinators. Larger floral displays are considered to be more attractive to pollinators (Ghazoul 2005), and this pollination facilitation may even take place when the floral display is made up of more than one species (Ghazoul 2006; Chapter 1). If the floral display is sufficiently large, there might be population-wide competition for pollinators. Ohashi & Yahara (1998) found that the visitation rate of bumblebees to *Cirsium purpuratum* did initially increase with density of flowers, but then decelerated. Similar to phenology and optimum flowering, it would be difficult to point to an optimal density for a type of plant, even more so for a particular species; it depends on the pollinator species and its behaviour, the local pollinator density, as well as the microhabitats in which the plants grow, as the resource availability and thus the capacity to produce flowers may vary.

The study presented in this chapter investigated the impacts of conspecific density and flowering phenology on seed production in *Hyacinthoides non-scripta*. Seed production is here used as a proxy for fitness, as the study was limited to one flowering season. As previously stated, plant systems are better suited for density studies than animal systems (Knight 2003; Bell et al. 2006; Nottebrock et al. 2013), that said, the most appropriate scale to measure density is not always clear. Both competition for resources and facilitation of pollination were investigated. If resource competition is limiting seed development, the plants can be expected to produce more seeds at lower plant densities. If larger floral displays are needed to attract pollinators, then seed production can be expected to be higher at higher densities. However, because of the

interplay between the two processes, and because flowers may compete for pollination at very high densities, we might expect the highest seed production at intermediate densities. Similarly, timing of flowering may impact seed production; plants flowering too early or too late might miss out on pollination opportunities, and so we might expect plants flowering in the middle of the flowering season to produce more seeds. The study was conducted on a local population of bluebells in northern England, UK, using naturally occurring patches of *H. non-scripta* that varied in plant densities. Previous studies on bluebells that have included density effects and phenology have been based on comparing habitats (Grabham & Packham 1983; Gonzales Sierra et al. 1996). In the present study the bluebells are compared within the same woodland.

Materials and Methods

Focal species and study area

H. non-scripta grows mainly in deciduous woodland, but can also be found in more open habitats. It typically starts growing in early spring (or as early as January if there is no snow cover), before the development of the tree canopy. Plants usually flower en-masse from April to early June, creating what is known as bluebell fields, i.e. high density patches (Grabham & Packham 1983; Kohn et al. 2009). The timing of these events has been shown to differ between habitats; Gonzales-Sierra et al. (1996) found that *H. non-scripta* flowered earlier in oak forest than beech forest, potentially because light levels (Photosynthetically Active Radiation, PAR) were much higher in the oak-site. They also found an initial temperature threshold for flowering, 11° Celsius, which was constant for both habitats. The bluebell's main pollinators are bumblebees (*Bombus* spp.), but potential lesser pollinators include hoverflies (Syrphidae) (Kohn et al. 2009). The flowers on a raceme open in sequence, starting from the bottom. The flowering season is several weeks long (6-8 weeks, personal observation) and individual flowers are usually open for two to three weeks (Corbet 1998, personal observation).

Data were collected around the School of Biological and Biomedical Sciences, of Durham University, in the North-East of England, UK (Figure 2.1). Data collection

started in the beginning of April when the flowering season began, and ended in late June 2015, after all the fruit had been collected and the seeds counted. See Chapter 2 for more details on the woodland sites and the focal species.

Sampling

100 clusters of five flowering *H. non-scripta* were chosen and tagged at the beginning of the flowering season, 14th-15th of April. Plant patches of different size and density were sampled throughout the woodland (i.e. stratified sampling). Randomly chosen plants within the patches were tagged, using a white strip of plastic tied around its base, along with its four closest flowering neighbours. 80 clusters were tagged in Great High Wood, and 20 in the smaller Little High Wood (Figure 2.1). A total of 46 plants suffered some form of mortality over the period of the experiment (trampled, broken, knocked over, etc.), leaving 454 plants and 3458 fruit for analysis.

Every plant was visited each day, or every other day, between the 14th of April and the 13th of June, and the opening date of each flower on the raceme was recorded. The density of surrounding plants and flowers was also measured in May. Plant density was quantified as the distance from the focal plant to the nearest neighbour (flowering or non-flowering). Minimum distance between neighbours was assumed to be strongly related to the degree of resource competition among plants. Flower density was measured by counting the flowering plants in a 1m by 1m quadrat around the focal plant. In denser patches a 50 cm by 50 cm quadrat was used and then an estimate was made for 1m by 1m. Estimates were also made for 2 m by 2 m, but with the exception of small patches (i.e. less than 2 m by 2 m) and spatially irregular patches, this measure was highly correlated with 1 m by 1 m, and so not required in the analysis.

Soil pH and soil moisture were measured at each patch, along with slope aspect. A total of three soil samples was taken from the ground around each of the 100 clusters of plants. The samples were taken *ca.* two weeks apart. Soil pH was measured using a pH meter (Hanna HI98128 pHep®5 pH and temperature tester; Hanna Instruments, Leighton Buzzard, UK). Soil moisture (ThetaProbe ML2x meter and reader; Delta T Devices, Burwell, Cambridge, UK) was also measured three times, again *ca.* two weeks apart, alternating with the pH measurement. The ThetaProbe measures soil moisture

in the upper 5 cm of soil, so measurements were not taken on rainy days as this gave disproportionately high near-surface soil moisture readings.

The data were analysed using general linear mixed effect and general linear models (GLMMs and GLMs) and the importance of factors was evaluated using likelihood ratio tests comparing the models with and without the factor of interest. Fruit production and seed production per fruit were analysed using GLMMs with Binomial and Poisson distributions respectively and plant ID as the random effect, while seed production per plant was analysed using a GLM with Poisson distribution. The factors used in the models were inflorescence size, density of surrounding flowers, minimum distance to neighbours, soil pH, soil moisture, slope aspect, and, for analysis of fruit development and seed production per fruit, flower position on raceme (raceme rank). Non-fruiting plants were removed from the dataset when investigating seed production.

Results

Flowering phenology

Flowering commenced a few days before the start of the data collection period (15th April) and the number of open flowers steadily increased over time. The date that the first flower on a raceme opened peaked on the 23rd of April (Julian day 113). After this early peak, the number of plants starting their flowering slowly declined (Figure 3.1a). The opening of individual flowers peaked twice, first on the 23rd April, due to the high number of plants beginning their flowering on that day, and second on the 9th of May (Julian day 129), approximately half-way through the flowering season (Figure 3.1b).

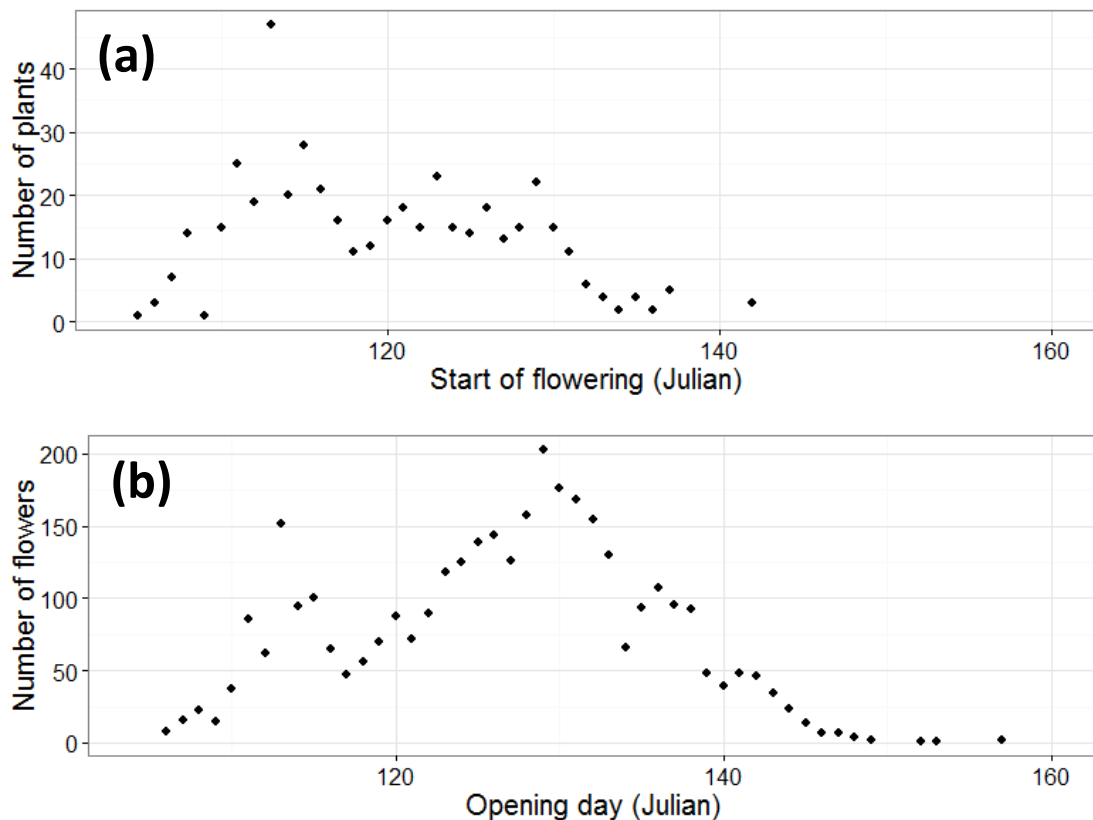


Figure 3.1: The start of flowering for each plant (a) and opening day of individual flowers (b).

Fruit production

The opening day of the flower had a highly significant effect upon fruit production (Table 3.1), flowers that opened earlier had a higher probability of being developed into a fruit (Figure 3.2a). Soil moisture had a significant effect upon fruit production; but there is only very weak evidence for a slight positive correlation (Figure 3.2b). Aspect also affected fruit production; plants growing on North-East or South-West facing slopes were more likely to produce fruit and plants growing on slopes facing West or North-West were the least likely to fruit (Figure 3.2c). Neither minimum distance to neighbours, soil pH and the density of the flowers around the focal plant, nor factors relating to the plant itself such as flower position and inflorescence size, had any statistically significant impact on fruit production (Table 3.1).

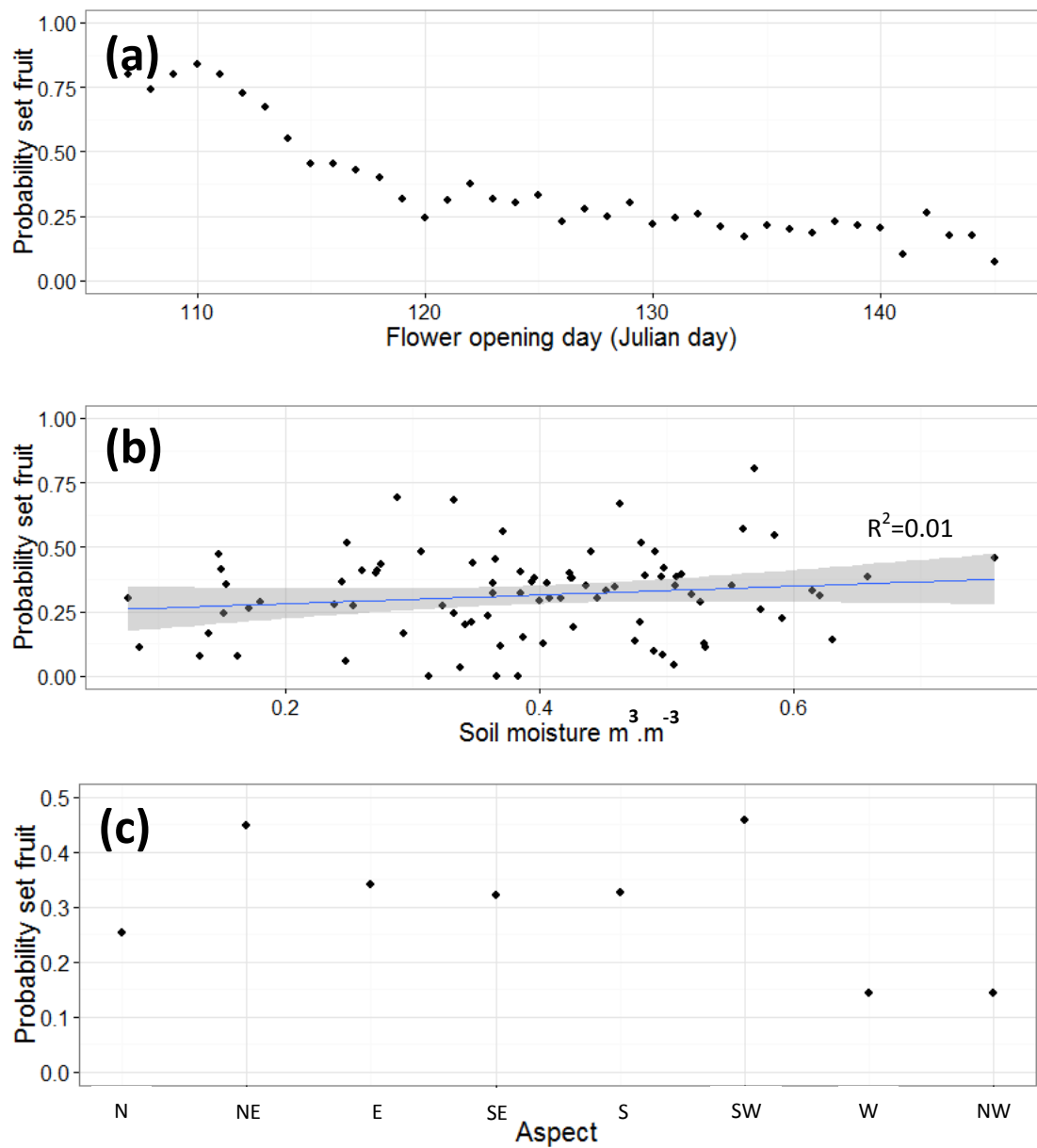


Figure 3.2: Probability of fruit set in relation to (a) flower opening day, (b) soil moisture and (c) aspect. The shaded area around the regression line in (b) represents the 95% confidence interval based on SE (estimated using R).

Table 3.1: Summary of statistical analysis investigating the effect of factors on fruit production per plant

| Factor | G | df | P-value | Effect size |
|--------------------|-------|----|------------------|-------------|
| Inflorescence size | 0.476 | 1 | 0.491 | 0.015 |
| Flower position | 3.224 | 1 | 0.073 | 0.037 |
| Flower opening day | 177.9 | 1 | <0.001 | -0.140 |
| Minimum distance | 0.895 | 1 | 0.344 | 0.023 |
| pH | 0.601 | 1 | 0.438 | 0.265 |
| Flower density | 0.128 | 1 | 0.721 | <0.000 |
| Soil Moisture | 5.233 | 1 | 0.022 | 1.565 |
| Aspect | 8.732 | 2 | 0.013 | -0.177 |

Seed production per plant

The number of flowers a plant had (inflorescence size) was positively correlated with the number of seeds produced per plant (Table 3.2, Figure 3.3a). Plants that opened their first flower earlier produced significantly more seeds in total (Table 3.2, Figure 3.3b). The minimum distance to neighbours (used here as a proxy for density) was statistically significant, but there was only a very slight negative correlation (Table 3.2, Figure 3.3c). Local flower density had a slight positive effect on total seed production (Table 3.2, Figure 3.3d). Soil pH had a significant impact on seed production per plant, but there was no evidence for a positive or negative correlation (Table 3.2, Figure 3.3e). Aspect was also important; plants growing on North-East facing slopes produced the most seeds overall, slightly more than plants growing on East-, South-East-, South- and South-West-facing slopes, while plants growing on slopes facing West produced the lowest number of seeds (Table 3.2, Figure 3.3f). Soil moisture was the only factor that did not have a significant impact on seed production per plant (Table 3.2).

Table 3.2: Summary of statistical analysis investigating the effect of factors on fruit production per plant.

| Factor | G | df | P-value | Effect size |
|--------------------------|--------|----|---------|-------------------------|
| Inflorescence size | 1077.4 | 1 | <0.001 | 6.878×10^{-2} |
| First flower opening day | 837.12 | 1 | <0.001 | -5.3×10^{-2} |
| Minimum distance | 10.817 | 1 | 0.001 | 1.036×10^{-2} |
| Flower density | 30.607 | 1 | <0.001 | 2.384×10^{-4} |
| pH | 29.333 | 1 | <0.001 | 2.044×10^{-1} |
| Soil Moisture | 0.338 | 1 | 0.561 | 4.783×10^{-2} |
| Aspect | 168.37 | 1 | <0.001 | -1.114×10^{-1} |

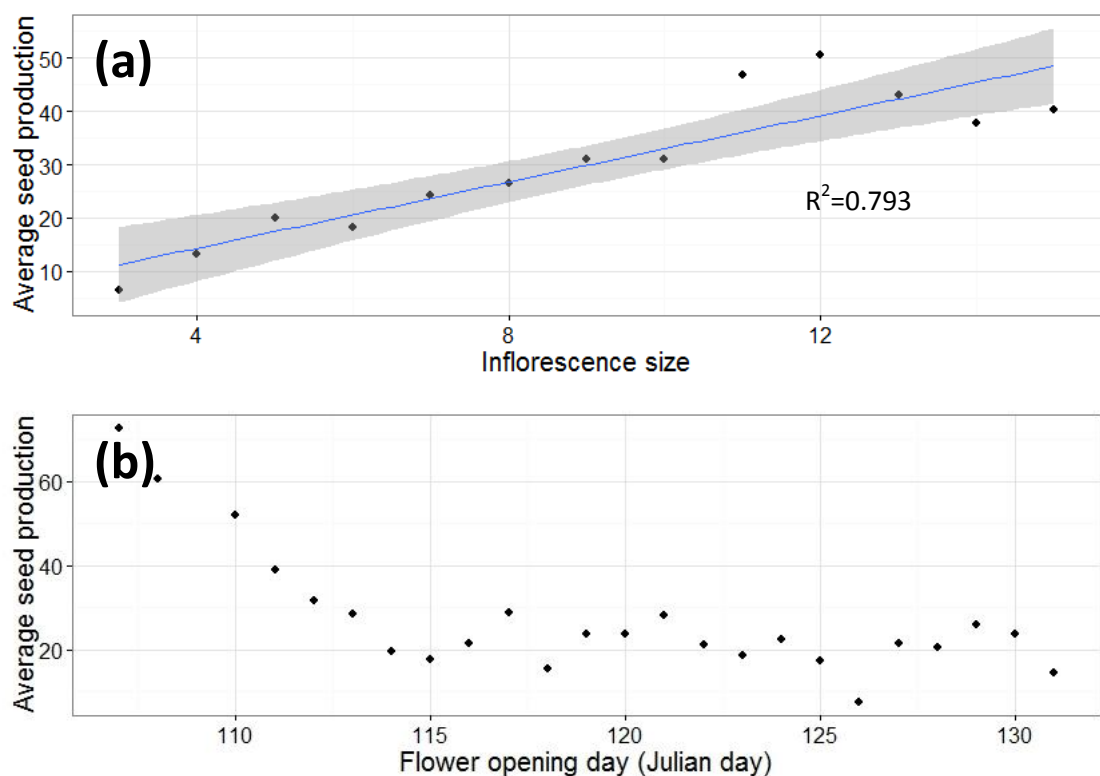


Figure 3.3: Average seed production per plant in relation to (a) inflorescence size, (b) start of flowering, (c) minimum distance to neighbours, (d) surrounding flower density, (e) soil pH and (f) aspect. The shaded areas around the regression lines in (a), (c), (d) and (e) represent the 95% confidence intervals based on SE (estimated using R). (NB: Plants growing on North-West-facing slopes are not included on the graph, due to the low sample size).

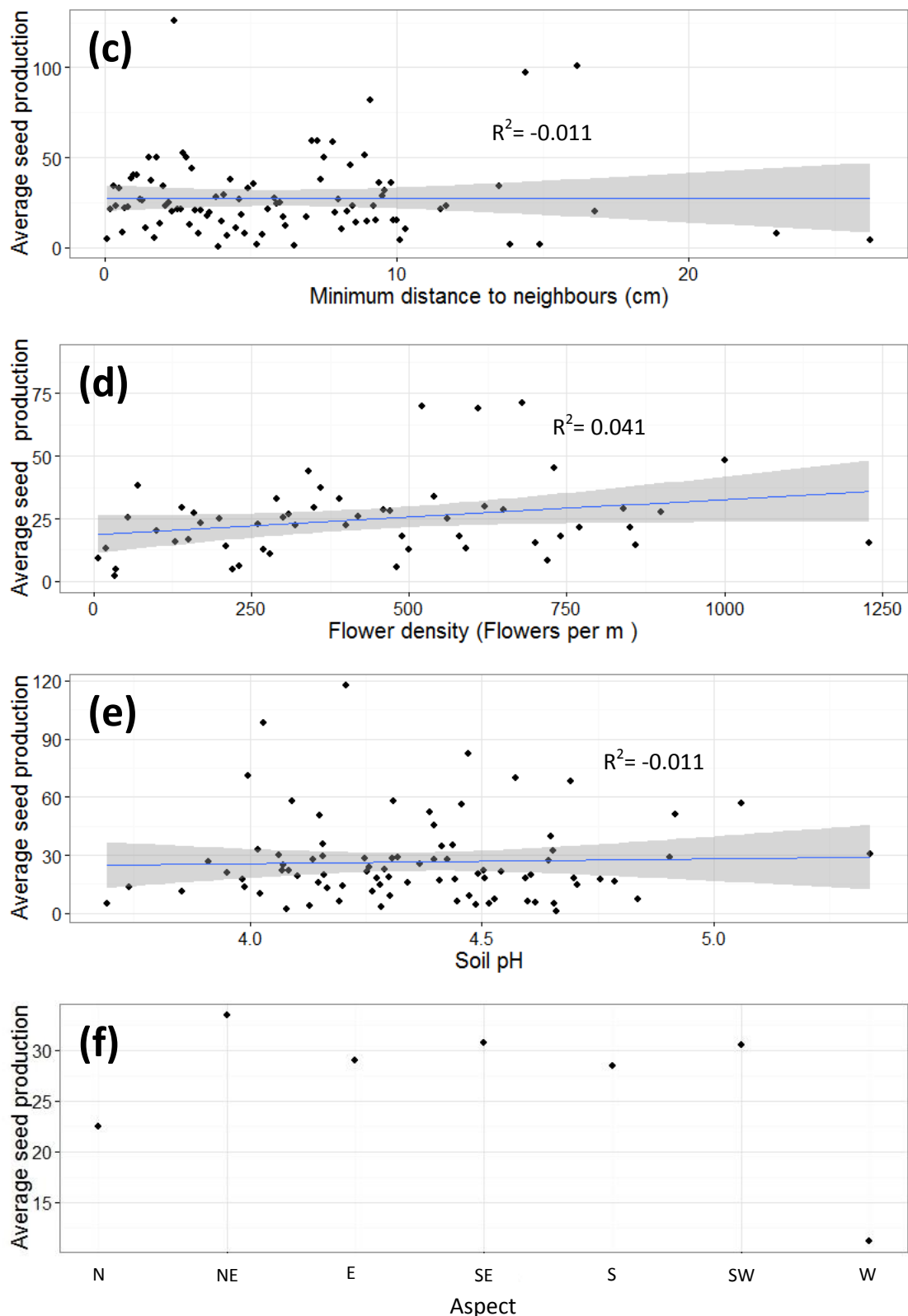


Figure 3.3 continued: Average seed production per plant in relation to (a) inflorescence size, (b) start of flowering, (c) minimum distance to neighbours, (d) surrounding flower density, (e) soil pH and (f) aspect. The shaded areas around the regression lines in (a), (c), (d) and (e) represent the 95% confidence intervals based on SE (estimated using R). (NB: Plants growing on North-West-facing slopes are not included on the graph, due to the low sample size).

Seed production per fruit

Flower position and flower opening day both had highly significant impacts upon seed production per fruit (Table 3.3). On average, fruit higher up on the raceme produced more seeds (Figure 3.4a). Flowers opening earlier produced on average more seeds than flowers that opened later (Figure 3.4b). Minimum distance to neighbours, soil pH, soil moisture, inflorescence size and the density of flowers surrounding the focal fruit had no effect on seed production (Table 3.3). However, aspect influenced seed production per fruit (Table 3.3), South- and South-West facing slopes were the most productive, albeit by a very slight margin, whilst Fruit growing on West and North-West facing slopes had very low seed production (less than 5 seeds on average, while all other fruit produced more than 7 seeds) (Figure 3.4c), a trend similar to that seen in seeds per plant.

Table 3.3: Summary of statistical analysis investigating the effect of factors on seed production per fruit

| Factor | G | df | P-value | Effect size |
|--------------------|----------|-----------|------------------|-------------------------|
| Inflorescence size | 0.473 | 1 | 0.492 | 9.687×10^{-3} |
| Flower position | 19.861 | 1 | <0.001 | 3.11×10^{-2} |
| Flower opening day | 20.907 | 1 | <0.001 | -1.803×10^{-2} |
| Minimum distance | 0.249 | 1 | 0.618 | -5.444×10^{-3} |
| pH | 3.556 | 2 | 0.169 | 2.331×10^{-1} |
| Flower density | 0.065 | 1 | 0.798 | 3.728×10^{-5} |
| Soil Moisture | 1.173 | 1 | 0.279 | -2.996×10^{-1} |
| Aspect | 4.527 | 1 | 0.033 | -5.653×10^{-2} |

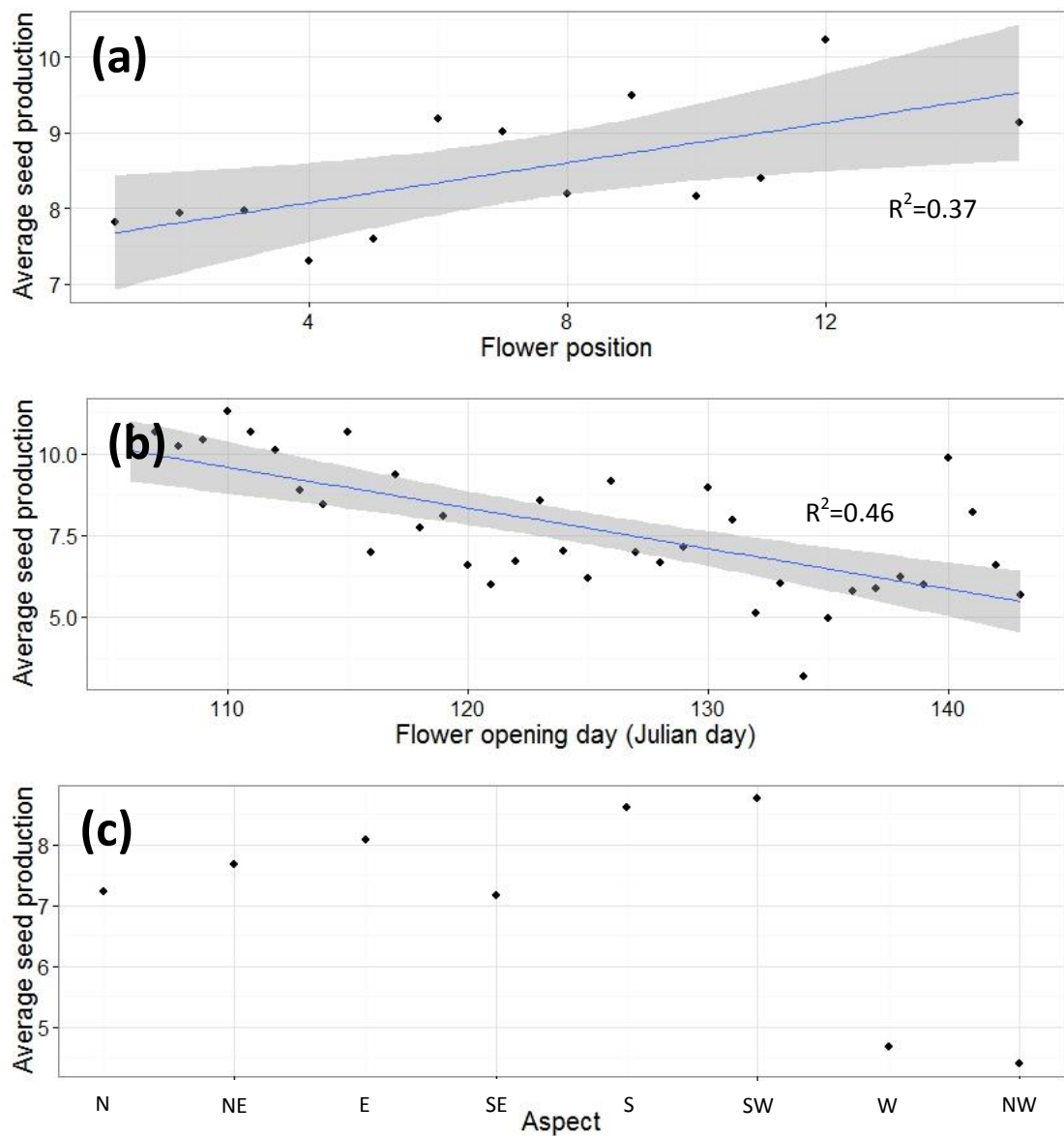


Figure 3.4: Average seed production per fruit in relation to (a) flower position, (b) flower opening day, and (c) aspect. The shaded area around the regression lines in (a) and (b) represent the 95% confidence intervals based on SE (estimated using R).

Discussion

Flower position

The physical location of a flower (within an inflorescence) can, according to Wyatt (1982), affect its chances of maturing into a fruit because of its vascular connection to the pool of resources and because of the time of its occurrence with respect to other developing fruits. The first flower to start developing into a fruit would have earlier

access to basal resources, and in the case of the bluebell (which flowers serially), would also be those closer to the bulb. A raceme also spreads the demand for resources over a longer period of time than, for instance, a solitary terminal flower (Wyatt 1982), this is especially true for long-flowering plants such as the English bluebell. Corbet (1998) suggests that, in bluebells, the fruits compete with the bulb, as well as with other fruit, for bulb resources. The renewal of the bulb and roots overlaps somewhat with the flowering and fruiting period, and while most of the assimilates for leaves and shoots are laid down in the autumn of the previous year (Daft et al. 1980), it is not unlikely that there is a trade-off between resources dedicated to fruit and seed development, and those dedicated to the development of the new bulb. Flower position was not found to be significant for fruit production for the plants investigated here, but there was a slight negative correlation with rank number (Appendix 1).

Corbet (1998) found lower-positioned successful fruit of *H. non-scripta* to produce more seeds, and attributed this to their flowers opening first and therefore having earlier access to assimilates, and because they are situated closer to the source of assimilates than fruit higher up. Here, the trend is reversed; once successfully developed, fruit higher up on the raceme produced more seeds on average than fruit lower down. However, Corbet (1998) investigated effect of raceme position only in pruned plants (i.e. all racemes retained only five fruits, spread across 9 ranks), whereas this study investigated entire plants.

Fruits of *H. non-scripta* are green and it is possible that they have capacity to supplement resource demands of the developing seeds. This would make the between-fruit competition for bulb-assimilates less intense. Fruit photosynthesis has been observed in many crop plants, such as coffee, soybean, pea, barley, orange, mandarin, apple and tomato, among others (Phan 1970; Flinn et al. 1977; Lopez et al. 2000; Carrara et al. 2001; Hiratsuka et al. 2012). Flinn et al. (1977) found that while the photosynthetic activity of the pea pod did contribute to the growth of the pod itself in the early stages of pod development, it did not supply any extra assimilates to the developing seeds. Physiological studies of the bluebell are scarce, and focus on roots and mycorrhizae (eg. Hendry 1987; Merryweather & Fitter 1995b) or its toxicity (eg. Watson et al. 1997; Kato et al. 1999). However, the fruit do grow in a phototropic manner, hinting at photosynthetic activity; during fruit development the raceme goes

from nodding to erect, and in larger flowers, where the weight of the fruit prevents the raceme from straightening, the fruit themselves grow towards the light (personal observation). Detailed studies on the respiration and carbon transport of bluebells would be needed to verify this.

Flower phenology

The date of first flowering was very important for fruit development and seed production. Plants that flowered earlier produced the most seeds, suggesting that the start of the flowering season occurred after the emergence of bumblebees. The probability of fruit set and average seed production per plant decreased rapidly after the beginning of the flowering period. The reason for the poorer output of later flowers could be due to changes in pollinator abundance and their foraging behaviour, the weather, or a combination of these factors. In a review by Elzinga et al. (2007) most pollinators were found to favour early or peak flowering. Unfortunately, no pollinator data were available on the local pollinators for this study. Weather data from Durham University Observatory (*ca.* 0.75 km from the study sites) reveal that April was slightly warmer than average (+1.3 degrees Celsius), and relatively dry. May, on the other hand, was the coolest since 2010 (although temperatures were close to average), and more than three times rainier than the previous month. April had a mean daily sunshine duration of 6.3 hours, whilst May only had 4.7 hours (Durham University Observatory, <http://community.dur.ac.uk/durham.weather/>). The decrease in available light due to increased cloud cover combined with the development of the forest canopy could have negatively affected plant growth. Bluebells are normally shade-tolerant plants, but according to Blackman and Rutter (1954) bluebells growing in beech woods are close to their shade-limit. Additionally, poor weather may have prevented plant-pollinator interactions during May. The observed increase in rain in May may have been detrimental to the main bluebell pollinators, *Bombus* spp., which normally shelter under vegetation or in their nests during rain (Lundberg 1980). Whilst *H. non-scripta* flowers for a long time (each flower having the potential to stay open for up to two weeks (Corbet 1998)), it is possible that the combination of lack of sunshine and poor weather did impact on their reproductive output.

Due to the time limit of only one flowering season for this project, there is no evidence for whether the observations made are representative of bluebells in the North-East of England, in general, or if the poor seed production of later flowering plants is an annual anomaly due to a combination of factors, such as unstable weather or pollinator behaviour. Many British plants have been shown to flower earlier and earlier due to increasing temperatures with climate change, spring-flowering plants especially (Fitter & Fitter 2002). This may also be the case for *H. non-scripta*; earlier flowering plants were found to produce more seeds than later flowering plants in this study, which could be an indication of selection pressure for earlier flowering. To verify this, however, longer-term studies than could be performed here are needed (Khanduri 2012).

Density: competition, facilitation and pollinator behaviour

Minimum distance (i.e. distance to the nearest neighbour), had a very slight negative effect on seed production for entire plants. Grabham and Packham (1983) found that at lower plant densities seed output per plant, and also plant size, were greater. Size often correlates with the amount of resources the plant has stored, which in turn can be used for seed development, and does therefore usually significantly impact seed production (e.g Lawrence 1993). This was also found in the present study; plants with larger inflorescences (correlated with bulb size, see Chapter 2) produced more seeds on average than did smaller plants. The size of the plant may also influence flowering dates; larger plants may have resources available for flowering sooner than smaller plants (Ollerton & Lack 1998, cf. Schmitt 1983a). Larger plants may also have the resources to increase the duration of flowering both at the flower and inflorescence scale (Schmitt 1983b; Ollerton & Lack 1998; Khanduri 2012). Longer flower duration is expected to increase the chance of flower pollination. The present study did not find any evidence of increased seed output at lower plant density, nor were plants larger at lower densities (Figure 3.3c, Appendix 2). It is possible that the low density patches studied by Grabham and Packham (1983) were well within foraging range of the bluebell pollinators, and that the plants in question produced enough flowers to attract pollinators, i.e. because of the greater size of the plants in low density patches investigated in their study, the flower density might have been high despite the plant

density being low. Additionally, bumblebees have large foraging ranges; Osborne et al. (1999) found the mean maximum distance of outward foraging tracks to be more than 300m, and the bumblebees in their study often overlooked foraging patches close to their nests and proceeded to patches further away. Therefore, while the patches themselves had low plant densities, they might not have been sufficiently isolated to experience significant pollination loss.

There was a weak positive correlation between the size of the local floral display and the seed output of entire plants. Heithaus et al. (1982) reported density dependent pollination success for *Bauhinia unguolata*; pollination success increased slowly as more flowers were open, but was reduced to almost zero when only 20 flowers or less were open. No evidence for a threshold was found in the present study; even plants growing at the lowest observed flower densities produced 10 or more seeds (Figure 3.3d). These plants may have self-fertilised, but the level of self-incompatibility in *H. non-scripta* is considered to be high; Blackman and Rutter (1954) claims it has no self-compatibility, while Corbet (1998) claims it may have some, as self-pollinated plants were found to initiate a low number of seeds. Thresholds are likely to vary for different plant-pollinator relationships. *B. unguolata* is pollinated mainly by bats, while *H. non-scripta* is pollinated mainly by bumblebees; these species are likely to have different foraging behaviours, and bumblebees may not exhibit density dependent foraging (Smithson & MacNair 1997).

Seed output was weakly, positively correlated with floral display size, suggesting pollinator facilitation (Figure 3.3d). Bumblebees have been found to prefer plants with larger display sizes (number of flowers) (Ohashi & Yahara 1998). The movement of pollinators within patches can also affect reproductive success. Pollinators tend to fly short distances within a patch, visiting neighbouring plants or several flowers on the same plant, especially in low density patches (Ghazoul 2005), which can reduce pollen-mediated gene dispersal. The effect of this foraging behaviour on seed production depends on the level of self-incompatibility in the plant; self-compatible plants may experience increased selfing with no negative effects, while self-incompatible plants may experience increased “clogging” of the stigma by incompatible pollen, or loss of siring opportunities (Mitchell et al. 2009). Further studies would therefore benefit

from accompanying studies of self-incompatibility, pollinator foraging behaviour, and their consequence for relatedness of neighbouring plants in the population.

Environmental factors

Of the environmental factors studied in this project, aspect was by far the most important determinant of seed production. Growing on slopes facing West or North-West consistently led to less reproductive output. These aspects are likely to receive less light, but there may be other factors, biotic or abiotic, that affect plants more on these slopes, such as temperature, soil moisture or the presence of competing plant species. Plants growing on North-East and South-West facing slopes were the more likely to set fruit, South-West slightly more so than North-East. However, plants growing on slopes facing North-East produced the most seeds, followed by plants facing South-West and South-East. These aspects may receive more light, or the surrounding vegetation might have benefitted the plants (e.g. fewer shrubs may have reduced competition for light).

Thomson (2010) found that the earliest flowering plants of the subalpine lily *Erythronium grandiflorum*, in western Colorado, were always growing on south-facing slopes, while the latest flowering individuals were growing in particularly shaded areas. Bluebell flowering started about a week earlier on East, South-East, South, and South-West facing slopes compared to plants growing on North and North-East facing slopes, and two weeks earlier compared to plants growing on West and North-West facing slopes (Appendix 3). All plants growing on West and North-West facing slopes started flowering in May, which seems to have been a generally unfavourable period for seed development for all plants investigated, so it is likely that the shift in weather and/or pollinator behaviour affected this group disproportionately more than the others.

Soil moisture was observed to be weakly positively correlated with fruit set. Soil moisture is important for nutrient uptake of roots, but the level of “optimum” moisture levels may differ for different species (Davidson 1969; Mackay & Barber 1985; Caldwell et al. 1998). The English bluebell prefers a well-balanced water regime (i.e. few fluctuations between drought and flooding), and avoids water-logged soils (Van der Veken et al. 2007). *H. non-scripta* was here found to grow at a range of

different moisture levels, though never in soils exceeding $0.8 \text{ m}^3 \text{ m}^{-3}$ and rarely in soil measuring less than $0.1 \text{ m}^3 \text{ m}^{-3}$. Mucilage in seeds (Villellas & García 2013) and bulbs of other species (Al-Tardeh et al. 2008) have been found to help reduce water deficit, and it is possible that this is also true for bluebells, and could explain why it was found in relatively dry conditions. Gonzales Sierra et al. (1996) also found that soil moisture significantly influenced *H. non-scripta*, especially in oak forest, where the soil was looser than in the beech forest at their study site. In their study peak flowering coincided with maximum soil moisture in oak forest and the number of flowers per plant increased with soil moisture, indicating that bluebells do better in moister environments. This pattern was not found here for *H. non-scripta*, however, soil moisture was not measured every day, which may have prevented its detection.

Soil pH was found to be significant for seed production for entire plants, but showed only a very slight positive correlation. Most bluebells in this study were found in soils ranging from 4.0 to 5.0, which is within the range presented by Blackman and Rutter (1954), if slightly on the acidic side. Like soil moisture, pH could be monitored every day in future studies and additionally the presence of different nutrients in the soil could be investigated, e.g. phosphate, which has been shown to be important for *H. non-scripta* (Merryweather & Fitter 1995a).

A limitation in this study is the fact that data were collected during a single flowering season. Bluebells renew their bulbs each year, and assimilates are laid down for the leaves and shoots the year before. This effectively means that plants are sensitive to conditions a year before flowering (Kohn et al. 2009). As no data were collected on the bluebells in previous years it is not possible to investigate long-term patterns of reproduction and their association with environmental factors.

Conclusion

Overall the most important factor for seed production was the date of first flowering. Plants starting their flowering in the first two weeks of the flowering season produced more than double the number of seeds produced by later plants, dwarfing the effect of the other factors, such as aspect or local flower display. The findings presented here suggest that flowering date is an important plant trait that is likely to be subject to

strong selective pressure. Future research could focus on the factors that place a limit on the earliest flowering date.

Chapter 4 – Synthesis

The preceding chapters have focused on the impact of density dependence and phenology on the ecology of *H. non-scripta*, from population spread and growth to seed production throughout the flowering season. This study, coupled with the literature, portray the English bluebell as a slow-growing, extremely slow-spreading plant, which has the ability to produce high numbers of seeds. However, few of those offspring are likely to reach maturity, as the model predicts very low seedling survival. Once maturity has been reached the plant is very robust; results from the model indicate very high adult survival, the renewal of the bulb each year means senescence is virtually non-existent, and bulbs growing deep in the soil are protected from fluctuating weather. The biggest threat to the survival of mature bluebells is likely excessive disturbance, e.g. rooting by wild boar (*Sus scrofa*) (Sims et al. 2014) or trampling by humans (Blackman & Rutter 1954; Littlemore & Barker 2001). As wild *S. scrofa* is not present in the North-East of England, trampling by humans and dogs was likely the main cause of mortality for mature plants at the study site. *H. non-scripta* appears vulnerable to changes in weather or pollinator behaviour when it comes to seed production, which plummeted two weeks into the flowering season (Chapter 3). The ability to produce clones might make the plants less dependent upon seeds for population growth (Merryweather & Fitter 1995a), but seed production is still important for dispersal (Van der Veken et al. 2007). No data on vegetative reproduction were collected in the present study.

The garden plant *H. hispanica* is considered to pose a threat to the native plant, albeit not an immediate one (Kohn et al. 2009). Knowledge of a species' ecology and general ecological principles, such as life-history traits and how the focal species is affected by its surrounding biotic and abiotic factors, are always helpful, and potentially necessary, when developing conservation plans. When considering invasive species, the effects of the focal species on its neighbours and its environment should also be investigated

(e.g. Sala et al. 2007). Ecological studies are therefore especially important in cases that involve threatened, or potentially threatened, species.

The present study sought to fill some of the gaps in the knowledge of bluebell ecology and investigate the effects of phenology, competition and facilitation, if any, on this system. Whilst insight has been gained into certain aspects, such as seed production over time and population growth and spread, others remain unclear. The bulb data showed great variation and no single size or age at flowering could be established, and neither facilitation nor competition could be established as the main process in the field. Whilst the model demonstrates what can be expected from the population in terms of negative and positive density dependence, this is difficult to tease apart in the field; the data only hint at the potential for facilitation, and no evidence could be found for significant competition between plants.

Model and Field Studies

The simulation model reported here was developed before the flowering season began, and thus the fieldwork period, using parameters based upon the literature. This was especially useful because it helped predict what data should be collected, and because it gave an idea of what to expect once the flowering season began. Some data were collected specifically to check the validity of the model, i.e. the transect data, whilst other aspects of the model were not explicitly verified, but supported by field observations, e.g. the carrying capacity of a patch.

The baseline model predicts a carrying capacity of 240 plants per m^2 , which is within the range found by Van der Veken et al. (2007) (76 plants – 317 plants per m^2). The total number of plants in a patch can be difficult to count in the field, especially in high density areas. Seedlings and very young plants (one small, grass-like leaf) are often hidden in the leaf litter and thus hard to find. Because of their size and position in the soil, the seedlings and young plants were considered unlikely to put great competitive pressure on the mature focal plants, whose bulbs were located much deeper in the soil, and total number of plants was therefore not counted. There were, however, patches ranging from 10 to 176 flowering plants per m^2 found in Great High Wood, so

a total of 240 plants per m² is likely not too unrealistic, even if somewhat on the high density end of the spectrum.

The transects in Little High Wood showed the decrease in total plant density towards an expanding edge, as predicted by the model, as well as showing the accompanying decrease of older, flowering plants and an increase in younger plants. These features were also observed, though not measured, in patches in Great High Wood. Non-expanding edges, e.g. where the patch was bordered by footpaths, did not exhibit this decrease in density and proportion of mature plants (personal observation). In both study areas the patches were usually surrounded by suitable habitat, indicating that the patch should continue to spread. Whilst there is no way to measure rate of spread, or the direction of patch expansion, within one field season, the observed patch features, i.e. the age- and spatial-structure of the expanding edge, and the large, adjacent areas of unoccupied habitat seem to support the slow-dispersal predicted by the model (personal observation). Furthermore, it seems unlikely that any patch would expand in a uniform manner, but again, this is difficult to quantify within one field season. The change in proportion of flowering plants along the transects also highlight the different density dependent processes that are likely taking place in the patch. For example, the model predicts that higher proportions of flowering plants are due to negative density dependence on seedling survival, seed development and flower production (Chapter 2). This could mean that high proportion of flowering plants in the field is an indicator of negative density dependence on at least one of these factors. The model aims to make predictions for all plants in the population, whilst data were only collected for flowering plants. Hence no data were available for analysis of seed and seedling survival, but this could be undertaken in the future.

Using the field data and observations to verify the model allows for fine tuning and corrections if necessary. New predictions could be made, which could be verified or disproven in the field and the new data would be added to the model, and so on. Given time and extended datasets, the model could be 'perfected', making its predictions more valuable. There is however a trade-off between simplicity and accuracy when developing models, which would have to be taken into account.

Future studies

The biggest improvement on this study would be to collect the same data over several years. The changes in fruit development success rate along the raceme, the changes in seed production over time, and if it is indeed beneficial to flower earlier could then be verified as actual trends (rather than anomalies), or disproven. Future research could also focus on the factors that place a limit on the earliest flowering date to investigate if there is a point at which early flowering becomes detrimental to the plant; by flowering before the emergence of pollinators, for instance, or suffering frost damage. Furthermore, the bluebells could be monitored from seed to flowering and the annual bulb growth rate could be measured. This would allow for mortality studies, e.g. investigation of seedling and young plant survival at different densities, and records of how many plants survive from seed to flowering (Merryweather and Fitter (1995a) found this to be less than 0.4%). Time of flowering, i.e. how long it takes for the plant to reach the mature stage, could also be recorded. Investigation of the juvenile stage may prove especially profitable, e.g. does the length of this stage depend on the speed of resource accumulation, and if so, do bluebells take longer to flower in harsher environments? The bulb study showed that some non-flowering bulbs were bigger than flowering bulbs, and so knowing the exact age of these bulbs would further facilitate a detailed analysis. Experimental designs to investigate density could also prove useful. Bluebells could be grown from seed at different densities, in similar conditions, and then hand pollinated to eliminate pollinator discrimination in competition studies. The same could be undertaken to study facilitation; the experimental densities could range from much lower than the densities found in the field for the present study, e.g. from a single plant to very high numbers per m². Experimental protocols could allow for manipulation of both the population size and density (Kunin 1997). Additionally, the closing date for each flower could be recorded and the longevity of each flower could be calculated. Longevity may influence the probability of pollination, as the longer a flower is open the bigger the chance of being found and visited by a pollinator. Limited data were collected on closing date and longevity in the present study, but due to time constraints this collection was not

prioritised and the data were not used for the main analysis. Preliminary analysis of these data shows increased seed production with longevity, for up to 20 days when the trend becomes less certain (unpublished data). Further data collection and analysis would be needed to confirm this. A more extensive dataset would also allow for fine tuning the population model and make it more nuanced. Studies investigating vegetative reproduction, and incorporating any significant results in the model, could also be undertaken.

Due to the significant impact of aspect of slope upon fruit and seed production this factor would be worth investigating further. As mentioned in Chapter 3, aspect of slope is likely to be a proxy for how much light is available to a plant. Measurements of light interception, both quality and quantity, at each site/cluster, could help determine the photosynthetically active radiation (PAR) available to the plants. These measurements should ideally be made continuously at each site.

Vegetation surveys could be undertaken and measurements of the leaf-area index (L.A.I. is the leaf area per ground surface area and is a method used for characterizing the plant canopy) could be taken to complement the light-data, as the vegetation surrounding the focal plant can also influence the light available for photosynthesis at different times of the day, and may vary throughout the flowering season.

Furthermore, the angle/incline of the slopes was not measured here due to time constraints, but could be determined to accompany the light-data in future studies. The angle of the slope can influence light availability, soil water availability and the availability of other nutrients, as well as the soil stability (Armstrong 1974). The topography of both areas used in the present study is varied, with slopes ranging from gentle to quite steep.

As well as measuring the prevailing light regime, other abiotic factors could also be recorded, such as soil moisture and pH. Gonzales Sierra et al. (1996) found flowering of *H. non-scripta* to coincide with peak soil moisture. Measuring these factors continuously would allow for more detailed analysis and a record of the variations of these factors across both space and time. Soil moisture, for instance, is likely to change rapidly in the upper layers with changes in the weather. These changes are not likely to be reflected in just one measurement, or in three such measurements as in the

present study. As soil moisture did have a statistically significant impact on fruit and seed measurement, this nature of this relationship is worthy of further, more detailed, investigation. Measurements of the availability of nutrients in the soil would also benefit future studies that seek to investigate the environmental similarities of patches. Phosphate in particular has been shown to be of great importance to *H. non-scripta*; the bulb's contractile root pulls it deeper into the soil as it ages, moving it from a nutrient-rich to a nutrient-poor environment (Merryweather & Fitter 1995a; Merryweather & Fitter 1995b).

The photosynthetic abilities of bluebell fruit, which is hinted at by their seemingly phototropic growth and chlorophyll content (personal observation), and whether this phototropic activity benefits the developing seeds significantly, could also be investigated. Studies could also investigate genetic diversity within a patch, as this would be of interest when studying the fitness of outcross-pollinated plants versus self-pollinated plants. Flight distance between plants is important in mediating gene flow, and this is reportedly low in *Bombus* spp.; bumblebees usually collect pollen from several flowers on a plant in one visit, before moving on to visit close neighbours (Schmitt 1980). Cresswell et al. (2002) found that the pollen carryover from a single *Brassica napus* flower, i.e. the 'paternity shadow', when foraged on by a bumblebee was a maximum of 20 flowers, with decreasing deposition of the focal pollen with each visit. Combined with the short seed dispersal of *H. non-scripta*, this could mean that patches often consist of highly related plants or that within larger patches there are groups of close relatives. Bumblebees often follow physical features in nature when they forage (Cranmer et al. 2012), which could mean that they visit only two or three patches in one foraging trip, or that they only forage on one side of a footpath during each foraging bout. This could mean that there is relatively little gene flow between plants growing on either side of a footpath. Bumblebees also exhibit high site fidelity (Cresswell et al. 2002), which may contribute to a potentially low gene flow between sites. Future studies could investigate both pollinator behavior and the genetic diversity between patches and sites, and whether a public footpath represents a barrier to gene flow. Genetic studies could also look for the presence of hybrids and gene flow between *H. non-scripta* and *H. hispanica*. The ecology of the competitors, including the hybrid *H. x massartiana*, should also be investigated.

Conclusion

The present study has shown that the unspecialized manner of bluebell dispersal leads to extremely slow spread and influences the spatial structure of the population. The model highlighted the importance of seedling survival and fertilisation for population growth and spread, and the necessity for high adult survival for population existence. Data from the field show that the early-flowering plants produced significantly more seeds than did later-flowering plants. More data from several years are needed to verify the model further, and determine if the trends seen in the data are common for the English bluebell in the North-East of England, UK, or are the result of a non-optimal growth season.

Appendix 1

Flower position was slightly negatively correlated with rank number (or raceme rank); fruits were more likely to develop at lower ranks, i.e. closer to the bulb, than higher up. Despite this, flower position was not significant for fruit production for the plants investigated here (Figure A.1).

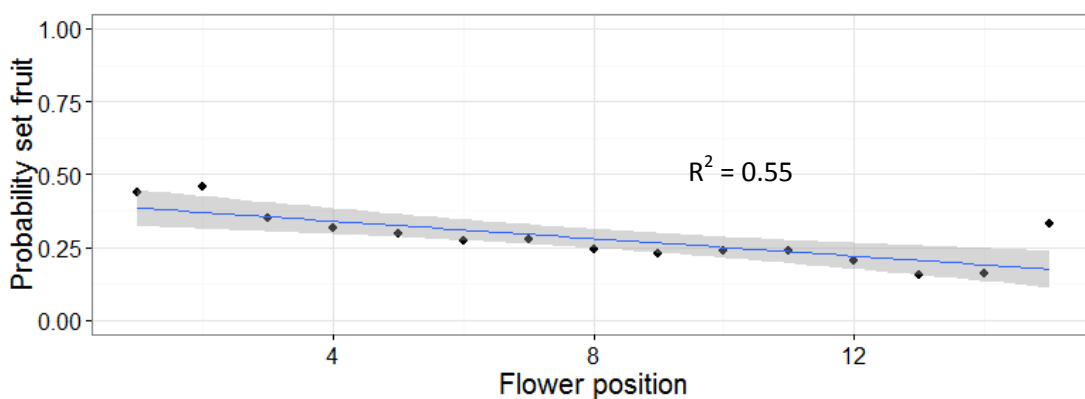


Figure A.1: Probability of setting fruit based on flower position on the raceme, 1 being the lowest rank. The shaded area around the regression line represents the 95% confidence intervals based on SE (estimated using R).

Appendix 2

The inflorescence size (total number of flowers on a plant) varied, but was not found to increase with decreasing density, as was found by Grabham and Packham (1983). There was, however, a slight decrease in inflorescence size at lower densities (Figure A.2).

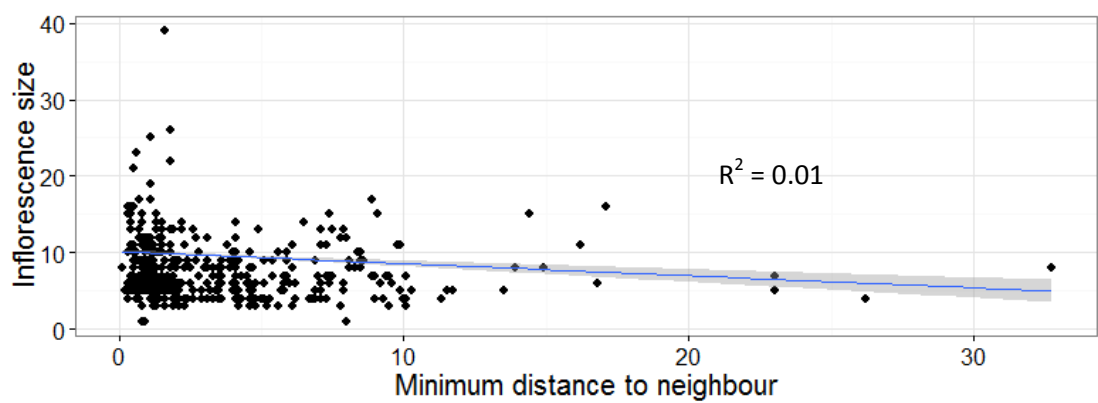


Figure A.2: *Inflorescence size decreased with decreased density. The shaded area around the regression line represents the 95% confidence intervals based on SE (estimated using R).*

Appendix 3

Flowering started about a week earlier on East, South-East, South, and South-West facing slopes compared to plants growing on North and North-East facing slopes, and two weeks earlier compared to plants growing on West and North-West facing slopes. Additionally, the length of the flowering season differed between the slopes, at least for the plants investigated in this study; the flowering season was longest on East, South-East and South facing slopes, followed by North, North-East and South-West facing slopes. The flowering season was shortest on West and North-West facing slopes (FigureA.3).

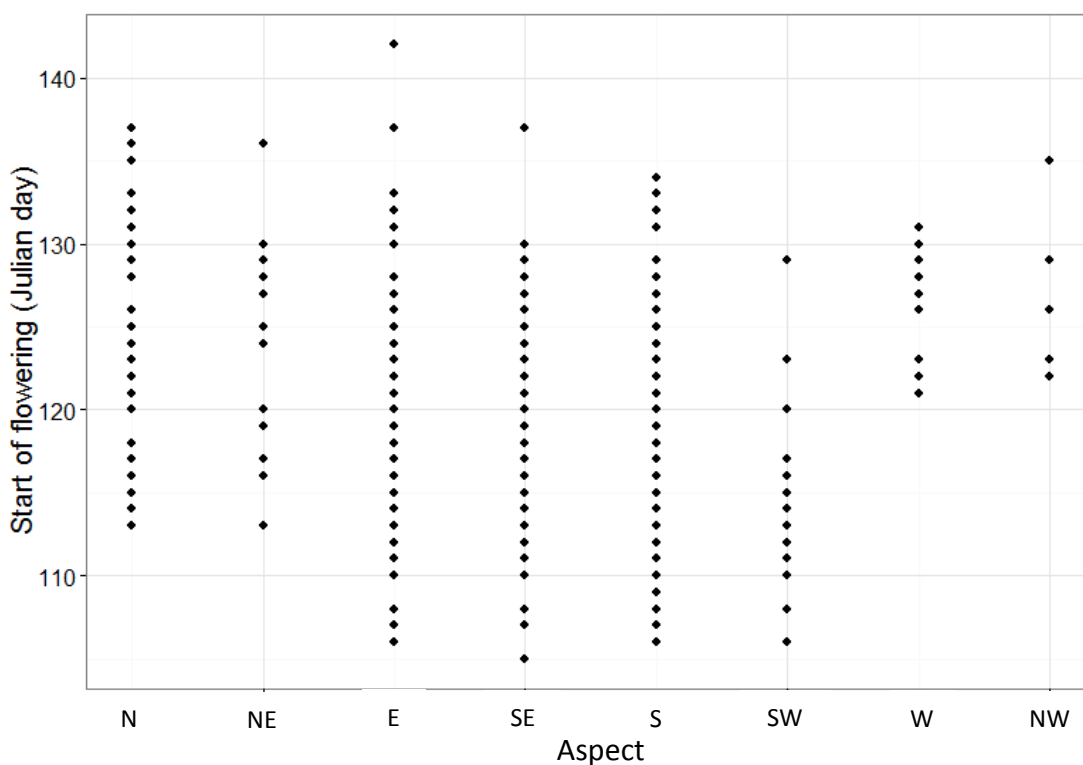


Figure A.3: The start of flowering for the first flower on each plant, at different slopes.

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