Patterns and fitness impacts of phenology shifts in pollination networks

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Dedicated to Flora

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"There is a time for everything,
and a season for every activity:
a time to be born and a time to die,
a time to plant and a time to uproot"

Pete Seeger / Ecclesiastes 3:1-2

•••

"Flowers' closing time: bee lurches
Across the hayfield, singing
And feeling its drunken way
Round the air's invisible corners…
Something has been completed
That everything is a part of"

Norman MacCaig

Abstract

Phenology shifts are one of the most prevalent responses to climate change across taxa. Temperature increases have resulted in many species shifting the timing of seasonal behaviours such as bud-burst or nest-building. Interspecific variation in the level of response has given rise to previously interacting species diverging in their phenologies, leading to the possibility of temporal mismatch. One system in which this has been predicted, and in some cases evidenced, is pollination networks.

There are several underdeveloped areas of understanding that restrict our ability to anticipate and accurately predict the impacts of phenology shift in pollination networks. There is limited research quantifying the demographic fitness impacts of mismatch, which hinders understanding of the risks of mismatch. The drivers of interspecific variation in phenological sensitivity to temperature are uncertain, limiting capacity to predict which species may undergo divergent rates of shift. Finally, current methods do not account for how phenology shifts may also change how species population are distributed through time, which affects predictions of mismatch. This thesis seeks to examine each of these knowledge gaps.

First, a manipulative field experiment was conducted using a generalist plant to quantify how continuous variation in pollinator phenology impacts on seed-set. Pollinator abundance and species composition was found to vary significantly over the field season, resulting in large variation in seed-set depending on flowering phenology.

Second, the phenology of a group of key pollinators, Syrphidae (hoverflies), was examined using a UK recording scheme from 1980 onwards. Syrphidae were generally found to be advancing their flight phenologies, although response was asymmetrical over their flight period and first date of flight advanced at a greater rate than peak abundance date or last date of flight. Life history traits that were used

to predict phenology response in Lepidoptera were applied to the data set but were not found to be predictive in Syrphidae, potentially indicating that these traits are not general predictors of phenology response in arthropods.

Finally, simulations of phenology shift in pollination networks were run using an individual-based model in which the shape of population distributions were also allowed to shift in response to temperature. These predicted large rates of species loss in networks and demonstrated that accounting for the shape of phenology events results in larger rates of species loss, indicating that current predictions may underestimate risk.

These findings advance understanding of phenology shifts by showing that they can have severe fitness impacts and that it is necessary to model phenology in a way that takes account of the shape of phenology events.

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Chapter One
General introduction

The precise configuration of the average pollination network exists within the slimmest confines of possibility space. The exact links between interacting species and the timings of those interactions are one small part of all possible combinations of these moving parts. However, pollination networks are not the product of chance. They are the product of mutualistic species associations moulded by selective pressure and adaptation over decades, centuries and millennia. Time has allowed the complexity of these networks to develop and time remains integral to their functioning. The annual window during which a plant flowers or their pollinating insect flies is limited and the timing of it is governed by a suite of environmental cues. Synchronising this timing with interaction partners is crucial, with both plant and insect needing to co-occur temporally as well as spatially for pollination and feeding to occur. As the environmental cues that mediate the timing of species' activities alter as a response to climate change, it is critical to ask how species will respond and how their responses will affect the functioning of species interactions in systems such as pollination networks.

Phenology shifts

Phenology, the study of the timing of life-history events (Lieth 1974), is integral to ecosystem functioning. Organisms are exposed to significant variance in biotic and abiotic risks and rewards depending on the timing of these life history events. Theoretically there should exist an optimal window for mammals entering and waking from hibernation, for birds to nest, for plants to bloom (Visser & Gienapp 2019). Therefore, selection should act on phenology of behaviours such as emergence from overwintering, nesting or flowering so that the timing of these behaviours maximises fitness (Kronfeld-Schor *et al.* 2017).

In order to calibrate the timing of behaviours such as nest-building, organisms' phenology responds to abiotic cues (Elzinga *et al.* 2007, Hodgson *et al.* 2011, Tang *et al.* 2016). If there is shift in these abiotic cues, then the phenology of organisms can also be expected to shift. Until recent decades, the study of phenology and its variance was undertaken primarily from the perspective of mapping organism natural history (e.g. Ollerton & Lack 1992, Brody 1997) until phenology shifts were characterised as one of the most conspicuous responses of organisms to climate change (Walther *et al.* 2002). A series of landmark papers in the early 2000s synthesised observed phenology shifts across taxa (Parmesan & Yohe 2003, Root *et al.* 2003, Parmesan 2007) and ascribed them to climate change, with the result that phenology was listed as one of the most important fields of study for understanding the biotic effects of climate change by the IPCC (2007).

Phenology shifts are still principally studied within temperate environments – while there is an increasing body of research studying seasonality and phenology drivers in tropical ecosystems (Abernethy *et al.* 2018) these are highly complex systems and trends are less well understood. Therefore the focus of this thesis is exclusively on phenology shifts in temperate environments. Within temperate environments, meta-analyses conducted in the early 2000s found that rising temperatures are leading many species to advance their phenologies (Parmesan & Yohe 2003, Root *et al.*

2003, Parmesan 2007). Spring events such as bud burst, bird/amphibian breeding and insect flight are advancing at an overall rate of 2.3 days per decade across Europe and North America (Parmesan 2007), primarily attributed to rising global temperatures. However, phenology shifts are not synchronous between or within taxa, with interspecific variation in both rate and direction of shift (Parmesan 2007, Duchenne *et al.* 2020). This heterogeneity in response means that phenologies of species which used to be synchronised, or overlap, may diverge, leading to phenological mismatch (Gordo and Sanz 2005).

Phenological mismatch

A phenological mismatch may be thought of as a temporal decoupling of previously interacting species, where there is a reduced rate of co-occurrence, or none (Figure 1.1). In this context, synchrony and mismatch are not binary conditions and there is a spectrum of decreasing degrees of temporal overlap between full synchrony and complete decoupling of species (Forrest & Miller-Rushing *et al.* 2010). Often, although not exclusively, phenological research focusses on mismatches between consumer and resource (Visser and Gienapp 2019). Mismatches between consumer and resource appear to be increasing with climate change: Visser and Both (2005) found evidence of emerging mismatches in 8 of 11 focal species studied across different taxa, where the consumers' rate of phenological change was either too great or too little to remain synchronous with the phenology of their food source.

Under circumstances where mismatch with resources has a high impact on individual fitness, it can be expected that mismatch will have a measurable impact on population demographics (Iler *et al.* 2021). Demographic consequences of phenology shifts have been described across taxa and ecosystems. Increasing levels of mismatch with their plant food resource have decreased offspring production in both arctic geese (*Chen rossii*) (Ross *et al.* 2017) and caribou (*Rangifer tarandus*) (Post & Forchhammer 2008) while fish spawning becoming increasingly mismatched from phytoplankton blooms has decreased fish farm stock (Asch, Stock & Sarmiento 2019). The long-term demographic effects of phenologically-induced mismatch are not always net negative, however. While mismatch between the blue tit (*Parsus*

major) and its caterpillar food source results in within-season decreases in offspring produced, when considered across seasons the lower number of offspring results in lower density-dependent winter mortality (Reed *et al.* 2013). Due to the balancing effect of recruitment, the phenologically-induced reduction in offspring production overall had a negligible effect on population demography.

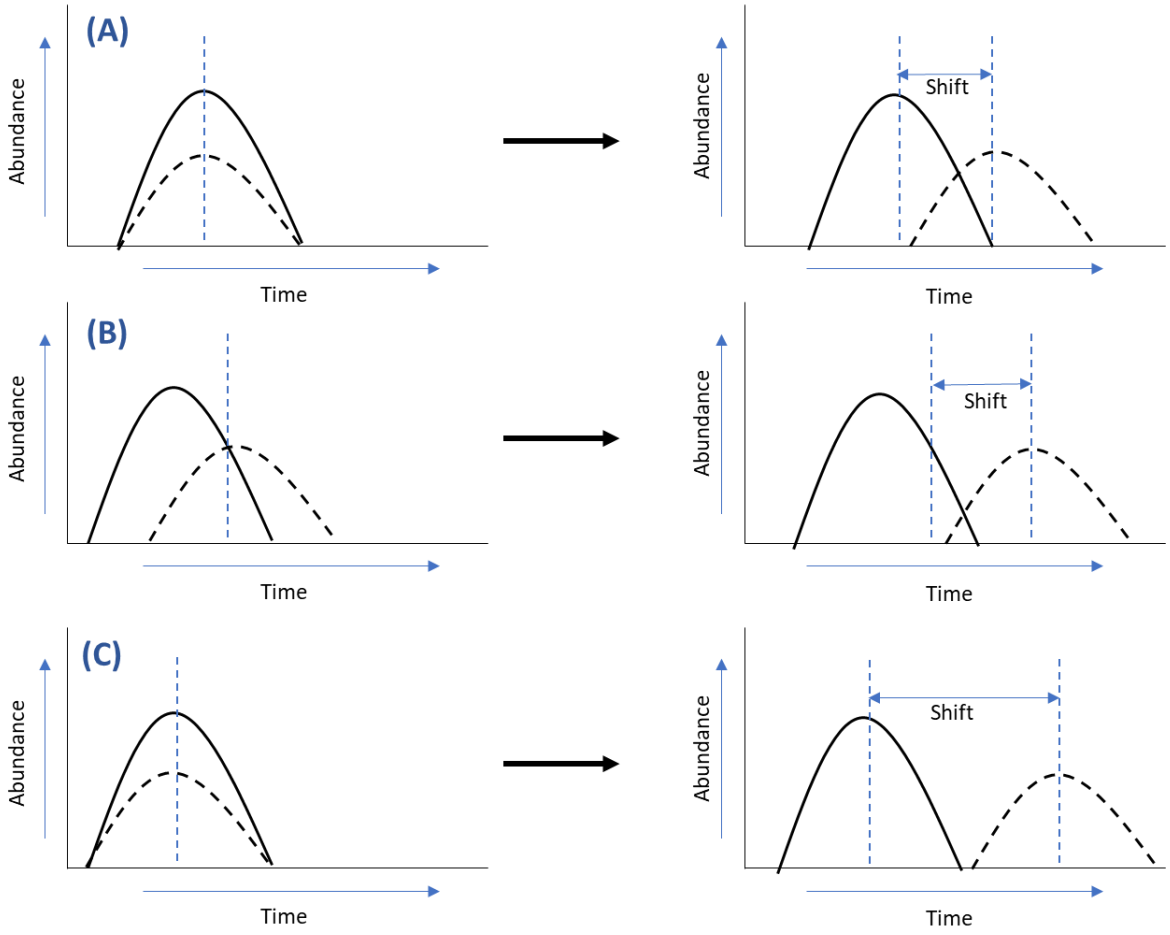

Figure 1.1: Illustrations of temporal mismatch generated through phenology shifts. Species 1 is represented by the black dashed line, with its population peak represented by the blue dashed line. Species 2 is represented by the black bold line. Row (A) illustrates species 1 shifting its phenology from a point of baseline synchrony with species 2, where population peaks are matched, to create a partial mismatch. Row (B) illustrates a similar scenario of increasing level of mismatch but does not assume a perfect phenological match between species 1 and 2 to start with – instead they start from a position of baseline asynchrony. Scenario (A) would be more likely in systems where a species is primarily reliant on a single other species as a resource and where resource acquisition is the primary selective pressure (as opposed to, for example, predator avoidance). Scenario (B) is the likely scenario between any single species-species pairing in a system where species have multiple interaction partners, or have stronger selective pressures on them than synchrony with resources. Both (A) and (B) exhibit partial mismatch, whereas (C) illustrates total phenological mismatch.

Historically there has been a focus on phenological mismatch between consumers and resources (Visser & Gienapp 2019) but in recent years study of mismatch has broadened out to other systems of interaction, particularly the interacting

communities that comprise pollination networks (Hegland *et al.* 2009, Forrest 2016, Rafferty 2017). Pollination networks pose interesting challenges for assessing phenological mismatch as interactions are not simply pairwise, except in examples of extreme specialisation (e.g. Robbirt *et al.* 2014), with match and mismatch occurring between multiple overlapping populations of different species. Despite this challenge they are fertile ground for phenological questions, with both plants and pollinators undergoing phenology shifts with high levels of interspecific variation (Memmott *et al.* 2007) and clear hypothetical fitness consequences for mismatch (Forrest 2016). It is for these reasons that in this thesis I use pollination networks as a study system for phenological questions.

Study system: Pollination networks

Pollination networks are key ecosystem services, with over 85% of flowering plants worldwide animal-pollinated (Ollerton, Winfree & Tarrant 2011) and approximately 75% of globally-significant crops (Klein *et al*. 2007, Potts *et al.* 2016) reliant on pollination to some degree. Pollination networks also help support a wide range of vertebrate and invertebrate diversity (Garibaldi *et al.* 2013). The presence and impact of phenological mismatches has been identified as a potential threat to pollination networks for 40 years (Willmer 2012) and the idea of emerging mismatches appears to have been borne out by recent research. The phenology of flowering (Sparks, Jeffree & Jeffree 2000, Miller-Rushing 2006) and flight windows of pollinators (Roy & Sparks 2000, Bartomeus *et al.* 2011, Duchenne *et al.* 2020) are asynchronously shifting in temperate habitats worldwide. Given the fact that the species making up pollination networks display heterogeneous phenological responses to climate change, phenological mismatches could emerge between pollinators and flowers (Hegland *et al.* 2009, Willlmer 2012, Kudo & Ida 2013). Therefore, emerging mismatches in pollination networks have increasingly been an area of active research (Hegland *et al.* 2009, Rafferty 2017), particularly since the advent of simulations projecting extensive future disruption (Memmott *et al.* 2007).

Within pollination networks, phenological mismatches may give rise to a number of adverse effects including, but not limited to, reduced pollination services impacting plant seed-set and reproduction (Thomson 2010); reduction of floral resources impacting pollinator survival (Memott *et al.* 2007); and weakening of pollination networks, increasing susceptibility to future perturbations (Burkle, Marling & Knight 2013, Ramos-Jiliberto *et al.* 2018). Weaking of network robustness is of particular significance given the range of threats to which pollination networks are currently subject, with pollinator abundance declining through insecticide-use, habitat reduction and invasive pests (Potts *et al.* 2016).

Despite the clear evidence for phenology shifts and the strong theoretical basis for their impacts on pollination networks there is a distinct lack of consensus on both the trends and impacts of mismatches. Typically, modelling approaches have produced stark predictions of species loss (e.g. Memmott *et al.* 2007, Burkle, Marling & Knight 2013) while fieldwork in both controlled and open systems has produced more equivocal results (e.g. Parsche *et al.* 2011, Rafferty & Ives 2011). Here I shall discuss the evidence for plant-pollinator mismatches and their impacts, along with future work that could be performed to better quantify them. First, however, I shall describe the trends in phenology shifts and the abiotic cues and life history traits that may drive them.

Trends of phenology shift in plants and pollinator

Phenology studies are still strongly wedded to the methodology of characterising phenological events through specific focal dates (Inouye *et al.* 2019). Frequently, but not exclusively, these are 'first' dates – first dates of bloom or flight (Visser & Gienapp 2019). By this measure, both plants and pollinating insects have exhibited general advances in the timing of flowering and flight activity respectively, although there is a high rate of interspecific variation within this general trend. Studies primarily using data from temperate regions in Europe and the USA have shown that since 1950 there have been marked advances in flowering phenology correlating with temperature rises, with the rate of the advances rapidly increasing since 1970

(Menzel *et al.* 2000, Sparks *et al.* 2000, Fitter & Fitter 2002, Menzel *et al.* 2006, Parmesan 2007, Menzel *et al.* 2020, Buntgen *et al.* 2022). As methods have been refined, estimates of the size of this shift have typically increased: early analysis across 19 European countries found plant flowering times to have advanced by a mean 0.14 days / year, 6.3 days from 1951 to 1996 (Menzel *et al.* 2000, Parmesan 2007). However recent studies have found much higher rates of advance of 0.32 days / year, with UK plants blooming 21.4 days earlier across a similar time period of 1952 to 2019 (Buntgen *et al.* 2022).

Compared against the literature for plants, analyses of phenology shift across insects tend to be restricted to more specific taxonomic groups (Langowska *et al.* 2017, Buntgen *et al.* 2022). A recent exception analysed shifts in four groups of pollinators across Europe (Lepidoptera, Diptera, Coleoptera, Hymenoptera) which displayed a mean advance in onset of flight activity of six days over the last 60 years (Duchenne *et al.* 2020). Lepidoptera were over-represented compared to other groups in this study (1,427 of 2,248 species, 63.4%) and they are one of the most popular insect groups for studying phenology shifts, with patterns of advance in first flight date and peak abundance (Roy & Sparks 2000, Altermatt 2010, Diamond *et al.* 2011, Hodgson *et al.* 2011, Kharouba *et al.* 2014). Outside of Lepidoptera, work has been performed on honeybee populations (*Apis mellifera*) in Poland and Spain, where date of first flight negatively covaries with increases in spring (February-April) temperatures (Gordo & Sanz 2006, Langowska *et al.* 2017). Wild Hymenoptera species have also exhibited phenological advance. In the USA, comparison of museum specimen collection dates have estimated advances in emergence time of 0.08 days / year, 10.4 days over the past 130 years (Bartomeus *et al.* 2011, 2013). This is strikingly similar to the advances predicted through comparison of field observations to historic records, where wild bee emergence had advanced an average of 0.09 days / year, a total of 11 days since the 1870s (Burkle, Marlin & Knight 2013).

Summary statistics show that, over time, both plants and pollinators have broadly advanced phenologies, but these headline figures do not capture the substantial variance in response frequently exhibited in these studies. For example, in a study

looking at a suite of phenological events in European plants (germination, leaf-burst and flower bloom) the majority (75%) of the 542 species showed phenological advance in all these events from 1970 to 2000 (Menzel *et al.* 2006). However, 22% of the plant species exhibited no response, and 3% of species delayed their phenologies. Similar diversity exists within trends in pollinator phenology – from 2,248 European pollinator species, 57% exhibited advance in first flight dates, but 13% delayed flight and 30% had no significant response (Duchenne *et al.* 2020). This variation is sometimes tied to broad categories of species – for example insect-pollinated plants often exhibit higher rates of shift than wind-pollinated plants (Menzel *et al.* 2020, Buntgen *et al.* 2022) - but often there is significant variance even within taxonomic groups. For example, in Diptera, interspecific variation in response ranged from advancing phenology at a rate of 2 days/year to delaying it at a rate of 1 day/year (Duchenne *et al.* 2020) and comparable variation can be seen within Lepidoptera (Diamond *et al.* 2011, Kharouba *et al.* 2014, Duchenne *et al.* 2020). Understanding which underlying factors predicts species' variance in response is desirable in order to anticipate which species may be most at risk of mismatch. However, to understand phenological responses it is first necessary to consider the abiotic cues and species traits which mediate these responses.

Effect of cues & life history traits on phenology shift

Temperature is the primary abiotic variable considered in phenology studies, whether implicitly or explicitly (Visser 2022), with one meta-analysis finding that 35 of the 38 phenology studies in plants inferred a causal link between temperature rises and phenology shifts in plants (Franks, Weber & Aitken 2013). However, response to temperature varies depending on which phenological variable is being measured and in which species it is being measured. For example, both date of leaf burst and date of first flower typically covary negatively with early-year temperatures, but the trends in bud burst and flowering do not necessarily extend to the phenology of fruiting (Sandor *et al.* 2021). Temperate plants can respond to temperature in complex ways - while the onset of flowering is closely tied to temperature many plants also require an extended chilling period of vernalisation before the onset of flowering can trigger (Simpson & Dean 2002, Henderson *et al.* 2003). As flowering phenology is mediated both by cold winters and warm springs, rising global

temperatures may result in a confounding effect on cues for flowering phenology as it gives rise to both warmer winters and springs (Forest 2016).

While some groups, such as bumblebees, are able to exhibit facultative endothermy (Heinrich 1975), insects are predominantly small-bodied poikilotherms. The phenology, growth and activity rates of insects are likely to be driven by temperature, at least in temperate regions (Hegland *et al.* 2009). As such, phenological changes in insect eclosion are also almost exclusively interpreted with reference to temperature as the abiotic driver (Rafferty 2017). The effect of temperature on insect phenology is complex because temperature both promotes emergence from diapause (Bale & Hayward 2010), as well as hastening development across life stages (Bennett *et al.* 2015, Hassall, Owen & Gilbert 2017). While climate change will probably raise both development temperature and eclosion cues, these two factors may be affected differently, and the phenologies of different species may vary in the relative importance of each.

Plants and pollinators are therefore thought to respond to temperature cues in broadly similar ways, but the details in individual cases will give rise to some of the interspecific variation observed. For example, one field experiment demonstrated the differing responses of a plant-pollinator species pairing to temperature (Forrest 2010). Using reciprocal transplants of *Bombus* nesting boxes in the Rocky Mountains, in order to assess the response of insect populations independently of their local plant populations, Forrest measured the emergence date of insects and onset of blooming in the surrounding plants. Although the phenology of both emergence and bloom responded strongly to temperature, insect emergence had a higher degree-day requirement than flower bloom, indicating that while insect and plant phenology is broadly dictated by temperature cues, subtle differences in sensitivity could give rise to the emerging mismatches.

Methodologically, it is common for studies to characterise phenology shifts in terms of days per year (e.g. Menzel *et al.* 2006, Duchenne *et al.* 2020, Buntgen *et al.* 2022). The link between phenology shifts and temperature is often inferred by citing the link between temperature and phenology and evidencing that mean temperatures have risen over the study's time period, but temperature is not always used as a

predictor of phenology shift. That temperature is not explicitly included as a predictor is in part due to the difficulties of attributing phenological shifts to temperature on broad geographic scales, as local variance in temperature can cause strong variance in response. Particularly with insects, which are often highly mobile, plastically shifting phenology is not the only response to changing climate available to organisms. If an organism is not able to shift its phenology to temporally track changing climates, selection may act on dispersed populations to functionally shift their ranges, resulting in spatial shifts as the population tracks climate niche (Socolar *et al.* 2017). Range shifts in response to climate change are frequently reported, with many species expanding their latitudinal range poleward as temperatures have risen (Walther *et al.* 2002, Hickling *et al.* 2006, Vasquez *et al.* 2017). These movements are one mechanism by which species that have low plasticity in their phenological response may still exhibit the effects of phenological constraints in their population dynamics. For example, in UK butterflies those that had not altered their phenology in responses to temperature rises since 1973 had been subject to range shifts (Hodgson *et al.* 2011).

A second common abiotic cue linked with phenology is photoperiod, which is static in the face of climate change (at least in the absence of populations undergoing significant latitudinal range shifts). Many insects are thought to be sensitive to photoperiod as a cue for initiating diapause (Bradshaw & Holzapfel 2007, Bale & Hayward 2010). Plants also track seasons through photoperiod length and some species appear to be more highly reliant on it for senescence than for flowering onset or leaf burst (Singh *et al.* 2016). The apparent reliance on different cues for onset and ending of phenological activity may help to explain some of the asymmetries observed in phenological response, with last dates of activity typically less responsive to temperature than first dates (Forrest 2016, Duchenne *et al.* 2020).

Overall, the fine detail of the abiotic cues used to govern phenological response in species is poorly resolved even while the broad trends are clear (Forrest & Miller-Rushing 2010), which makes anticipating interspecific variation challenging. An alternative approach is to consider the life history and behavioural traits of species and ask how they may shape phenology and phenological shifts (Chmura *et al.*

2019). In insects, Altermatt (2010) studied how butterfly life history traits might be predictive of phenology shifts, hypothesising that species with greater larval diet breadth would demonstrate less phenological plasticity as there is less selective pressure to align with specific host plants. Since then, other research has generated further hypotheses regarding how life history traits should influence phenological response in insects. Additional life history traits that may affect phenology include overwintering stage (Diamond *et al.* 2011, Forrest 2016), historic timing of flight season (Pau *et al.* 2011), voltinism (Tobin *et al.* 2008, Forrest 2016) and migratory status (Kharouba *et al.* 2014). While there is some support for these traits influencing phenology, and the effect of these life history traits on phenology is often framed as generalisable across taxa (Chmura *et al.* 2019), most research has been conducted on Lepidoptera (Altermatt 2010, Diamond *et al.* 2011, Kharouba *et al.* 2014, Brooks *et al.* 2017, but see Hassall, Owen & Gilbert 2017 for an exception). The extent to which these patterns apply outside butterflies and moths remains unclear, but life history traits provide one way in which interspecific variation in phenological response may be understood.

The interspecific variation observed in phenological responses is therefore likely to be driven by which cues species are responsive to and how responsive they are to those cues. In turn, the scale of response may be driven by life history traits. Understanding this variation may lead to the potential for anticipating emerging phenological mismatches (Chmura *et al.* 2019).

Measurement of mismatches

Research to identify and measure emerging mismatches often relies on a correlative approach (Visser & Gienapp 2019), comparing single-date measures of phenology for interacting species and quantifying their level of overlap. One of the first studies to apply this method to plants and pollinators, using data records of species emergence from the Mediterranean, showed that 4 pollinating insect species exhibited an increasing degree of mismatch with their food plants from 1970 onwards (Gordo and Sanz 2005). Emerging mismatch has been identified in multiple pollinator taxa. In British Columbia an examination of flowering in 59 plants across

130 years found that flowering date advanced at more than double the rate of phenologies for the 187 butterfly species that feed on them (Kharouba and Vellend 2015). A specific study of a species-to-species pairing in Japan found early onset of spring, characterised by date of snowmelt, causes earlier blossoming of *Corydalis ambigua* than the *Bombus* species that exclusively pollinate it (Kudo & Ida 2014).

Not all studies comparing phenology in terms of calendar days find evidence for mismatch (Renner & Zohner 2018). In a study using museum specimens, the phenology of 10 *Bombus* species in north-eastern USA had advanced their first-flight phenologies at a largely synchronous rate to their food plants over the past 100 years, with mismatches emerging at a mean rate between -0.08 to 0.024 days per year during the study period (Bartomeus 2011). Their conclusion was that this mismatch is unlikely to cause significant fitness costs for interacting species, although whether this conclusion holds true depends both on the scale of fitness costs incurred with marginal mismatch and on whether comparison of calendar dates in this manner correctly reflects the underlying interaction potential of species' populations.

The above studies emphasise first dates of bloom or flight as relevant estimates of population phenology, and the mismatches they describe are based on comparisons of these first dates, although full duration of bloom and flight events can last weeks or months and plant-pollinator interaction takes place throughout this period (Straka & Starzomski 2014). One method for projecting how mismatches may emerge over a full season is the use of simulation approaches that assess plant-pollinator interactions over the full period of a phenological event. Typically, these simulations suggest the possibility for large levels of mismatch once shifts over decades are accounted for. A landmark paper (Memmott *et al.* 2007) simulated the phenology shifts of 1,419 pollinating insects and 429 plant species from historic data of a pollination network in Illinois (described in 1929). Depending on model assumptions, 17-50% of pollinator species suffered some level of food disruption during their total flight period under predicted temperature increases of 3.5 °C – 5°C. A separate study in Illinois studied a recorded historic pollination network from 1890 and revisited the area it was taken from to confirm how many of the interactions still existed (Burkle, Marlin & Knight 2013). They found a massive loss of network interactions, with 76% of original interactions absent in the present-day

study site. While habitat loss and range shifts were deemed responsible for the majority of these losses, simulations reconstructing phenology shift estimated that up to 13% of the interactions had been lost purely due to phenological mismatches. Although these simulations assess mismatches in the context of total blooming or flight period rather than dates of first event, their approach still primarily examines phenology through dates and time windows. Comparison of dates alone has the potential to underestimate the true scale of mismatches (Kharouba & Wolkovich 2020). Once population distributions are taken into account across windows of activity, predictions of mismatch could be substantially altered, but characterising the distribution shape of a species' phenology is currently not a common approach in the literature (Inouye, Ehrlen & Underwood 2019).

Impact of Mismatches

Given the evidence for potential emergence of phenological mismatch, understanding the impact on species requires measurements of the demographic effect of these mismatches (Iler *et al.* 2021). However, both quantifying this impact and attributing it to mismatches has proved troublesome (Miller-Rushing 2010, Forrest 2015, Renner & Zohner 2018, Iler *et al.* 2021) despite the strong theoretical basis for how mismatch could affect plant and pollinator demography.

In plants, phenological mismatches with pollinators may give rise to a lower level of seed production, which could ultimately impact population demographics. Pollen limitation is a plant trait that describes how seed set is constrained by the level of pollination received by a flower (Knight *et al.* 2005). One meta-analysis found 62% of 258 plant species displayed some level of pollen limitation, often significantly influencing seed set (Ashman *et al.* 2004); pollen limitation is also affected by pollinator visitation rates and species composition in open systems (e.g. Liu & Koptur 2003). It is therefore possible that phenological changes may reduce pollination service received by plants, limiting seed set. Mismatch causing reproductive impacts is demonstrated in a spring ephemeral, *Corydalis ambigua*, where seed set decreases with advanced onset of spring, which increases the

mismatch between the plant's flowering period and the peak abundance of the *Bombus* species which pollinate it (Kudo & Ida 2013).

Although determining the impact on seed set is critical, fieldwork that measures plant mismatch with pollinators and then ties that mismatch to a demographic effect is rare. A field manipulation in which plant species were induced to flower in different weeks before being placed in an open setting found that many early-flowering plants did not suffer a reduction in pollinator visitation, and certain species experienced a higher rate of visitation (Rafferty & Ives 2011). However, follow-up work showed that in the two species of wildflower for which they artificially manipulated onset of blooming, mean seed set produced per single pollinator visit was lower during advanced blooming times (Rafferty & Ives 2012). The variance in seed set was driven both by changes in species composition of pollinators and variability in effectiveness of pollinating species over time. That single-visit seed-set reduced in phenologically advanced plants demonstrates that even while visitation rates were unaffected, or improved, by phenological advancement, the quality of pollination service received could have been significantly reduced.

Phenological mismatches therefore probably have real impacts on plant reproduction and demography, at least when measured under experimental conditions. However, in open systems, seed set and survival rate will also be governed by factors such as resource limitation or herbivore abundance, which can confound the effect of mismatch with pollinators. For example, in experimental plant populations with manipulated flowering time, lower pollinator visitation in early-flowering plants was offset by release from herbivorous antagonists (Parsche, Frund and Tscharntke 2011). The abundance of pest species was lower at early-flowering times, leading to increased seed-set compared to later-flowering populations despite a lower level of pollination. Determining the partial effect of pollinator mismatch on plant seed set therefore requires consideration of these factors, ideally through the use of well-designed field experiments.

The impact of phenological mismatches directly affects food resources for pollinators given that flowers provide food in the form of both nectar and pollen for visitors (Willmer 2011). Greater access to these food resources has a demonstrable impact on reproductive success in pollinators; bumblebee colonies in field settings that are given pollen and nectar supplements exhibit growth respectively 51% and 81% greater than control colonies (Pelletier & McNeil 2003), while high levels of floral diversity and abundance increase local bumblebee colony mass (Goulson, Hughes & Derwent 2001). Despite these links, the effect of mismatches on pollinator populations is understudied, partly due to the difficulties of consistently monitoring population dynamics of insects, particularly non-social insects, in field settings (Forrest 2015). One attempt to quantify the fitness impacts of phenology shifts on pollinators showed that under controlled conditions and using flight cages, mismatches of three days or more between solitary bee species and their food flowers can harm insect fitness (Schenk, Krauss & Holzschuh 2018). From three bee species studied, two suffered significantly decreased brood size after mismatches of three days; at mismatches of six days all three species exhibited high levels of mortality. That bees showed reproductive fitness impacts at three to six days of mismatch demonstrates that for some species even low levels of mismatch between pollinators and associated flowers can quickly lower reproductive fitness.

Phenology shifts and mismatches in context

While controlled experiments can evidence potential impacts in principle, in natural settings specific plant-pollinator mutualisms do not occur in a vacuum, but instead take place as part of a wider structure of interactions in a pollination network. The structure of these networks may serve to buffer some of the effects of these mismatches (Forrest 2015, Hegland *et al.* 2009, Rafferty 2017); pollination networks are structured to maximise coexistence of interacting species under a range of conditions (Waser *et al.* 1996, Rohr, Saavedra & Bascompte 2014) which indicates that even in the case of specific interactions undergoing decoupling, plants or pollinators may be able to rely on alternative interaction partners. One possible reason why simulation approaches predict a greater prevalence of mismatch than appears to have been found in studies of recorded data, aside from the differing methodologies used to characterise phenology, is that simulation approaches

assume plant and pollinator phenological responses will occur independently of each other (Hegland *et al.* 2009, Straka & Starzomski 2014). However, it is possible that plants and pollinators may be able to alter their phenology in order to track their respective interaction partners. Indeed, a high proportion of species within pollination networks are generalist (Waser *et al.* 1996) and individuals tend to interact with both generalist and specialist species, giving a degree of redundancy to specific pairings (Bascompte *et al.* 2007, Olesen *et al.* 2011). Loss of certain interactions may therefore be offset by the ability of species to rely on alternative partners; even within a given season pollination networks are dynamic, with a high rate of interaction turnover (CaraDonna *et al.* 2017). Alternatively, species may form novel interactions, with host-shifts in pollinating species already being observed. *Aricia agestis* butterflies have expanded their range due to climate-driven rises in abundance of a novel host plant (Pateman *et al.* 2012) while two bumblebee species have evolved shorter tongues over the last 40 years to become more generalist after temporal decoupling from their previous, specialist interaction partner (Miller-Struttmann *et al.* 2015). Specialist species that are unable to adapt and have an especially limited range of interactions will be most at risk, such as the orchid *Ophrys sphegodes* which is undergoing increasing mismatch from its sole pollinator, a single species of solitary bee (Robbirt *et al.* 2014).

Despite the clear risks to specialists, it seems unlikely that, for many species, phenological mismatches will be so complete or the consequences so severe that they will directly lead to significant population loss within a short timescale (Forrest 2015, Rafferty 2017). However, over time, these affects have the potential to restructure pollination networks (Duchenne *et al.* 2020). Loss, or rewiring, of pollination network interactions could occur as species become increasingly mismatched with their historic pollination partners, and are only partially able to compensate with new interactions (Burkle, Marling & Knight 2013). Although pollination networks are typically robust to perturbations (Bascompte *et al.* 2007, Song, Rohr & Saavedra 2017), loss of interactions or interacting species can incrementally affect the functioning of these networks (Winfree *et al.* 2018) and heighten their susceptibility to future disturbances. The demographic impacts of mismatches on plants (Kameyama & Kudo 2009, Kudo & Ida 2013) and pollinators (Schenk, Krauss & Holzschuh 2018) need to be understood as a contributing

aggravating factor to what has been termed a pollination 'crisis' (Levy 2011, Martin 2015).

Research objectives

Given that variance in phenology can give rise to temporal mismatches which harm the dynamics of pollination networks, I wanted to examine the trends and impacts of these mismatches in three ways. First, I aimed to quantify the fitness impacts of variation in pollinator phenology on seed-set to understand the continuous effect of mismatches on reproductive fitness. Second, I aimed to describe phenology shifts in Syrphidae as a response to temperature and understand whether their phenological response is predictable using species life history traits. Third, I aimed to test how accounting for the shape of phenology events can affect predictions of mismatch in pollination networks.

Chapter Two: Phenological mismatch between plant and pollinator is often hypothesised to lead to reduction in plant reproductive effort. Despite this, experiments quantifying how phenological variation in pollination service affects seed set are few, and frequently confined to specialist plant species. In this chapter I measure phenological variation in pollinator abundance in a field setting, and link it to seed set produced in an experimental array of rat-tailed radish (*Raphanus raphanistrum subsp. sativus var. caudatus*), a generalist self-incompatible plant visited by a wide range of pollinators. I modelled seasonal fluctuations in pollinator abundance and modelled pollinator abundance and diversity as predictors of seed-set produced. To control for confounding effects on individual plant seed-set, I included positive (hand-pollinated) and negative (pollinator-excluded) control flowers on each plant. I aimed to quantify continuous variation in pollinator phenology on seed-set and whether this variation led to a quantifiable effect on seed-set.

Chapter Three: Interspecific variation in phenology shifts gives rise to the potential for phenological mismatch. Previous research has attempted to explain phenological variation in response to temperature based on insect life history traits, but this research is largely restricted to Lepidoptera. To test whether these explanations are generalisable to other taxa, I applied them to a key group of pollinators, the hoverflies (Diptera: Syrphidae). Using data from the UK Hoverfly Recording Scheme (HRS), I tested for the size of phenology shifts since 1980 in first emergence, peak emergence and last flight dates as a response to local temperatures. I hypothesised that specialist species would exhibit more phenological plasticity than generalists, that non-migratory species would exhibit a greater rate of shift than migratory species and that species with more advanced overwintering stages would exhibit greater shift. I also quantified seasonal voltinism and hypothesised that multivoltine species would exhibit greater rates of voltinism under higher temperatures.

Chapter Four: The shape of abundance distributions across species' phenologies affects their interaction strength and the prevalence of mismatches. However, these factors are not typically considered when assessing mismatch in pollination networks despite evidence that rising temperatures may also affect the shape of these distributions. To test how accounting for abundance distribution impacts predictions of phenological mismatch, I created an individual based model. This simulated a pollination network experiencing increases in temperature, where the shape of species' distribution across their phenologies was sometimes allowed to change in response to temperature. I hypothesised that if abundance distribution changed across a phenology window, this would result in variance in the negative effects, such as species loss, of phenological mismatch.

Chapter Two

Effect of phenological variation in bloom time on the reproductive fitness of *Raphanus raphanistrum subsp. sativus var. caudatus*

Abstract

Phenological responses to climate change show high levels of interspecific variation within the interacting species of pollination networks. One result of this variation is increasing levels of phenological mismatch between plants and their pollinators, both in terms of occurrence and abundance. This is typically hypothesised to lead to fitness costs, but the number of studies quantifying the fitness cost of phenological variation is limited. To quantify the demographic impact of phenology shift on plants I examined seed set in experimental groups of *Raphanus raphanistrum subsp. sativus var. caudatus* that were induced to flower at a range of times during the spring and summer across 2 years of fieldwork. Pollinator activity over the period was measured using pan trapping and direct observation. I found that pollinator activity varied over the fieldwork season both in total abundance and in relative abundance of different pollinator groups. I found that pollinator activity positively varied with both seed pod development and number of seeds produced by the experimental plants. *Bombus* species and *Apis mellifera* were found to have a greater contribution to this effect than solitary bee and Syrphidae species. These results point to partial phenological mismatch between plants and pollinators exerting a significant impact on seed recruitment and indicates that both the abundance and identity of pollinators co-occurring with a plant's blooming period can have large and significant effects on the seed-set produced.

Introduction

Phenology shifts are prevalent across taxa worldwide, with many species in temperate environments advancing the seasonal phenology of life-history events as a result of increasing temperatures (Parmesan & Yohe 2003, Parmesan 2007). While the overall picture is one of phenological advancement, responses are heterogeneous and display considerable interspecific variability in both the direction and the extent of shifts (Fitter & Fitter 2003, Gordo & Sanz 2005, Miller-Rushing & Primack 2008, Duchenne *et al.* 2020). A possible outcome of this heterogeneity is that previously interacting species experience divergent rates of phenology shift, limiting both their temporal overlap and potential for interaction in a move away from prior synchrony (Visser & Both 2005, Thackeray *et al.* 2016, Kharouba & Wolkovich 2020). While phenological mismatches have the potential to impact interacting species' fitness, with consequences for demography (Miller-Rushing *et al.* 2010), studies explicitly measuring the fitness impact of partial mismatch are limited (Evans & Pearce-Higgins 2021).

Pollination networks are an increasingly well-study system for assessing the fitness impacts of phenological mismatch. With 85% of flowering plants reliant to some degree on animal pollination for reproduction (Ollerton, Winfree & Tarrant 2011) and pollinators frequently dependent on floral food resources (Ogilvie *et al.* 2017, Timberlake, Vaughan & Memmott 2019), phenological mismatches are likely to give rise to measurable fitness impacts. Modelling approaches have found potentially severe consequences of phenology shifts in pollination networks (Burkle, Marlin & Knight 2013), with up to 50% of pollinator species experiencing food gaps under certain scenarios (Memmott *et al.* 2007), but observational and experimental data have produced more equivocal findings (Forrest 2015). This may reflect the propensity of model assumptions to overemphasise the likelihood of complete decoupling of interactions – while this may occur spatially in the form of climate-induced range-shifts of species (Richman *et al.* 2019), strong evidence for complete temporal decoupling of interactions has not been observed either in long-term data studies (Bartomeus *et al.* 2011, Iler *et al.* 2013) or in experimental phenology shifts

(Rafferty & Ives 2011, Forrest *et al.* 2011, Gallagher & Campbell 2020). It therefore appears likely that divergent rates of phenology shifts in pollination networks will give rise to partial rather than total mismatches. With pollen limitation prevalent among flowering plants (Ashman et al, 2004, Knight *et al.* 2005), more research research is needed on the fitness impact on plants of continuous variation in temporal co-occurrence of pollinators.

Research looking to capture the effect of partial phenological mismatches on plant fitness has found evidence of variation in pollination service received. Long-term data on the spring-blooming *Corydalis ambigua* has found seed-set to decrease with advanced blooming dates, linked with increasing degree of mismatch from its bumblebee pollinators (Kudo & Ida 2013, Kudo & Cooper 2019). As *C. ambigua* is primarily pollinated by bumblebees a pronounced effect may be anticipated as plants pollinated by more specialised interactions are more likely to be vulnerable to the effects of phenology shifts (Willmer 2012, Forrest 2015). Generalist interaction partners, as thought to comprise the majority of pollination networks (Waser *et al.* 1996, Rohr, Saavedra & Bascompte 2014), are likely to have a high degree of redundancy in their interactions and therefore be more robust to disruption of specific interactions due to phenology shifts (Bartomeous 2013).

The fitness consequences of shifts in flowering time for generalist plants have proved harder to identify. Experimental manipulation of flowering time in 14 generalist species did not find evidence of phenology shifts affecting pollinator visitation rate (Rafferty & Ives 2011). Those plant species with a historic trend of advancing flowering dates were found to be both visited by the same species of pollinators at experimentally advanced flowering times as at their natural flowering time; these plants were also subject to increased pollinator visitation rates. That the plants which had undergone phenology shifts do not suffer a significant reduction in pollination service indicates that those species which are advancing their flowering phenologies may be doing so because there is no adverse impact on fitness at advanced dates. Although in these experiments pollinator visitation rate was not adversely affected, subsequent work on 2 of these species showed that pollinator efficacy, measured through seed-set from single flower visits, was lower at advanced

flowering dates. Therefore overall pollination service received could be impacted from advanced flowering times even if visitation rates were not adversely affected, possibly as a result of increased levels of heterospecific pollen transfer (Rafferty & Ives 2012).

Visitation rate and single-visit pollinator efficacy are strong indicative measures of pollination service received though recent studies have measured phenology impacts on total seed-set over a plant's blooming period, a more direct measure of plant reproductive fitness. Kehrberger & Holzschuh (2020) found *Pusaltilla vulgaris* seed set positively correlated with pollinator visitation, with seed-set and visitation rates decreasing at later blooming times due to increased levels of floral competition. Conversely, experimental shifts in flowering phenology were not found to affect seed-set in *Mertensia ciliata*, as lower pollinator visitation-rates in late-flowering plants were compensated for by an increase in the proportion of visits by *Bombus* species, which were found to be more effective pollinators than other visitors (Gallagher & Campbell 2020). A common finding in research on pollinator efficacy is that certain pollinator taxa are more effective than others, which is a product of both morphological traits and interaction specificity (e.g. Sahli & Conner 2007, Rafferty & Ives 2012, Ballantyne, Baldock & Willmer 2015, Richman *et al*. 2020). Therefore, a key determinant in plant seed-set is not overall pollinator availability at different blooming phenologies but the relative abundance of different pollinator taxa at those phenologies and their contribution to pollination service.

Despite the strong theoretical basis for phenology shifts in plants and pollinators impacting on plant reproductive fitness, attributing variation in plant reproductive fitness purely to altered pollinator activity has challenges in open field settings. Plant reproduction is not purely driven by pollinator activity and is subject to several confounding factors that can themselves be attributed to phenological variation. In Parsche *et al*. (2011), plants with experimentally-advanced flowering received fewer pollinator visits but this did not result in a reduced seed-set, which the authors attributed to the advanced phenologies allowing a release from herbivorous pests. Phenology shifts also change the abiotic conditions experienced by plants, particularly in temperate species, with early phenologies often associated with

unfavourable conditions which may influence reproduction (Kudo & Cooper 2019). Within-plant investment in reproduction has been found to decline across the blooming season, with later flowers less likely to set seed even with comparable levels of pollination (Austen, Forrest & Weis 2014) and flowers commonly display both within-and between-plant variation in morphological characters such as colour and petal size which can influence attractiveness to pollinators (Williams & Conner 2001). When measuring reproductive success many pollination experiments account for seasonal variation in response by including control individuals for comparison, however few studies control for between-plant variation in response.

To determine to what extent altering the flowering phenology of a generalist plant, *Raphanus raphanistrum subsp. sativus var. caudatus,* could impact on its reproductive fitness, I aimed to quantify the effect of phenological variation measured through seed-set. I induced variation in timing of flowering to expose plants to both differing total numbers of pollinators and differing relative abundances of pollinator taxa and quantified how variation in these factors impacted both the likelihood of plants setting seed and the number of seeds produced. To achieve this I artificially induced flowering in plants to record seed-set produced at different times of the year, controlling for between-plant variation in response by including a positive control (hand-pollinated flowers) and negative control (pollinator-excluded flowers) alongside experimental flowers on each individual plant. I then reconstructed the seasonal phenology of pollinator abundance and tested it as a predictor of seed set in the studied flowers.

Methods

Study plant selection and growth

Cultivated radish (*Raphanus raphanistrum subsp. sativus var. caudatus*) is an annual root vegetable of the family Brassicaceae. As members of a self-incompatible genus, *Raphanus* species rely on insect pollinators for reproduction and are visited by a

broad range of pollinators including syrphid flies, butterflies, solitary and social bees (Conner & Rush 1996). The rat-tail variety of radish (*Raphanus raphanistrum subsp. sativus var. caudatus*) was selected for use in this experiment as the variety with the shortest flowering time (Charbonneau *et al.* 2018). It should be noted that *caudatus* is a cultivar of *Raphanus raphanistrum* and not all cultivars have been found to be fully self-incompatible like the wild species (Hawlader & Mian 1997). However levels of selfing have typically been very restricted and this behaviour has not been evidenced in *caudatus* specifically.

Commercially-bought *caudatus* seeds were sown in groups of 30 and grown in a greenhouse in Stirling. All plants used in the experiment were sown at 4-week intervals from 9 April 2019 to 8 September 2019 with the exception of one group, which were sown on 27 August 2018. Seeds were initially sown in seed tray cells (40mm wide x 55mm deep) filled with seed compost (Sinclair Seed Growing Medium, Lincoln, UK) before being re-potted into 3.5-litre plastic plant pots filled with commercial plant soil (John Innes No 2, Dungannon, Ireland). All seedlings were re-potted within 2 weeks of sowing, once first true leaves had grown.

All plants were assigned a unique alphanumeric identification and grown under controlled conditions to encourage short time to first bloom regardless of season. Conditions were maintained at artificial summer levels with temperature set to an average of 18°C (max 20°C, min 12°C) and supplemental lighting used for 14-hour days (6am - 8pm).

Pollination treatment

Plants typically flowered at a density sufficient to initiate field work (minimum 60 individual buds and/or blooms per plant) within 6 weeks of planting. Open flowers were removed from the plant 5 days before commencement of field work so that only newly-opened flowers were used in pollination treatments. Pollination treatments were assigned 1 day in advance of fieldwork and were applied to entire inflorescences. Inflorescences were ranked in order of number of flowers and then

sequentially assigned to one of three pollination treatment groups - *open-pollinated*, *hand-pollinated* or *excluded* - with sequence order randomised for each plant. I aimed to include 100 flowers treated with each pollination treatment type per experimental group but in some groups this was constrained by flower availability. Once all inflorescences had been assigned a pollination type coloured string was loosely tied to the base of the infloresence to represent treatment group and the relevant treatment was applied on the same day:

Excluded inflorescences were covered with light plastic mesh (ca. 2 mm mesh size) to exclude pollinator access to the flowers, secured at the base of the inflorescence with a light elastic band. Elastic bands were also attached to the base of inflorescences in other treatment types to allow for constant conditions across treatments.

Hand-pollinated inflorescences were left exposed for pollinator access but had supplementary pollination applied by hand. Supplementary pollination for each flower consisted of direct anther-to-stamen contact with two other flowers, each taken from separate donor plants. This treatment was applied once per day over two days, 1 day in advance of field work and on the first day of field work.

Open-pollinated inflorescences were left exposed for pollinator access and had no supplementary pollination performed.

Field setting

The day after pollination treatments were initiated, plants were taken to the field. Plants were placed outside at 0700 hours on the first day and remained outside for three days (the approximate blooming time of an individual flower) until being collected at 1900 hours on the third day and returned to the greenhouse. Timings of

placing outside and collection remained constant across experimental groups despite daylight hours varying across groups.

Experimental plants were placed 30 - 50 cm apart in a 3 x 3 m array. A group of 5 plants that had not undergone any pollination treatment were placed in a similar array 2 m away from the experimental group, both to act as pollen donors and to increase the floral display for pollinators. Direct pollinator observations were conducted for the three days during which the experimental group remained outdoors.

Pollinator abundance and diversity

Two methods of measuring pollinator abundance and diversity were employed: direct observation of visitors to the experimental plot and passive collection of specimens using pan traps. Passive collection allows for insect diversity and abundance to be tracked throughout the season at all hours of the day. Pan traps typically produce higher species coverage and detection rate than transect walks or netting (Westphal *et al.* 2008) but provide little information on which fauna visit a specific plant (Cane *et al.* 2000, Roulston, Smith & Brewster 2007), therefore trap collections were supplemented with direct observations of pollinators.

Pollinator observations

Direct pollinator observations were performed on each of the 3 days plants were in the field. On each day 8 observation windows were performed on consecutive hours, with an individual observation window lasting 15 minutes, for a total of 6 observation hours across the 3 days. Observations were characterised as 'morning' (8 windows starting on 0700 hours or sunrise, whichever came first), 'afternoon' (8 windows starting on 1000 hours) or 'evening' (8 windows counting back from 1900 hours or sunset, whichever came last). Each 3-day experiment contained a morning,

afternoon and evening observation session although which day these were performed on was randomised between experimental groups.

During a 15-minute observation window, 3 focal plants were observed for 5 minutes each. For each focal plant pollinator visitations were recorded. A visit was included if a pollinator visited a flower on the focal plant and made contact with the flower's anther, stigma or both. Number of visitations was recorded for each pollinator along with species identification. Field identifications were made with reference to field guides (Ball & Morris 2015, Falk & Lewington 2015) and were predominantly performed to genus or morphospecies level, although species-level identification was performed where possible. Voucher specimens were netted and stored in 80% ethanol solution for identification to species in the lab.

Pan traps

Pollinators were passively collected using pan traps throughout the entire field work period, regardless of whether an experimental group of plants was in the field. Pan traps were set up in the same plot in which experimental plant groups were placed and consisted of 40 plastic bowls (20.8 X 12.8 X 12.4 cm) arrayed within a 4 x 2 m area. Each bowl had a 129 cm^2 surface area and contained 300 ml water with 1 mm unscented washing detergent to break surface tension. As insects display considerable preferences towards colour of bowls, 20 non-fluorescent white bowls and 20 non-fluorescent yellow bowls were employed to maximise the diversity of insects trapped, as these two colours provide the highest species coverage (Saunders & Luck 2012). Pan trap contents were collected on a daily basis from Monday to Friday of each week, with pan traps completely emptied and refilled and collected specimens stored in 80% ethanol solution.

Collected specimens were identified under a microscope with several keys used in combination (Stubbs & Falk 1983, Williams 2012, Falk & Lewington 2015). A conservative approach was taken to identification, and where species-level detail

was uncertain this was noted and genus used for further analysis. Voucher specimens have been stored and retained at University of Stirling.

Seed development and measurement

After being collected from the field, plants were returned to the greenhouse where they were kept in constant conditions and left to develop seed pods. Seed pods were classed as fully developed when they had fully dried and seeds could be heard rattling inside the pod when shaken.

At full development, the number of developed seeds pods on each plant was counted, grouped by pollination treatment type and pods were removed from the plant. Each pod was then split using a scalpel and the number of individual seeds per pod counted and recorded. Seeds were then weighed and the mean weight of seed by plant and pollination treatment type calculated.

Statistical analyses

Pollinator abundance

Pollinator abundance was estimated using data from both constant, passive sampling (daily pan-trap catches over 156 continuous days) and irregular, active sampling (direct observation windows on 27 days when plant test groups were active). Pollinators were split into five taxonomic groups representative of different pollinator characteristics, primarily size (Sahili & Conner 2007, Rafferty & Ives 2011): (1) large Hymenoptera (9 morphospecies, all *Bombus*), (2) small, solitary Hymenoptera (9 morphospecies, primarily *Andrena* and *Halictus*), (3) Diptera (14 morphospecies, all Syrphidae), (4) Lepidoptera (5 morphospecies). The fifth group, (5) Apidae (1 species, *Apis mellifera*), was used to separate domesticated honeybees from wild pollinators.

A hierarchical generalised additive model (HGAM) was used to assess nonlinear trends in pollinator abundance across the 2019 season using the pan-trap catch data. Pollinator taxonomic groups were included as hierarchical groups within the same model, which used a thin plate spline smoother class (Pederson *et al.* 2019). For the purposes of modelling, days on which no pollinators were recorded from a given group were treated as a structural zero. The Lepidoptera taxonomic group was omitted from this analysis entirely as it was comprised of 14 counts over the 166 sample days and did not have adequate data to allow for HGAM fit. All HGAM fitting was performed using R version 3.6.2 (R Core Team) and the mgcv package (Wood 2011). The data contained a high number of zeroes and therefore several distribution families were fitted to account for this (Poisson, negative binomial and zero-inflated Poisson), with the superior candidate model selected through comparison of Akaike's information criteria (AIC). Knot (k) values were automatically selected through mgcv's functionality and model diagnostics checked to confirm that these values were adequate.

Seed-set models

Seed set was measured per flower as a proportional response. The highest seed count observed for each plant for hand-pollinated flowers was used as the measurement base for maximum seed set. Number of seeds per flower were then recorded as successes (1) and the difference between this value and the maximum seed set recorded as failures (0). If a flower did not produce a seed pod it was recorded as zero seeds produced.

To quantify the effect of pollination service on seed production I used zero-inflated binomial (ZIB) models, fitted using the package glmmTMB (Brooks *et al.* 2017). Using a ZIB allowed us to account for the high level of zero inflation in the seed set data while accounting for uncertainty in the cause of zero counts; flowers not setting seed could be explained either through the predictor variables (pollinator

abundance or service) or due to biological causes not captured in the measurements (e.g. resource allocation by plants).

In total two candidate models were fitted which all included seed number as the response variable, pollination treatment type as a categorical variable and plant ID as a random effect, but differed by their measurement of pollinator activity. Pollinator activity was variably measured as (1) abundance by pollinator group from direct observations; (2) abundance by pollinator group taken from fitted HGAM predictions.

Each full model initially included all higher-order terms and was simplified by stepwise comparison and dropping of nonsignificant higher-order terms, with models compared through AIC. All main effects were retained. Once all models had been simplified, candidate models were compared by AIC.

Predictions of seed-set

In order to see how the observed variation in pollinator phenology over the field season could affect seed-set, predictions of seed set were calculated using the measures of pollinator abundance derived from the fitted HGAM models.

Results

Pollinator Community Abundance and Composition Varies Over Time

Pan traps were collected on 117 of 156 calendar days through the period 5th May 2019 – 7th October 2019 with pollinators collected on 105 of those days, comprising 922 individuals across 41 morphospecies. *Bombus* were markedly the most abundant and most present group, with 557 specimens recorded over 89 days, while solitary bees were recorded 115 times over 51 days, *Apis mellifera* (*Apis*) 100 times over 61 days and Syrphidae 94 times over 62 days (Table 2.1).

Table 2.1: Breakdown of pan-trap catches by pollinator group over field season. Days present is taken from a total number of days in the field during which pan traps were present (117)

Pollinator group	Total	Days Present	Mean (Among Days Present)	Mean (Among All Days)
Bombus	557	89	6.3	3.6
Apis	100	61	1.6	0.6
Syrphidae	94	62	1.5	0.6
Solitary	115	51	2.3	0.7

Table 2.2 Breakdown of pollinator observations by pollinator group during field season. Days present is taken from a total number of 18 days during which pollinator observations were conducted

Pollinator group	Total	Days Present	Mean (Among Days Present)	Mean (Among All Days)
Bombus	38	9	4.2	2.1
Apis	29	14	2.1	1.6
Syrphidae	289	17	16.5	15.6
Solitary	7	2	3.5	1.4

The top-ranking HGAM used a Poisson distribution with a log link, although the shape and curvature of the fitted model were robust across variations of distribution family used (Figure 2.1). The pollinator community at the outset of the study period through May was comprised of solitary bee species (primarily *Andrena*) and *Apis,* with *Bombus* species largely absent until a rise in abundance to a peak in early summer (June), during which they were the most abundant pollinator group. *Apis* and Syrphidae groups remained relatively constant through much of the summer until a peak in solitary bee abundance in early autumn (start of September), after which all four pollinator groups consistently declined in abundance through to the end of the of the study period.

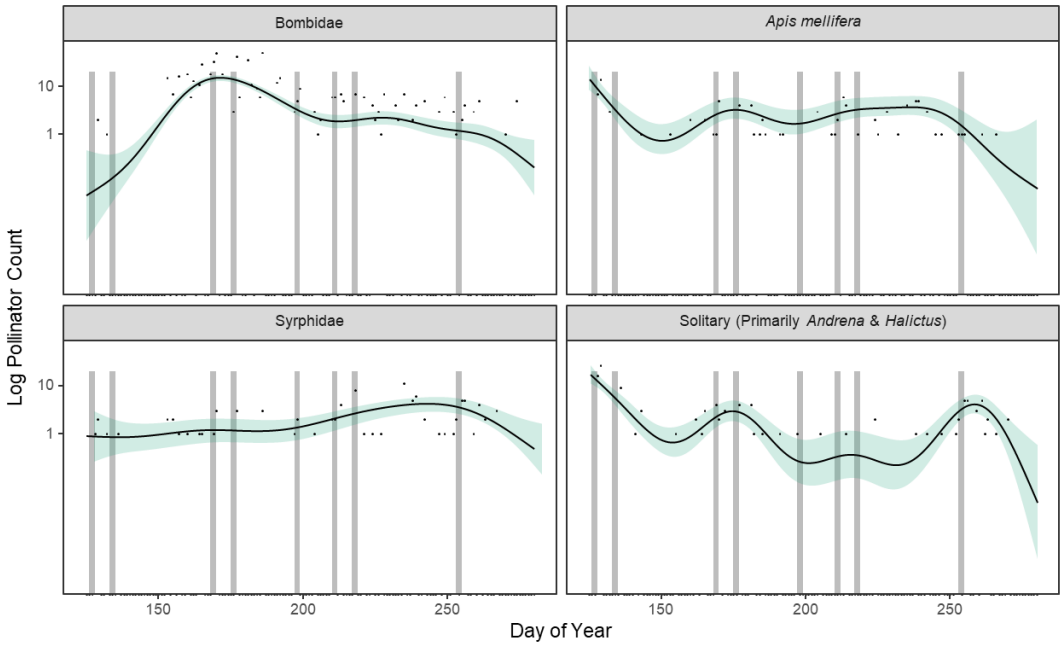

Figure 2.1: HGAM predictions of pollinator abundance with 95% confidence intervals by Julian day, split by the four pollinator groups used. Pollinator predicted abundance is presented, with raw data points for pan trap count on a log transformed y-axis. Shaded vertical bars represent the 3-day segments during which test groups of plants were in the field

Pollination Model Selection

In total 2,617 flowers were studied belonging to 36 individual plants, with 866 of these flowers producing seed pods yielding a total of 3,193 seeds (Table 2.3). The

top-ranked candidate model by AIC comparison used the HGAM-derived abundance per pollinator group as its predictors, having a lower AIC than models using observation-derived pollinator abundance (Table 2.4). Interaction terms were initially fitted to both the binomial and conditional elements of the ZIP models, but interaction effects were not significant for the binomial response and the simplified models excluded them.

Table 2.3: Summary data of experimental flower and seed populations

Treatment	Flowers	Seed pods	Total seeds	Mean seeds per pod
Excluded	837	20	39	2.0
Hand	869	505	2,063	4.1
Open	911	341	1,091	3.2

The following discussion focusses on the output of the model using HGAM-derived abundance for its measure of pollinator activity as this was the favoured model by AIC selection. However, all model outputs described a similar positive relationship between pollinator activity and seed production, though effect sizes and significance varied slightly between models.

Table 2.4: Comparison of simplified candidate models by AIC. Full model terms are split by the terms included in the conditional part of the zero-inflated model (modelling number of seeds produced) and the binomial part of the model (modelling whether seeds were produced or not). In the HGAM model pollinator group abundances were derived from the HGAM predictions of pollinator abundance. In the Observed model abundances were derived from directly observed pollinator abundance

Model type	AIC	dAIC	df	Conditional terms	Binomial
HGAM Abundance	2,332	0	16	Treatment * (Bombus + Apis) + Syrphidae + Solitary + 1|Plant ID	Treatment + Bombus + Apis + Syrphidae + Solitary + 1|Plant ID
Observed Abundance	2,344	12	22	Treatment * (Bombus + Apis + Syrphidae) + Solitary + 1|Plant ID	Treatment + Bombus + Apis + Syrphidae + Solitary + 1|Plant ID

Effect of Pollinator Activity on Seed Development

Open-pollinated flowers were significantly more likely to develop into seed pods (Figure 2.2) and developed a greater seed-set (Figure 2.3) than pollinator-excluded flowers, although the magnitude of these differences depended on variation in pollinator abundance.

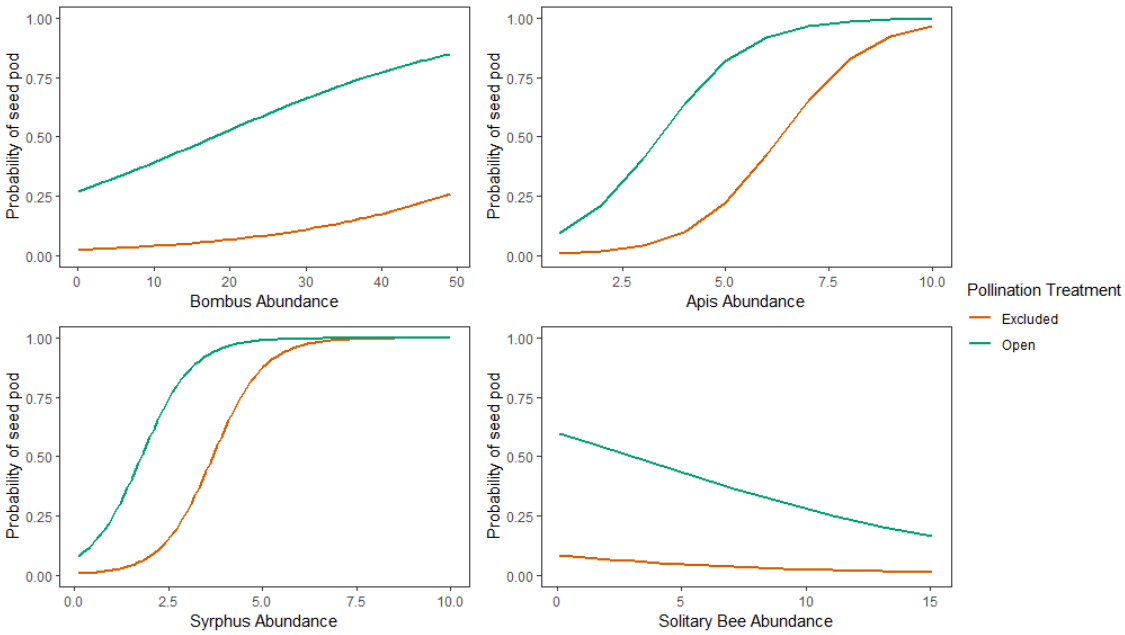

Figure 2.2: Zero-inflated mixed model binomial predictions for probability of seed pod (a single flower developing into a pod) by pollinator abundance and pollination treatment group, split by pollinator group; N = 1,748 flowers, (Excluded N = 837, Open N = 911, random effect levels = 36 plants). Hand-pollinated treatment group is not presented as measurement of seed-set is derived from it (see Methods – Seed set models)

Three of the four pollinator groups (*Bombus*, *Apis*, Syrphidae) exhibited a positive and significant association between their abundance and the probability of seed pods developing (Table 2.5). Solitary bee abundance had the inverse effect, showing a negative correlation with probability of seed pod development, although this effect was not significant. An exploratory model which looked at the effect of solitary bee abundance in isolation found a positive effect of increased abundance on seed pod development, so the negative association in the full model may be attributable to covariance with the presence of other pollinators.

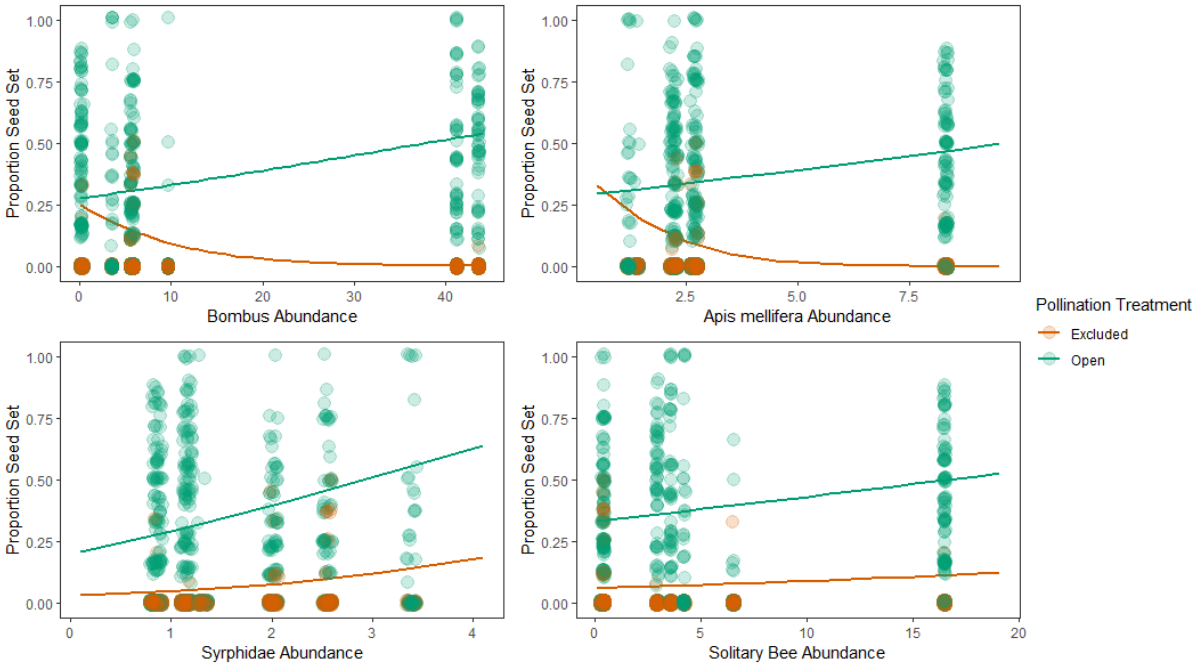

Figure 2.3: Zero-inflated model conditional predictions for seed set developed per flower against pollinator abundance, split by pollinator group plotted with raw data. N = 1,748 (Excluded N = 837, Open N = 911, random effect levels = 36 plants). Hand-pollinated treatment group is not presented as measurement of seed-set is derived from it (see Methods – Seed set models)

As expected, pollinator abundance increased seed set per-flower in the open-treated flowers but had negligible effects in the pollinator-excluded group (Figure 2.3, Table 2.5). In all four pollinator groups, increased abundance led to an increase in seed production per flower, this effect being highly significant for *Bombus* and *Apis*, and marginally significant for Syrphidae (Table 2.5). At high levels of pollinator abundance, the model predictions displayed a saturation effect, where predicted seed-set reached a plateau at 1.0, but this threshold was not reached at any of the HGAM-derived measures of pollinator abundance across the field season.

Table 2.5: Coefficient table for HGAM Abundance model, giving impact of pollination treatment type (Excluded or Open) and pollinator group (Apis, Bombus, Syphidae, Solitary) abundance on seed development. Fitted as a zero-inflated binomial using glmmTMB, see Table 2.4 for model terms

Predictors	Seed set		
	Log-Odds	*CI*	*p*
Count Model			
(Intercept – Treatment [Excluded])	0.25	-1.63 – 2.13	0.792
Apis GAMM	-0.74	-1.18 – -0.30	**0.001**
Bombus GAMM	-0.12	-0.17 – -0.06	**<0.001**
Treatment [Open]	-2.47	-3.68 – -1.26	**<0.001**
Solitary GAMM	0.04	-0.05 – 0.14	0.395
Syrphidae GAMM	0.47	0.01 – 0.94	**0.043**
Apis GAMM * Treatment [Open]	0.83	0.49 – 1.18	**<0.001**
Bombus GAMM * Treatment [Open]	0.14	0.09 – 0.20	**<0.001**
Zero-Inflated Model			
(Intercept – Treatment [Excluded])	8.58	5.83 – 11.33	**<0.001**
Apis GAMM	-0.95	-1.53 – -0.37	**0.001**
Bombus GAMM	-0.06	-0.09 – -0.02	**0.003**
Syrphidae GAMM	-1.46	-2.37 – -0.55	**0.002**
Treatment [Open]	-2.78	-3.39 – -2.16	**<0.001**
Solitary GAMM	0.13	-0.10 – 0.37	0.269
Random Effects			
σ^2	3.29		
$\tau_{00\ plantid}$	0.14		
ICC	0.04		
$N_{\ plantid}$	36		
Observations	1748		
Marginal R^2 / Conditional R^2	0.523 / 0.543		

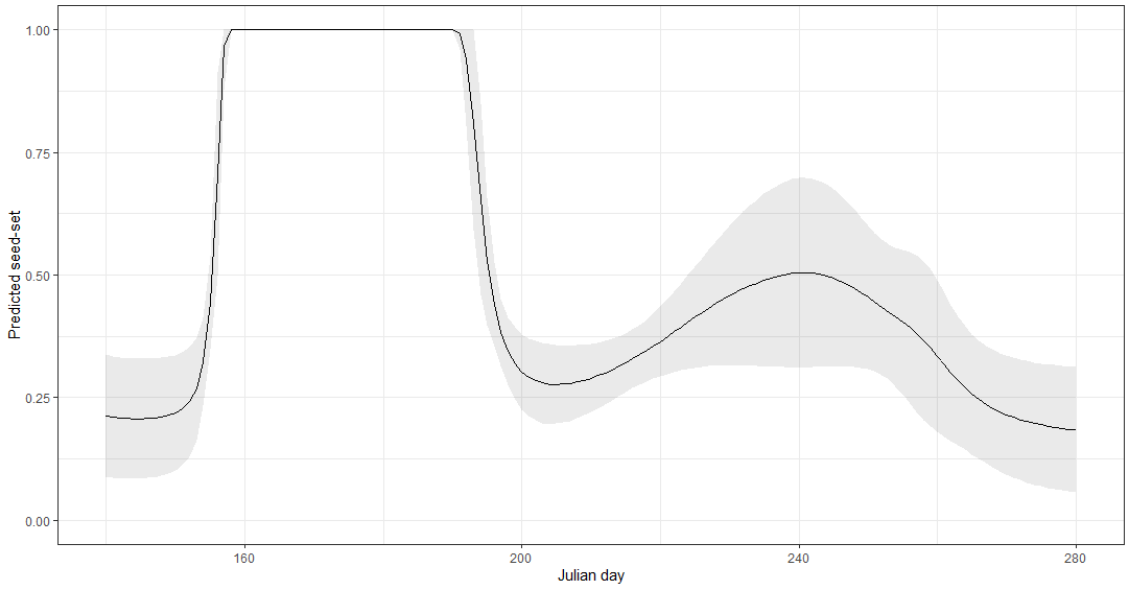

Figure 2.4: Model predictions of seed-set per flower across season, predicted using estimated pollinator abundances from HGAM models (see Figure 2.1). Random effect was set to null for model predictions, grey band represents 95% confidence intervals. The predictions are the product of both the binomial and continuous parts of the model, so predicted seed-set represents the combined likelihood of a flower fruiting and the expected seed-set from that fruit.

Impact of pollinator phenology on seed-set

Using predictions from the reported model (Table 2.5) I constructed a speculative picture of how the observed variation in pollinator phenology may impact on plant seed-set (Figure 2.4). Model predictions show that there is a high level of variance in predicted seed-set throughout the field season (Julian day 130 – 280), with an early peak of maximum seed-set lasting from day 125 – 193, is driven by high Bombus abundance. After day 193 there is a sharp decline in predicted seed-set from 100% to 30%, with this drop-off taking place over 20 days. This indicates that variance in flowering phenology of even a few days either side of this peak could have a high level of impact on plant reproductive fitness. There is a late-season rise to a peak of 50% seed-set at day 241, primarily driven by a rise in abundance of solitary bee species and Syrphidae at that time. After this late-season peak predicted seed-set declines. These predictions are overstated as they do not take account of random effect levels, which would have significantly decreased predicted seed-set for individual plants, but nevertheless illustrate how natural variation in pollinator abundance and diversity can potentially affect seed-set over the course of the year.

Discussion

Our results show that, even in a generalist plant, natural phenological variation in the abundance and diversity of distinct pollinator groups can affect plant reproduction. Through the inclusion of positive and negative controls on individual plants, I reduced the impact of seasonal variation in plant seed set due to biotic and abiotic variables in order to draw out the partial effect of pollinators on seed set. This revealed that the abundance of pollinators affects both seed pod development and seed set per pod, with the effect varying across pollinator groups.

Pollinator community composition varied across the field season as the abundance of pollinator groups fluctuated, with these fluctuations in pollinator activity having a discernible impact on plant reproductive effort. Over the course of the field season, model predictions showed large variance in seed set depending on the abundance and type of pollinator present, with seed set predictions varying from 25% to 100%. These results indicate that changes in blooming time of even a few days have the potential to cause a significant increase or reduction in seed-set depending on pollinator phenology.

Pollinator impact on seed set

The relative performance of the pollination treatment groups confirmed *Raphanus* as a pollen-limited plant, with insect pollination the controlling factor in seed-set for flowers that had not been supplemented by hand-pollination. At low levels of pollinator abundance, open flowers performed comparably to flowers which had pollinators excluded, but as pollinator abundance increased open flowers produced seed sets that matched those of hand-pollinated plants. Overall, model predictions indicated that plants are exposed to substantial variation in pollination service throughout the field with seed-set predictions varying by up to 75% depending on the time of year. It is not expected that these model predictions directly map to observed seed-set but they are nevertheless strongly indicative of large levels of variation in pollination service over time.

While all pollinator group abundances had a positive relationship with seed set, the contribution of different pollinator groups was not equal. *Bombus* and *Apis* were the groups which exhibited a significant trend and had the strongest impact on seed-set. However the finding that *Bombus* and *Apis* contribute more to pollination service than small, solitary bees or syrphids is consistent with previous pollination research in both generalist plant species (e.g. Ballantyne *et al.* 2017, Jacobs *et al.* 2009) and specifically with *Raphanus* species (Sahli & Conner 2007) which have measured deposition rates.

Measuring the contribution of pollinators to seed set can be confounded by other seasonal variables. When measuring the impact of experimental phenological shifts on seed set, release from herbivore pests has been found to offset reduced pollinator presence (Parsche, Frund & Tscharntke 2011) and individual plants can vary their level of investment in reproductive effort across a season regardless of pollinator activity (Obeso 2002, Brookes, Jesson & Burd 2010). By applying all three pollination treatments to each plant used in the research, I have controlled for individual-level variation in reproduction but are still subject to seasonal variation in pollinator efficacy (Gallagher & Campbell 2020). A primary driver of intraseasonal variation in pollinator efficacy in phenological manipulations is the abundance of conspecific blooms, variation in which affects the level of viable pollen carried by pollinator and therefore efficacy. In the experimental setting number of conspecifics should be limited to those in the test population as wild *Raphanus* does not grow at the test site. I controlled for conspecific presence by including the number of blooms as an explanatory variable in initial modelling, but as it exhibited only a minor, nonsignificant positive correlation with seed set, it was removed during model simplification.

Implications for climate-driven mismatch

These results link pollinator abundance with plant seed set, indicating that climate-induced disruption of pollinator presence, abundance or species composition can affect plant reproductive effort. Complete decoupling of specific plant-pollinator interactions was a hypothesised outcome of phenology shifts (Memmott 2007) but a growing body of work suggests that total mismatch is unlikely to occur (Burkle, Marlin & Knight 2013) and in many cases phenological shifts of plants and pollinators are broadly synchronous (Bartomeous *et al.* 2011, Benadi *et al.* 2014, Sevenello, Sargent & Forrest 2020). As long-term studies often focus on dates of phenological events, the degree to which plant blooming time is synchronised with a specific level of pollinator activity, rather than co-occurrence, is unclear in many cases. My findings suggest that even if temporal co-occurrence of interaction partners is maintained, plant reproduction can still be impacted if the level of pollination service it is exposed to is reduced from historic levels. Therefore co-occurrence alone does not determine seed set, but also the abundance and efficacy of pollinators a plant is exposed to.

Climate change is also predicted to alter species makeup of pollination networks, whether through precipitating temporal uncoupling (Burkle, Marlin & Knight 2013) or from climate-associated range shifts (Rafferty 2017). Many of the interactions in pollination networks are known to be generalist (Bascompte *et al.* 2003) with rapid turnover of interactions (CaraDonna *et al.* 2017). The changes to pollinator assemblages and loss of interactions caused by climate effects are therefore often portrayed as reducing redundancy or robustness of networks (Revila *et al.* 2015, Duchenne *et al.* 2020). While this is undoubtedly true, my research findings, along with others (e.g. Richman *et al.* 2020), indicate that connections within these networks are not equal. Therefore, climatic changes that affect certain pollinator groups more acutely than others could have disproportionate effects on the functioning of pollination networks.

Pollinator abundance

Pollination research typically emphasises measurement of pollinator activity through direct observation of flower visitors (e.g. Rafferty & Ives 2011, Kehrberger & Holzschuh 2019, Gallagher & Campbell 2020) or transect surveys (e.g. Kudo & Cooper 2019, Richman *et al.* 2020). These approaches tend to be preferred over flower-independent measures of pollinator abundance such as pan-trap data, as local abundance does not necessarily correlate with flower visitation rate (Herrera 1989). Despite this, measures of pollinator abundance can be effective in explaining plant seed-set (Woodcock *et al.* 2019) and pan-trap data is less subject to diurnal variation in pollinator activity and climatic effects than data collected during observation windows. Data from passive trapping therefore lends itself to consistent long-term sampling of pollinator presence which is preferable when trying to capture information about pollinator phenology.

There was disparity between pan-trap collections and visitation data, with bias toward certain pollinator groups a known feature of pan-trap collecting (Popic, Davila & Wardle 2013). Notably, Syrphidae abundance, but not diversity, was underrepresented in pan-trap data compared with visitation. Despite this, *Bombus* and *Apis* were found to be the significant pollinator groups for seed set across models whether using pan-trap or visitation data, indicating that importance of pollinator groups is robust to the measurement of activity used.

Conclusion

Our results show that seasonal variation in the local abundance of pollinators positively covaries with both seed-pod production and seed-set produced in a generalist plant species. As interspecific variation in phenological responses to climate change expose plants to novel communities of pollinators, or blooming at times of year where pollinator abundance is lower, my findings demonstrate that such changes could have dramatic demographic impacts, with model predictions estimating variance of up to 75% of seed-set throughout the season. As the contribution to seed production varies among pollinator groups, these results also highlight the potential risk to pollination service provided by the predicted restructuring of pollination networks subject to phenology shifts.

Chapter Three
Patterns of phenology shift in UK Syrphidae

Abstract

Insect taxa are undergoing widespread phenology shifts as a result of temperature rises due to climate change, but there is marked interspecific variation in the magnitude and direction of responses. Previous studies have sought to explain this variation by tying phenological response to species life history traits, but these studies have been taxonomically restricted, primarily focussing on Lepidoptera. Here I apply these methods to a different group of pollinators, Syrphidae, using records from the UK-based Hoverfly Recording Scheme. Combining this dataset with geographic and climate records, I estimated both phenology shifts and changes in voltinism for 103 species from 1980 onwards. I then combined these data with a database of Syrphidae life history traits to assess the impact of life history traits on phenological sensitivity. I found that UK Syrphidae are advancing their date of emergence and peak flight date as temperatures increase, but that the final dates of flight activity have no significant relationship with temperature changes. I found little evidence that life history traits are predictive of phenological response, although species which overwinter as adults displayed a higher rate of phenology shift than species which did not. I found no evidence of increased voltinism as a result of temperature increases. Species which emerged earlier in the year displayed a considerably higher rate of phenological response than species which emerged later. These results show that Syrphidae are undergoing similar trends in phenology shift to other pollinator groups but challenge the generality of previously links between life history traits and phenological response in pollinators.

Introduction

Changes in the phenology of organisms - the timing of periodic biological phenomena such as migration or breeding – are some of the best-documented biological responses to the rising temperatures caused by climate change (Fitter & Fitter 2002, Parmesan & Yohe 2003, Gordo & Sanz 2005, Duchenne et al 2020). Across taxa there has been an overall advance in the Julian date of phenological events in temperate species (Parmesan & Yohe 2003, Parmesan 2007, Thackeray et al 2010), but increasingly research focusses on interspecific variation in the direction and magnitude of phenological response and the drivers of this variation (Thackeray et al 2016, Chmura et a 2019, Duchenne et al 2020).

Interspecific variation in phenological sensitivity is a key driver of potential temporal mismatches arising in populations of interacting species (Visser & Both 2005, Kharouba et al 2018, Damien & Tougeron 2019). Mismatches can have demographic impacts on species as peak activity periods desynchronise with peak resource availability (Burkle, Marlin & Knight 2013, Renner & Zohner 2018) and can restructure species assemblages over time (Duchenne et al 2020). Improving understanding of traits that can predict variation in phenological response is therefore key to understanding which species and ecosystems are most susceptible to emerging mismatches.

Insects are frequently used as study systems for assessing both phenology shifts and their mechanisms for several reasons. As short-lived ectotherms, they are generally highly responsive to variation in temperature (Bennet et al 2015, Forrest 2016, but see Ellwood et al 2012) but also show significant interspecific variation in these responses (Duchenne et al 2020). Extensive long-term records of occurrence of specific insect taxa exist, particularly in North American and Europe, due to the prevalence of recording schemes (Altermatt 2010, Hassall, Owen & Gilbert 2017) and museum collections (Roy & Sparks 2000, Bartomeus et al 2011), allowing for analysis of long-term phenological trends. Finally, the importance of many insect taxa as pollinators (Ollerton, Winfree & Tarrant 2011), as crop pests (Sutherst et al 2011) and as food sources (Damien & Tougeron 2019) mean that potential phenological mismatches could have repercussions for ecosystem functioning (Hegland et al 2009).

The mechanisms that underlie variation in phenology can be divided into two main categories (Chmura et al 2019). Environmental mechanisms are spatial or temporal measures that covary with the abiotic cues used for initiation or cessation of phenological events; for example, organisms in higher-latitude temperate environments often exhibit greater degrees of phenology shift than those at lower-latitude as they are subject to greater variation in temperature (Parmesan 2007). Organismal mechanisms are the species traits that predict phenological responses to these abiotic cues; for example, European butterflies which emerge earlier in the year exhibit greater phenological sensitivity to temperature cues than those which emerge later in the year (Fric, Rindos & Konvicka 2020).

Studies of insect taxa have highlighted several possible traits or organismal mechanisms that can be used to predict phenological response. Species which overwinter at an advanced development stage typically exhibit a greater rate of phenological advancement (Diamond et al 2011, Kharouba et al 2014), presumably as they have a less time until eclosion (or have already undergone eclosion) and are thus more able to plastically respond to favourable conditions for emergence. Larval diet and oviposition site also appear to influence phenological sensitivity, with generalist species typically less sensitive to temperature shifts and therefore less likely to advance emergence phenology in response to temperature rises (Altermatt 2010, Diamond et al 2011, Cayton et al 2015). Emergence date itself affects phenological sensitivity as early-flying species seem to follow temperature cues more tightly than late-flying species, which may follow other environmental cues such as photoperiod (Forrest 2016), with the result that early species display greater rates of shift (Pau et al 2011).

While studies have identified traits that can help predict phenological response, the majority of these studies are conducted on a small number of insect taxa (primarily Lepidoptera, with a minority focussing on Hymentoptera (see Stemkovski et al 2020)). To determine whether these traits elicit similar phenological responses in another insect taxon I used a long-running citizen science dataset, the UK Hoverfly Recording Scheme (HRS), to describe and analyse phenology shifts in UK Syrphidae. Hoverflies are an important pollinator group in temperate and arctic environments (Doyle et al 2020) that

exhibit substantial interspecific variation in traits such as larval diet, habitat, migratory behaviour and overwintering stage. Using data from the HRS, I used the occurrence data to extract estimates of voltinism and then assessed the rate of phenology shift at start, peak and end of the flight period. I hypothesised that the following life history traits would affect response:

Adult body length: Insect size is often inversely associated with voltinism; larger body sizes require a longer development time from egg to adult (Buckley et al 2017; Cizek, Frik & Koncika 2006) and therefore limits the number of potential generations within a flight season. As a result, species with longer body lengths, or larger wingspans, are less likely to display multivoltinism (Zeuss, Brunzel & Brandl 2017; Cizek, Frik & Koncika 2006). Hoverflies with longer body lengths were therefore expected to be less likely to respond to warming temperatures with increased voltinism.

Overwintering phase: A key limiting factor on adult emergence is the length of the development phases from egg to adult. Species which overwinter in later life-cycle stages, and particularly those that overwinter as adults, have a reduced development period to undergo before being able to emerge as an adult (Diamond et al 2011, Kharouba et al 2014, Chmura et al 2019). They are therefore expected to show greater sensitivity to, and therefore exhibit a greater rate of phenological shift in response to, variation in temperature.

Larval habitat breadth: In insects, flight phenologies should align with the phenologies of larval habitat and food resource availability (Forrest & Miller-Rushing 2010, Kharouba & Wolkovich 2020). In butterflies, species with a broader range of larval host plants exhibit more plastic phenologies than those with a restricted host range (Altermatt 2010). Whether habitat breadth similarly covaries with phenological plasticity in hoverflies is uncertain; in Lepidoptera, plants used for egg-laying typically constitute the larval food source and are therefore highly indicative of larval specificity. To test this in Syrphidae, I summed the number of habitats each species was associated with for egg-laying as a measure of larval habitat breadth. The relationship between hoverfly larval habitat and diet specialisation is less direct than in Lepidoptera, but it is reasonable to hypothesise

that species with a wider range of suitable larval habitats are less resource-restricted and therefore may exhibit greater phenological plasticity than those with a limited range of larval habitats.

Migratory status: Several hoverfly species are known to migrate to the UK from continental Europe, although these typically also have a permanent domestic population or breed in the UK within their flight season (Wotton et al 2019). While UK temperatures generally co-vary with those on continental Europe, the relationship is not exact. The phenology of migratory species is therefore predicted to be less sensitive to the UK temperature measures used in this study.

Methods

Data Set

The UK Hoverfly Recording Scheme (HRS) is a citizen-science recording effort initiated in 1960 that comprised 1,072,472 records of 292 hoverfly species at the date of access (15/10/2018). Data are collected in the form of ad-hoc records of hoverfly sightings contributed by members of the public. Historically these records were predominantly obtained through specimen-collection, while in the past two decades, particularly since the inception of the UK Hoverfly Facebook site in 2013, records are overwhelmingly from photographs. The basic information in each record is a British National Grid (BNG) location, a species name and the recording date; while additional data fields are present in the dataset, they were not used for the purposes of this analysis. Each record is converted into correct formatting and goes through a series of checks by one of a small group of experienced recorders before being added to the HRS data base. Species identification is confirmed against the known UK species list and, if possible, through a direct check of photographic evidence or specimen (if available); the date of the sighting is checked against historic phenology of the species; and grid reference is sense-checked against BNG.

Sparse recording can lead to unreliable estimations of population-level events as greater weight is given to individual records, which may be outliers or the result of a single recorder's activity. Therefore, to reliably estimate within-year adult flight phenology, in-scope data were restricted to years in which a given species had 50 or more records. This meant that data was effectively restricted to records from 1980 onwards due to the low number of records per annum before that year. To better identify interannual trends, data were also restricted to species that had at least 3 separate years of records to allow for sufficient comparisons between years. As phenology can also vary with latitude, the geographic area from which the records were drawn was also restricted to those occurring below a latitude of 53° North. After these restrictions, the dataset used comprised 623,726 records of 128 species. For each species in each year an emergence date (5^{th}-percentile of records) and end date (95^{th}-percentile of records) was calculated, with percentiles being used to reduce the weight of outlying data points.

Estimating voltinism

Kernel density estimates of the Julian recording days for each species/year combination were performed using R version 4.02 (R Development Core Team 2008), to extrapolate record information in order to produce a smoothed estimate of species abundance within each year (Figure 3.1). I then extracted peak density and the Julian day of peaks for each mode within each abundance estimate, with each mode taken to represent a separate period of voltinism.

Kernel density estimation is a non-parametric method of estimating a probability density function for a variable. It requires selection of a bandwidth relating to the x-axis variable from within which data points are considered relevant to density estimates - the specified bandwidth governs the distance of datapoints across which probability estimates are derived. Lower bandwidths therefore result in reduced smoothing, and resultingly higher numbers of modes. Previous studies using these methods to estimate voltinism in Lepidoptera have used default bandwidth settings in R (Altermatt 2010) and found these sufficient. However, I found that R's default bandwidth selection led to high numbers of modes being identified for Syrphidae species relative to known levels of voltinism (for example, over 40 modes estimated in some species). In order to control for the overestimation of modes, bandwidth was directly constrained for each species to a biologically-relevant range, defined as the lowest estimate of development time (in days) from egg to adult for each species. I reasoned that fluctuation in observed abundance of individuals within this timeframe is likely to represent variation in activity from a single generation (generated by staggered emergence or response to weather conditions) while observations outside of this timeframe might represent distinct generations.

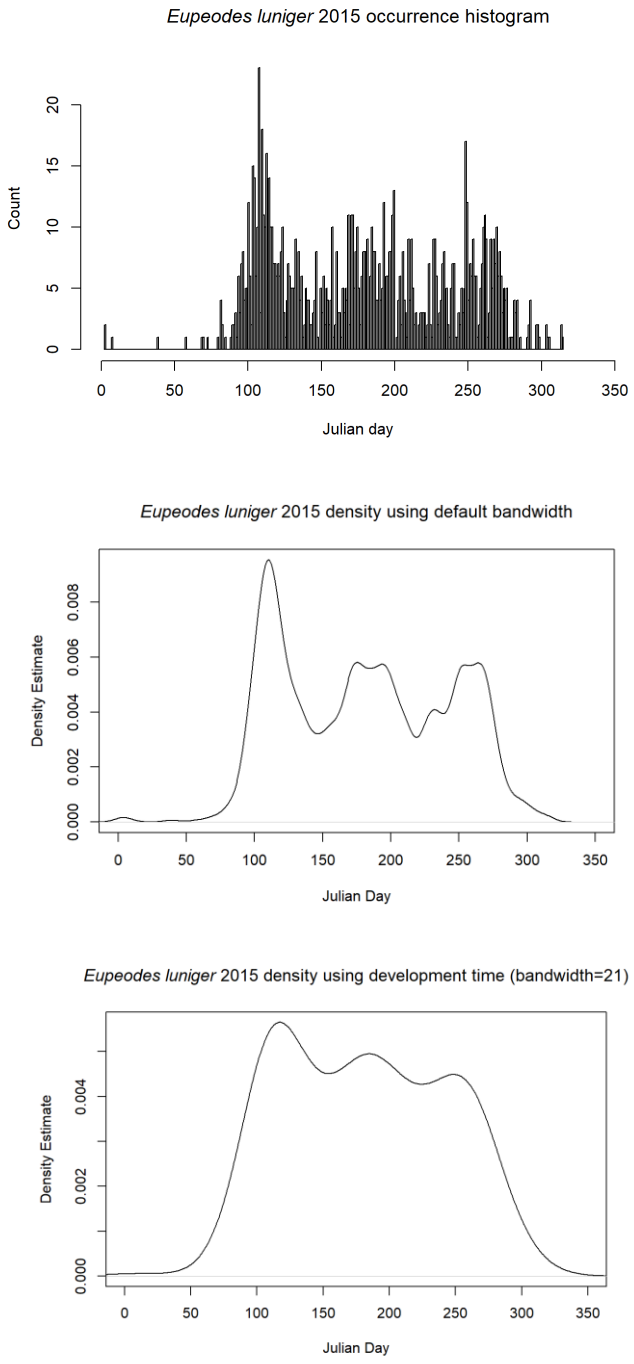

Figure 3.1: Examples of bandwidth selection and mode estimation using data from a single year/species combination (Eupeodes luniger 2015). From top to bottom: Occurrence histogram of species records in 2015, showing raw (N = 1,109); Density plot using default bandwidth estimation identifying 8 modes; Density plot using species development time as bandwidth (21), identifying 3 modes. The *bottom plot illustrates the final* method used

I obtained Syrphidae development times from Hassall, Owen & Gilbert (2017), which included data from 153 studies on development within the family. This dataset was incomplete on a species level, with 37 species out of 110 having complete development

data from egg to adult and the remaining 73 having partial information on any combination of egg, larva or pupa development stages. To compensate for the missing data, I calculated the mean duration of each development stage at different levels of classification – genus, tribe, subfamily and family. If an individual species was missing development information at a certain stage of development, the mean duration for that phase was taken from the lowest taxonomic level from which that information was available. By imputing partial species-level development data with means for their taxonomic groups, an estimate of total development time for all species was calculated and used for bandwidth (see Figure 3.1).

In order to estimate of the number of periods of voltinism for each species, I then extracted the number of modes per species per year from the density plots. Density estimates of each modal peak were also extracted from these plots, and the highest density peak for each species within a year was used as its peak abundance date for that year.

Temperature Measurements

Local temperature measurements were extracted from the Met Office's HadUK-Grid datasheets, a resource that interpolates weather station readings to provide daily weather readings at a spatial resolution of 1x1km across the UK (Hollis *et al* 2019). These readings were matched to the restricted geographic area from which I took hoverfly recording scheme records, but this was not done on a gridded basis.

Annual mean temperature (°C) is frequently used as a predictive measure of phenological response (e.g Altermatt 2010, Hassall, Owen & Gilbert 2017). However, one consequence of using annual means is that this measure encompasses a wide range of time including the temperature of periods that had no causal effect on the phenology of earlier events within the year. For example, if a species emerges in March, a mean annual temperature will include readings from April – December even though these can have had no causative effect on emergence date.

Therefore, for each year, I considered only temperatures occurring until the mean date of the phenological event being analysed (emergence date, peak abundance date, end date). To take the example of emergence date, the mean annual emergence date for each hoverfly species was calculated. The average temperature up to this mean date was then used as the temperature measurement for analysis of emergence date. The same approach was used to compute the mean temperature to peak abundance date and for mean temperature to end date.

Life history trait selection

Life history traits were taken from the Syrph the Net (StN) database (Speight 2019) of European hoverfly traits compiled from species accounts, reference books and personal communication from recorders (Table 3.1). Adult body length was taken as mean of the minimum and maximum size stated for each species (mm).

Table 3.1: Summary of selected life-history traits for species in the Hoverfly Recording Scheme (HRS) as extracted from Syrph the Net (Speight 2019)

Trait type	Subcategory	Species Count
Larval food source	Commensal	4
Larval food source	Living animals	56
Larval food source	Living plants	17
Larval food source	Micro-organisms	34
Larval food source	Saproxylic	12
Larval food source	Unknown	5
		128
Larval macrohabitat	Aquatic	20
Larval macrohabitat	Terrestrial	103
Larval macrohabitat	Unknown	5
		128
Migratory	Migratory	25
Migratory	Non-migratory	98
Migratory	Unknown	5
		128
Overwintering stage	Adult	4
Overwintering stage	Larva	99
Overwintering stage	Pupa	19
Overwintering stage	Unknown	6
		128
Larval microhabitat	Non-seasonal	3
Larval microhabitat	Seasonal	120
Larval microhabitat	Unknown	5
		128
Overwintering habitat	Earth	1
Overwintering habitat	Emergent plants	14
Overwintering habitat	Humus	34
Overwintering habitat	Nests	1
Overwintering habitat	Roots	40
Overwintering habitat	Water bodies	25
Overwintering habitat	Unknown	13
		128

Model fitting

Initial models were fitted as mixed models using lme4 (Bates et al 2015). Response variables were 5th-percentile emergence date (Julian day), 95th-percentile end date (Julian day) or date of peak abundance (Julian day). Maximal models included mean temperature (continuous) as a main effect. Visualisation of preliminary model-fitting indicated that

there was species-level variation in both the strength and direction of phenological responses to temperature so this interaction term was retained across all models. Species was fitted as a random effect crossed with the main effect of temperature to allow species-level variation in both intercepts and slopes. However in the models for peak abundance and 95th – percentile date this term constrained model fit and so the higher-order interaction was removed. Life history traits included as main effects were species overwintering stage (categorical with 3 levels), migratory status (categorical with 3 levels) and larval habitat breadth (continuous). All were fitted with interaction terms with mean temperature.

The voltinism model was fitted with separate predictors as these life-history traits were not hypothesised to affect voltinism. Voltinism was modelled as a binomial response (either univoltine, 0, or bi/multivoltine, 1) with mean annual temperature and hoverfly body length (mm) as main effects with an interaction term. Species was included as a random effect.

Seasonal sensitivity

A common feature of phenology shifts is that species which emerge earlier in the year display greater phenological sensitivity than those which emerge later (Forrest 2016, Fric, Rindos & Konvicka 2020). To test this for the HRS species, an estimate of phenological sensitivity was calculated by generating emergence date predictions using the final emergence model and the minimum and maximum temperatures across the timeframe of the dataset (3.99 ºC to 8.41 ºC). Phenological sensitivity was then defined as the difference between the emergence date predictions (in Julian days) at maximum and minimum observed temperatures. Species-level phenological sensitivity was then used as the response in a linear mixed model, fitted using lme4 (Bates et al 2015), to species-level mean emergence date.

Results

Temperature trends across study period

Consistent with global trends, annual mean temperatures (°C) in the study area increased over time (annual increase = 0.0259 ± 0.0002, p <0.001) (Figure 3.2). Annual mean temperature was not the predictor used in any of the phenology models as temperature was tied to phenology dates (see Methods – Temperature Measurements). However, the measures of temperature used were strongly correlated with annual temperature. Therefore annual temperature trends are strongly indicative of temperature trends in the models and Figure 3.2 provides a summary of these trends over time.

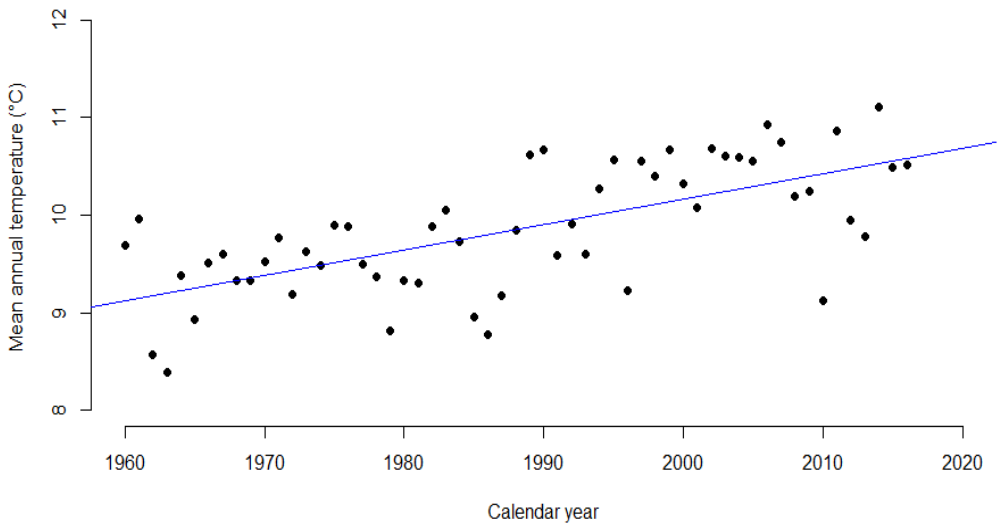

Figure 3.2: UK mean annual temperature (°C), constrained to UK areas below a latitude of 53° North, against calendar year (N=67) with fitted linear response line. 1960 was selected as the start date as this is when the first data was collected for the Hoverfly Recording Scheme, although only data from 1980 onwards were used in modelling (see Methods – Data Set)

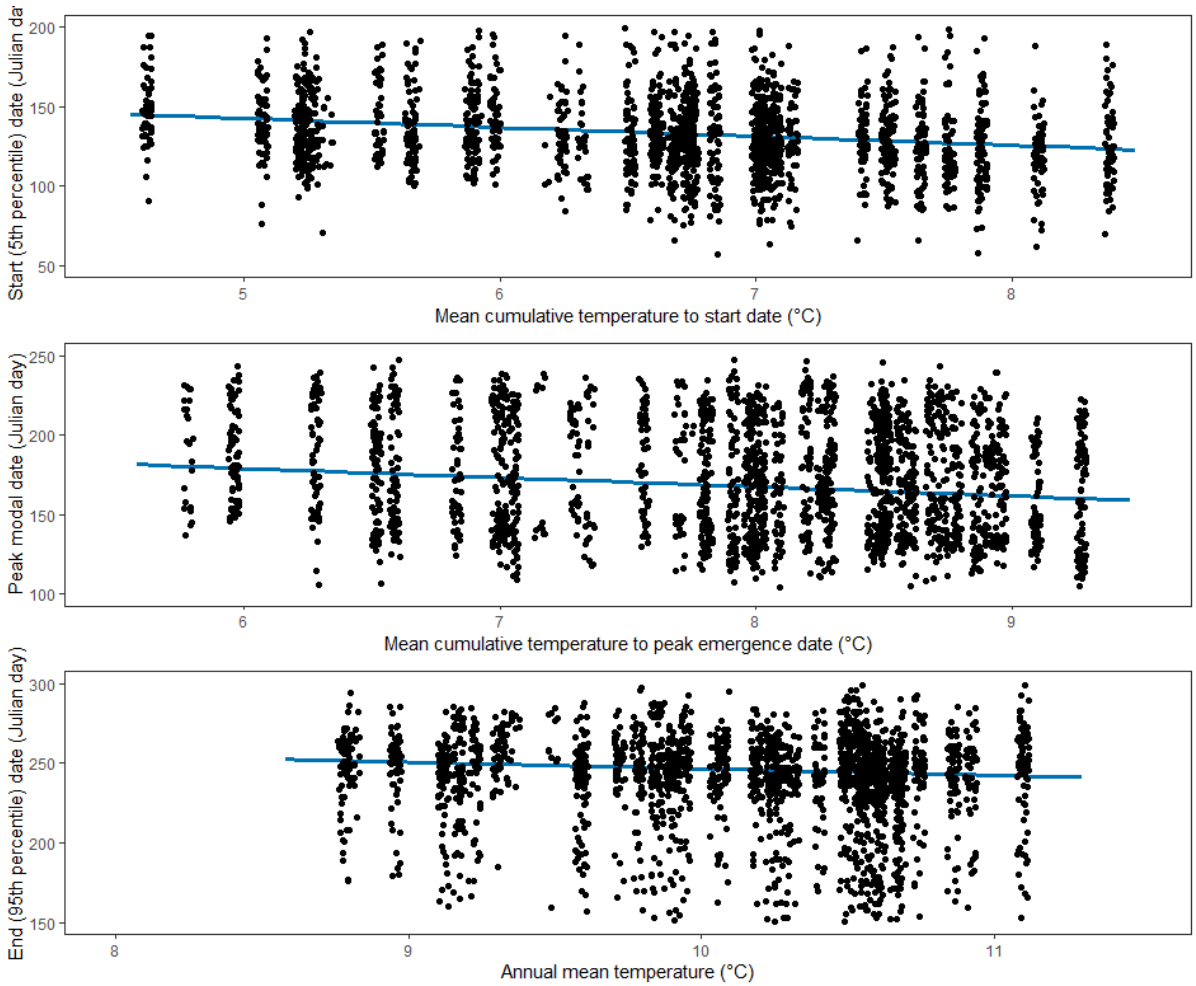

Figure 3.3: Marginal effect of temperature (°C) on each of the three measures of phenology (from top-bottom 5th-percentile, peak mode and 95th-percentile dates). When generating predictions for the fitted line, main effects apart from temperature are fixed to their most frequent levels in the dataset – overwinter state was set to larva, habitat breadth to dataset mean (3.7) and migratory status to non-migratory. N = 2475.

Life-history trait effect on phenology shift

Across the HRS dataset the most common life history traits were larval overwintering stage and non-migratory species – the relationship between these stages and temperature are shown in Figure 3.3.

For 5th – percentile emergence date, the effect of mean temperature on emergence date was dependent on species overwintering stage with the interaction term significant. Species that overwintered as adults advanced their emergence date at the greatest rate in response to temperature (-14.7 days $^{o}C^{-1}$, X^2 >0.001), with both species overwintering as

larvae (-4.91 days ºC^{-1}, X^2 >0.001) and as pupae (-4.94 days ºC^{-1}, X^2 >0.001) advancing at notably slower rates (Table 3.2, Figure 3.4). However, all three stages exhibited a strong negative relationship between emergence date and temperature. Non-migratory species and species with broader larval habitats exhibited slower rates of phenology shift than migratory species and species with restricted habitats, but neither the interaction terms nor the main effects for these life history traits were significant. *Eupeodes luniger* advanced its 5th-percentile at the fastest rate (-27.06 days ºC^{-1}) while *Platycheirus pelatus* delayed its emergence at the highest rate (3.36 days ºC^{-1}).

Peak abundance date generally advanced in response to temperature, although at a slower rate than emergence date. Species which overwintered as adults again showed advances at the greatest rate (-7.24 days ºC^{-1}, p = 0.011). Following the trends observed in model for emergence date, species which overwintered as larvae (-4.02 days ºC^{-1}, X^2 = 0.450) and as pupae (-4.24 days ºC^{-1}, X^2 = 0.450) showed lower rates of shift although this interaction was not significant. Neither migratory status or habitat breadth were significant predictors of phenology response, either as main effects or their interaction terms. *Rhingia campestris* advanced its peak emergence at the greatest rate (-28.29 days ºC^{-1}) while *Helophilus hybridus* delayed its peak emergence (1.27 days ºC^{-1}).

95th- percentile end date showed no significant relationship with temperature and none of the life-history traits used in the model (overwintering stage, migratory status or habitat breadth) had any significant relationship with phenological response of end date (Table 3.2). *Leucozona lucorum* advanced its 95th-percentile date at the greatest rate (-8.90 days ºC^{-1}) while *Syrphus torvus* greatly delayed its 95-th percentile date in response to temperate (16.45 days ºC^{-1}).

Table 3.2: Output from final models fitted for 5th–percentile, peak and 95th–percentile emergence dates. The Mean Temperature measure used was different for all three models as it was a cumulative mean of temperature tied to the date of the response variable being tested (see 'Methods - Temperature measurements' for full explanation). Intercept levels were Adult for Overwintering Stage and Migratory for Migratory Status. The main effect structure was constant across all three models with Species as a random effect. The 5th–percentile model included a crossed random effect term between Species and Mean Temperature which was *not fitted for the* peak and 95th–percentile models *(see Methods)*. Full model structure for each model was as follows:

- 5th-percentile emergence ~ Mean temperature * (Overwinter + Habitat breadth + Migratory) + (1 + Mean temperature | Species)
- Peak emergence ~ Mean temperature * (Overwinter + Habitat breadth + Migratory) + (1 | Species)
- 95th-percentile emergence ~ Mean temperature * (Overwinter + Habitat breadth + Migratory) + (1 | Species)

Predictors	Response variable								
	5th - Percentile emergence			Peak emergence			95th-percentile emergence		
	Estimates	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
Intercept	212.05	183.10 – 240.99	**<0.001**	261.23	200.29 – 322.16	**<0.001**	249.52	182.98 – 316.05	**<0.001**
Mean temperature	-14.70	-18.09 – -11.32	**<0.001**	-7.24	-12.85 – -1.63	**0.011**	1.67	-3.39 – 6.73	0.518
Overwinter stage - larva	-44.31	-71.03 – -17.60	**0.001**	-57.52	-113.31 – -1.73	**0.043**	14.7	-46.00 – 75.39	0.635
Overwinter stage – puparium	-46.09	-74.05 – -18.13	**0.001**	-54.38	-112.92 – 4.15	0.069	14.02	-49.78 – 77.81	0.667
Habitat breadth	2.14	-1.46 – 5.74	0.243	7.48	-0.34 – 15.30	0.061	1.83	-6.81 – 10.48	0.677
Non-migratory	-5.1	-14.93 – 4.73	0.309	-17.99	-39.12 – 3.14	0.095	7.96	-15.24 – 31.15	0.501
Mean temperature * Overwinter - larva	9.79	6.72 – 12.87	**<0.001**	3.22	-1.78 – 8.23	0.207	-3.23	-7.74 – 1.27	0.16
Mean temperature * Overwinter - puparium	9.76	6.53 – 13.00	**<0.001**	3.04	-2.26 – 8.34	0.261	-3.17	-7.95 – 1.61	0.193
Mean temperature * Habitat breadth	-0.34	-0.79 – 0.10	0.132	-0.58	-1.36 – 0.21	0.149	0.07	-0.64 – 0.78	0.851
Mean temperature * Non-migratory	0.61	-0.57 – 1.79	0.313	0.44	-1.59 – 2.48	0.669	-3.49	-5.34 – -1.64	<0.001
Random Effects									
σ^2	109.2			383.37			143.49		
τ00 species	220.82			703.42			760.64		
$\tau_{11\ Species*\ Mean\ temperature}$	1.81			**NA**			**NA**		
$\rho_{01\ species}$	0.63			**NA**			**NA**		
ICC	0.81			0.65			0.84		
N species	103			103			103		
Observations	2369			2369			2369		
Marginal R2 / Conditional R2	0.086/0.827			0.114 / 0.687			0.178 / 0.870		

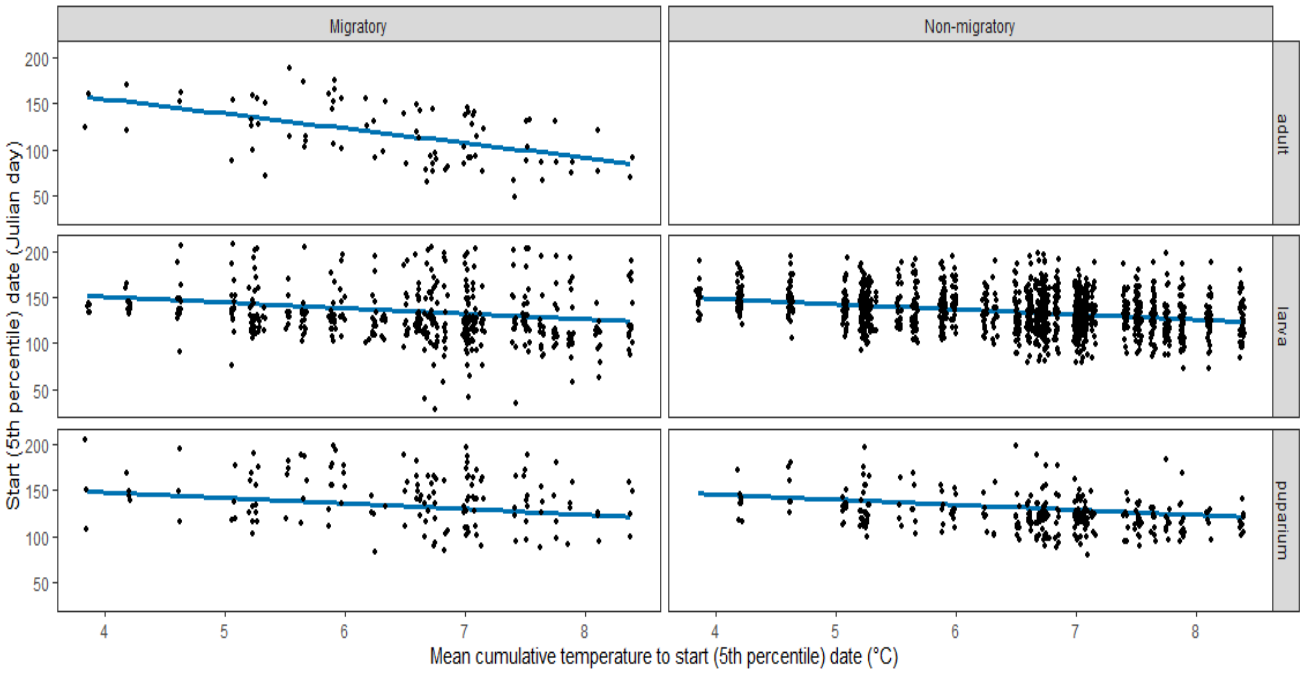

Figure 3.4: The effect of temperature (°C) on 5th-percentile emergence dates showing the effects of species overwintering stage (adult, puparium or larva) and migratory status (migratory or non-migratory). Points indicate raw data (N = 2475)

Phenological sensitivity

Phenological sensitivity decreased with increasing mean emergence date, such that those species that emerged later in the year had a reduced range of phenological shift to increasing temperatures (-0.23 days $^{o}C^{-1}$ ± 0.02, p <0.001, R^2 = 0.821) (Figure 3.5, Table 3.3).

Table 3.3: Output from phenological sensitivity model with species' phenological sensitivity modelled against mean emergence date

Predictors	Phenological sensitivity (days)		
	Estimates	*CI*	*p*
(Intercept)	55.06	52.23 – 57.88	**<0.001**
Mean emergence date	-0.23	-0.25 – -0.20	**<0.001**
Observations	103		
Marginal R2 / Adjusted R2	0.823/ 0.821		

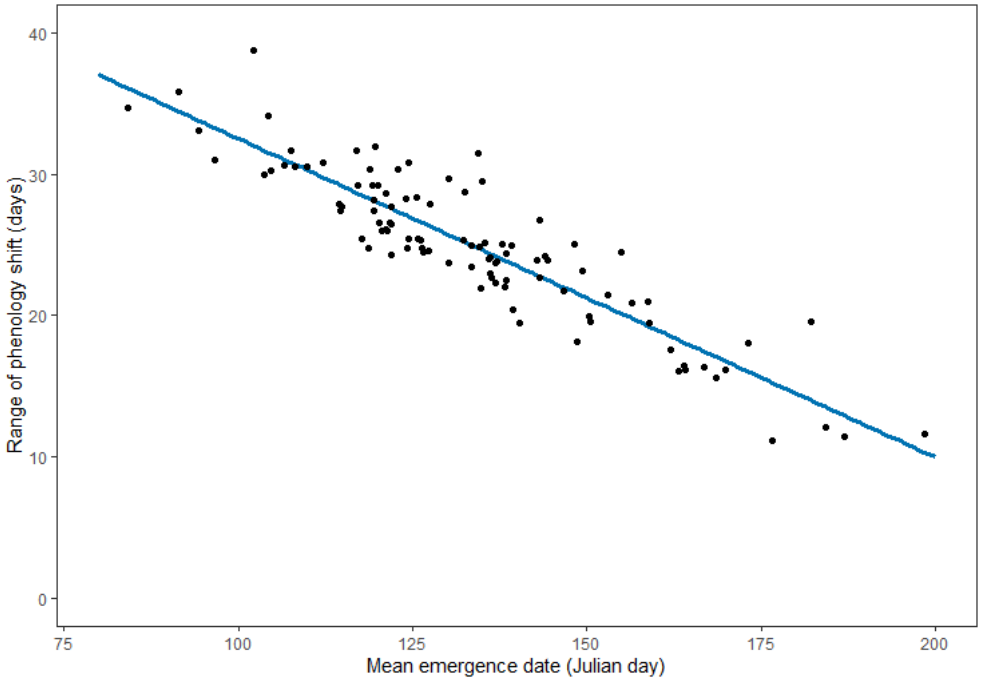

Figure 3.5: Effect of species mean emergence date (Julian day) on predicted range of phenological response (range for day emergence at maximum/minimum observed temperatures). N = 103.

Likelihood of multivoltinism

The likelihood of species displaying multivoltinism was hypothesised to increase with rising temperatures. I found no evidence that temperature had an overall effect on likelihood of multivoltinism (0.01 per ºC SE ± 0.07, p = 0.882) but found that, in line with theory, body length had a significant negative correlation with likelihood of multivoltinism (-1.26 per ºC, SE ± 0.45, p = 0.005). Body length had a marginally significant interaction with temperature, with larger hoverflies more likely to display multivoltinism at higher temperatures (0.18 per ºC, X^2 = 0.046). (Table 3.4).

Table 3.4: Binomial model output for likelihood of multivoltinism predicted by flight season length and species size (length in mm).

- Multivoltinism ~ Mean temperature (Celsius) * Length (mm) + (1| Species)

Predictors	Likelihood of Multivoltinism		
	Odds Ratios	*CI*	*p*
(Intercept)	0.07	0.03 – 0.18	**<0.001**
Mean temperature	1.01	0.88 – 1.17	0.882
Length (mm)	0.28	0.12 – 0.68	**0.005**
Mean temperature * Length	1.19	1.00 – 1.41	**0.048**
Random Effects			
σ2	3.29		
τ00 species	14.59		
ICC	0.82		
N species	103		
Observations	2369		
Marginal R2 / Conditional R2	0.082 / 0.832		

Discussion

This study described the patterns of phenology shift in a key pollinator taxon using a long-term citizen science dataset, and assessed covariance in these patterns with interspecific variation in life-history traits. I found evidence for a general advance in emergence date and peak flight date as a response to temperature, with end date showing no clear response to temperature. Because of the divergent responses for the start and end of flight, adult hoverfly flight activity has effectively lengthened in duration as a response to rising temperatures. Species which overwinter as adults had significantly higher rates of phenology shifts than those with different overwintering stages, however no other life history traits were found to significantly affect phenological response. Phenological sensitivity negatively covaried with average species' emergence date, showing that species which emerge earlier in the year have significantly greater phenological responses to temperature than those which emerge later. No evidence was found for a significant effect of temperature on the likelihood of voltinism. Overall I found little evidence that life history traits which have predicted phenology response in Lepidoptera are generalisable to Syrphidae, although I observed similar phenology responses to temperature as have been documented in studies of other pollinator groups.

Influence of life history traits on phenology

Of the life history traits included in the analysis, only overwintering stage had a significant effect on phenological sensitivity to temperature, with species overwintering as adults being highly responsive to temperature increases. The findings are consistent with the hypothesis that overwintering adults are more mobile than larvae or pupae and can quickly respond to climate cues (Forrest 2016, Chmura et al 2019). Species overwintering as adults exhibiting higher rates of shift also supports similar results from other taxa such as butterflies (Diamond et al 2011, Kharouba et al 2014) and bees (Bartomeus et al 2011). Despite being in line with theory, some caution should be exercised when interpreting these findings as they are driven by 4 species, the only hoverfly species which overwintered as adults from

the dataset, all of which were migratory. There is no comparison with non-migratory species with the same overwintering stage. This limitation is true of UK butterfly data as well (Diamond et al 2011), as species which overwinter as adults are rare in the UK. However, if phenological response increases with a more advanced overwintering stage, I might expect to see variation of response in other stages of development as well aside from adult. I found no incremental progression of phenological response through the overwintering stages (species overwintering as larvae are marginally more responsive to temperature than pupae, although the difference is not significant). That there is no progressive response through overwintering state may indicate that the pronounced response of those at adult stages may be driven by the particularities of the 4 species in that group rather than a more general response linked to overwintering stage.

Other than overwintering stage, I found no evidence to suggest that Syrphidae life history traits are strong predictors of phenological response. I found no difference in response between specialists and generalists using larval habitat breadth as a proxy for species' specialism/generalism. Larval habitat is only an approximate measure of specialisation – compared with Lepidoptera, the degree of specialism of Syrphidae species is harder to determine. Suitable oviposition sites are a constraining resource for hoverflies and are thought to determine their emergence and flight periods (Waldbauer 1988), but most hoverflies are associated with a considerable range of larval habitat types and species-specific associations are frequently only partially understood. One possible conclusion is that there is not sufficient variability within UK Syrphidae as a group to expect a specialism/generalism divide in phenological response. Another possibility is that levels of specialism are not understood in sufficient detail to draw out these differences.

An alternative conclusion is that these traits are simply inconsistent predictors of phenological responses across insect taxa. The clearest signal for traits influencing phenology in insect taxa is in studies focussing on Lepidoptera, and even within this group findings are inconsistent. For example: larval diet (polyphagy/oligophagy) and/or habitat specialism in Lepidoptera has been observed to generate greater phenological plasticity (Altermatt 2010, Diamond et al 2011). The mechanistic basis

posited is that specialists are more highly attuned to climatic cues in order to time emergence with host plants. When these climatic cues shift, specialists are therefore more sensitive to these shifts. However, the reverse hypothesis has also been argued and empirically supported (Zografou et al 2021), with the mechanistic rationale that specialists have greater selective pressure to emerge at a specific time of year and are therefore less likely to exhibit high phenological sensitivity. Even within a group with species knowledge as highly resolved as Lepidoptera, the relationship between life history traits and phenological response is often unclear, which makes generalising these hypotheses to taxa that are less well-described on a species level, such as Diptera or Coleoptera, rather difficult. My results indicate that, with the exception of overwintering stage, in Syrphidae there is not yet any evidence for a clear relationship between life history traits and phenological response.

Effect of temperature on voltinism

Hoverfly body length had a significantly negative relationship with likelihood of multivoltinism, indicating that potential for multivoltinism may be linked with development time, which positively covaries with body size. If voltinism is constrained by development time, then rising temperatures might speed up development and increase facultative multivoltinism. However, I found no evidence for this link: the likelihood of multivoltinism was not significantly associated with temperature. One possible explanation is that confounding effects of temperature compromised the potential to accurately determine separate periods of voltinism. For example, variation in temperature cues, particularly pre-emergence warming, can increase asynchronous emergence in insect populations (Forrest 2016), which would mean that late-emerging individuals from the first generation could extend into early second-generation emergence, reducing the ability of density-estimation methods to recognise these as separate generations due to modes becoming less distinct.

Temperature rises are known to increase voltinism in some butterfly species (Altermatt 2010) but increasing temperatures may not consistently do so across all insect taxa. While temperature rises can increase development rate, they can also

reduce size and fecundity at maturation (Kingsolver and Huey 2008). Moreover, temperature rises can also increase mortality in larval stages susceptible thanks to the reduced humidity that frequently accompanies heightened temperature, thus increasing intra-seasonal larval mortality rate (Gutierrez & Wilson 2020). The association between temperature rises and voltinism is therefore not necessarily consistent across taxa. My results reveal no evidence for heightened levels of voltinism in UK Syrphidae due to increased temperatures; further work on this and other taxa may clarify the generality of Altermatt's (2010) findings.

Lengthening of flight period

I found indirect evidence for a functional lengthening of the flight activity period for Syrphidae as conditions warm, with the end date of Syrphidae flight phenology less sensitive to temperature than emergence date. An extended flight period is typically observed in long-term studies of insect phenology shift (Roy & Sparks 2000, Brooks et al 2014, Buckley et al 2015, Hassall, Owen & Gilbert 2016), although some studies have failed to find support for this (Duchenne et al 2020).

One explanation for the lengthening of flight periods is that, as warming climates have given rise to milder autumns and longer flowering periods, there is simply a greater period during which conditions are favourable for Syrphidae and they have expanded their activity period accordingly. However a lengthening of flight period can be observed in species with flight windows throughout the year where, if this lengthening was purely driven by an increase in favourable flight conditions, it might be expected that this effect be limited to species whose flight activity is curbed by winter conditions.

Another possible explanation for longer periods of flight activity is multivoltinism: lengthening of the flight season is demographically driven by completion of more generations during the year (Altermatt 2010, Teder 2020), however I found no

evidence for increased levels of voltinism. An alternative explanation is that different cues govern the emergence of early and late generations, with late emerging generations having greater reliance on cues such as diurnal cycles (Forrest 2016) which are less subject to change. Intra-seasonal variation in how generations respond to phenology cues could result in an asymmetric phenological response to temperature rises at initiation and end of flight seasons, resulting in the observed lengthening of the flight period as first generations emerge earlier but later generations do not. If lengthening flight seasons in Syrphidae were caused by more generations, an increase in occurrence of multivoltinism in warmer years should be observed but, as discussed above, increases in multivoltinism were not found in the HRS data. My findings therefore appear to indicate that, in Syrphidae, lengthening flight seasons may not be driven by a rise in the number of generations but could instead be related to a more dispersed eclosion of generations or later generations shifting at a different rate to earlier generations.

Trends in phenological sensitivity

Our results support findings that species which emerge earlier in the year exhibit a greater degree of phenological plasticity than those which emerge later in the year (Parmesan 2006, Diamond et al 2011, Wolkovich et al 2012, Mazer et al 2013, Forrest 2016, Fric, Rindos & Konvicka 2020). Diamond et al (2011) posited that high phenological variability in early-emerging species is often a by-product of spring temperatures being more variable than other seasons; therefore early-emerging species show more variation in phenology as they are exposed to greater shifts in temperature. That higher rates of shift merely reflect higher rates of temperature variation is potentially true of phenological studies that do not include temperature as an explanatory variable – many measurements of phenology examine shift by year rather than temperature. However, as temperature was explicitly included as a predictor in modelling we can be confident that the results instead suggest late-emerging species are less phenologically sensitive to temperature change than early-emerging species. Divergent responses to shift in early- and late- season fliers could occur if late-season fliers are more responsive to cues other than temperature, such as photoperiod, that are less susceptible to variation (Valtonen et al 2011). If

late-emerging species are governed by cues other than temperature, then they are unlikely to experience significant phenological advance as a result of changing climate. When determining which species are most likely to be at risk from emerging phenological mismatches, one might therefore focus attention on species with early emergence dates.

Comparison of phenology shifts to other taxa

In keeping with general patterns observed across taxa (Parmesan & Yohe 2003, Parmesan 2006), increases in temperature appear to be advancing the flight phenologies of British Syrphidae. The rate observed for the advance in emergence date for hoverflies overwintering as adults (-14.7 days/°C) is markedly larger than those in similar studies of insect phenology in temperate climates (Roy & Sparks 2000, Bartomeus et al 2011, Kharouba 2014, Duchenne et al 2020). Notably, the rate I report is greater than that found in another study using the same dataset (Hassall, Owen & Gilbert 2016), which estimated the average rate of advance at -12.48 days/°C across all species. However, the majority of species in the HRS dataset overwintered as either larvae (98 species, 76.56% of the dataset) or pupae (19 species, 14.84% of the dataset), which both displayed significantly lower rates of phenological advance than the adult overwintering group (-4.94 days/°C and -4.91 days/°C respectively). Even these lower rates are greater than those found in studies of other insect taxa (Roy & Sparks 2000, Bartomeous et al 2011, Kharouba 2014), but are considerably lower than the mean responses across Syrphidae species found by Hassall, Owen & Gilbert (2016).

While the direction of effect is consistent with similar studies, with an overall advance in phenologies observed across insect taxa, variability in the magnitude of response is to be expected. However, given that Hassall, Owen & Gilbert (2016) derived their results from the same dataset, it is worth considering how the methodologies used may have resulted in differing values obtained for phenology shift. Aside from

differences in modelling approach the primary differences in methodology from Hassall, Owen & Gilbert are temperature measure used and geographic range encompassed. Hassall, Owen & Gilbert used annual mean temperature as their predictor and analysed HRS records for the entirety of the UK. In contrast, I used event-specific measures of temperature and a restricted geographic range of records to reduce the confounding effect of latitude on phenology. Using temperature measures from the months immediately preceding adult emergence can better estimate insect phenology shifts (Gutierrez & Wilson 2021), therefore it is not necessarily surprising that using event-specific temperature measures has resulted in a different estimate of phenology.

Plants in the UK (Fitter & Fitter 2002), and more widely in Europe (Menzel et al 2006), have also been found to be advancing first blooming phenology in response to increasing temperatures over time. The rate of advancement (-4.0 days/°C and -2.5 days/°C respectively) is marginally slower than that observed in the majority of UK Syrphidae in this study, which could support the prediction of varying rates of shift generating mismatches between plants and pollinators (Memmott 2007, Hegland et al 2009). However, overall comparisons of phenology dates such are merely indicative of mismatch: a full assessment of potential for emerging mismatches would require comparison of the phenologies of interacting species. Hoverflies are typically highly generalist (Doyle et al 2020), therefore phenology shifts in the plants they visit for feeding may not compromise their resource availability to the same degree as specialist pollinators due to the range of species they can interact with. The highest risk group are likely the subset of species that overwinter as adults, given that the rate of advance in their phenology is markedly higher than other Syrphidae groups.

Estimates of phenology from opportunistically collected recording scheme data such as the HRS frequently compare favourably with findings from standardised data-collection (Bishop et al 2013, Taylor et al 2019), however there are potential biases when examining the outlying dates of phenological events, such as start and end of flight period, with opportunistic data. Increased recorder effort typically results in an increased reporting in outlying observations, therefore an observed

advance in phenology can be driven by variation in recorders rather than the true movement of an underlying biological event (Diamond 2011). The HRS is certainly subject to fluctuations in recorder effort and there has been a large increase in both number of recorders and individual records submitted over the period studied (Ball & Morris 2012). I have sought to account for variation in recorder effort in the methodology by employing a percentile-based approach to observation of key dates, which decreases the potential for extreme outlying observations to influence measurement. Further, the responses of the start and end dates of the flight period provide further confidence that variation in recorder effort is unlikely to be an issue in accurately measuring underlying phenology shifts: if increased recorder effort had increased detection of outliers, then an attendant delay in the end date alongside an advance of start date should be observed; the fact that the end date has also advanced indicates that this effect is not occurring within the HRS data.

Conclusion

I have found that Syrphidae are generally experiencing advances in emergence and peak abundance date as a response to temperature, but that end date had no clear relationship with temperature increases. This has led to a functional lengthening of the flight activity period in response to temperature rises, but I did not see this reflected in evidence for multiple generations in the form of increased incidence of voltinism. Contrary to the findings in Lepidoptera, I have found few life-history traits that allow us to predict interspecific variance in the magnitude of phenology responses. Species which overwinter as adults and species that have early historic emergence dates display greater rates of shift, but I found no evidence of other life history traits such as migratory status or habitat breadth to be predictive of phenological sensitivity. This emphasises the importance of long-term monitoring schemes for understanding species-level trends in phenological responses.

Chapter Four

Realistic phenology functions increase consequences of mismatch within pollination networks

Abstract

Changes to phenology are some of the most visible effects of climate change. As plants and pollinators are currently undergoing divergent rates of phenology shift, interacting species could become mismatched in terms of their activity periods. Previous research has sought to understand the risk this poses to pollination networks by simulating projected rates of phenology shift to networks of pollinators and plants. However these simulations rarely account for changes to the shape of abundance distributions across phenologies. To understand this impact, I created an individual-based model of a pollination network in which I could independently modify different parameters contributing to the shape of species' abundance distribution. Modelling abundance distribution through time using an asymmetric logistic growth function, I altered the shape of the abundance distribution through making three parameters sensitive to temperature change: midpoint, rate and skew. I applied phenology shifts over 50 seasons using UK temperature predictions and allowed for phenology shifts to also shape species' abundance distribution by altering the model parameters of midpoint, rate and skew. Under phenology shifts, pollination networks became less species-rich over time, with specialist species and pollinators particularly vulnerable to risk of extinction. Accounting for shifts in parameters that affect shape resulted in considerably greater species loss than simulations that did not incorporate changes to shape. I conclude that there is a need for phenology research to characterise changes to the shape of abundance distributions within phenological events as well as changes to the temporal window in which they occur. Accounting for the shape of phenological events has pronounced consequences for predicting the effects of climate change, with reliance on single-date metrics underestimating the effect of phenology shifts.

Introduction

Shifts in the phenology of species' life events such as breeding, migration or emergence from hibernation are increasingly documented as responses to rising temperatures induced by climate change (Visser & Both 2005, Parmesan 2006, Stevenson *et al.* 2015, Buntgen *et al.* 2022). Due to the high level of interspecific variation in phenological response across taxa (Primack *et al.* 2009, Gutierrez & Wilson 2019, Duchenne *et al.* 2020) there is correspondingly high potential for phenology shifts to lead to mismatches in the timing of previously interacting species (Kharouba *et al.* 2018). Temporal mismatch generated by phenology shifts has the potential to lead to fitness impacts for affected species (Chapter Two, Memmott *et al.* 2007, Hegland *et al.* 2009).

As pollination networks are comprised of seasonal interactions between plant and pollinator populations, disruptions to species' phenology can severely impact interaction potential. It is therefore unsurprising that increasing rates of phenology shift in plants and pollinators have led to forecasts of severe loss of network interactions and potential species extinctions (Hegland *et al.* 2009, Forrest 2015). Understanding the impact that projected change to the climate will have on the functioning of pollination networks is critical to determining the level of risk posed to function loss, but there are relatively few studies that project future impacts of phenology shifts (Visser & Ginenapp 2019).

A common approach to characterising phenological mismatch in the literature is the comparison of calendar dates of activity periods for interacting species (e.g., Bartomeus *et al.* 2011, Kudo & Cooper 2019). Studies looking at mismatch use first and last bloom/flight dates for plant/pollinator as measures of pollinator phenology, with activity period described as the difference between these two dates. Mismatch is then defined as a period, in Julian days, when a plant or pollinator does not overlap with any of its historic interaction partners. By using this approach of comparing overlap in Julian days to describe match/mismatch, Memmott *et al.* (2007) forecast the impact of projected temperature change on the phenology of a North American pollination network. They extracted the rate of phenology shift for

first bloom/flight, in days, from other studies, and forecast expected phenology shifts for the study network. Memmott *et al.* estimated that 17-50% of pollinator species in the study network would experience critical levels of food disruption under current predicted temperature rises. A similar approach has also been applied to historic data: on revisiting a pollination network in 2010 that had been described in 1920, Burkle, Marlin and Knight (2013) recreated the phenology shifts that may have occurred to species within the intervening time period. They estimated that 15% of the observed species loss since 1920 was attributable to the impact of phenological mismatch. Therefore, existing simulation approaches to phenology shift in pollination networks have predicted high levels of species disruption or extinction but these predictions could differ if simulations account for changes in the shape of phenological events. Simulations which look at phenological mismatch through comparison of calendar dates cannot account for the two-dimensional nature of population abundance over time (Figure 4.1).

The implicit assumption in studies which look at temporal mismatches in terms of overlapping time periods is that population abundance is constant over the activity period, but flower and pollinator abundances are demonstrably uneven across their phenologies (Balfour *et al.* 2018). Assuming static abundance can potentially lead to error in either direction, over- or under-estimating impacts on species' demographic responses. Currently there is not a wealth of data that precisely describes how variation in climate affects the shape of abundance distributions (Inouye, Ehrlen & Underwood 2019), but there is ample evidence to infer that it does through asymmetric phenological responses at the onset and end of activity periods (Thackeray *et al.* 2012, Forrest 2015). As phenology shifts appear to affect within-season abundance distribution as well as key measurement dates, accounting for this within-season variation when simulating phenology shifts is key to understanding the susceptibility of species to phenological shifts and mismatches.

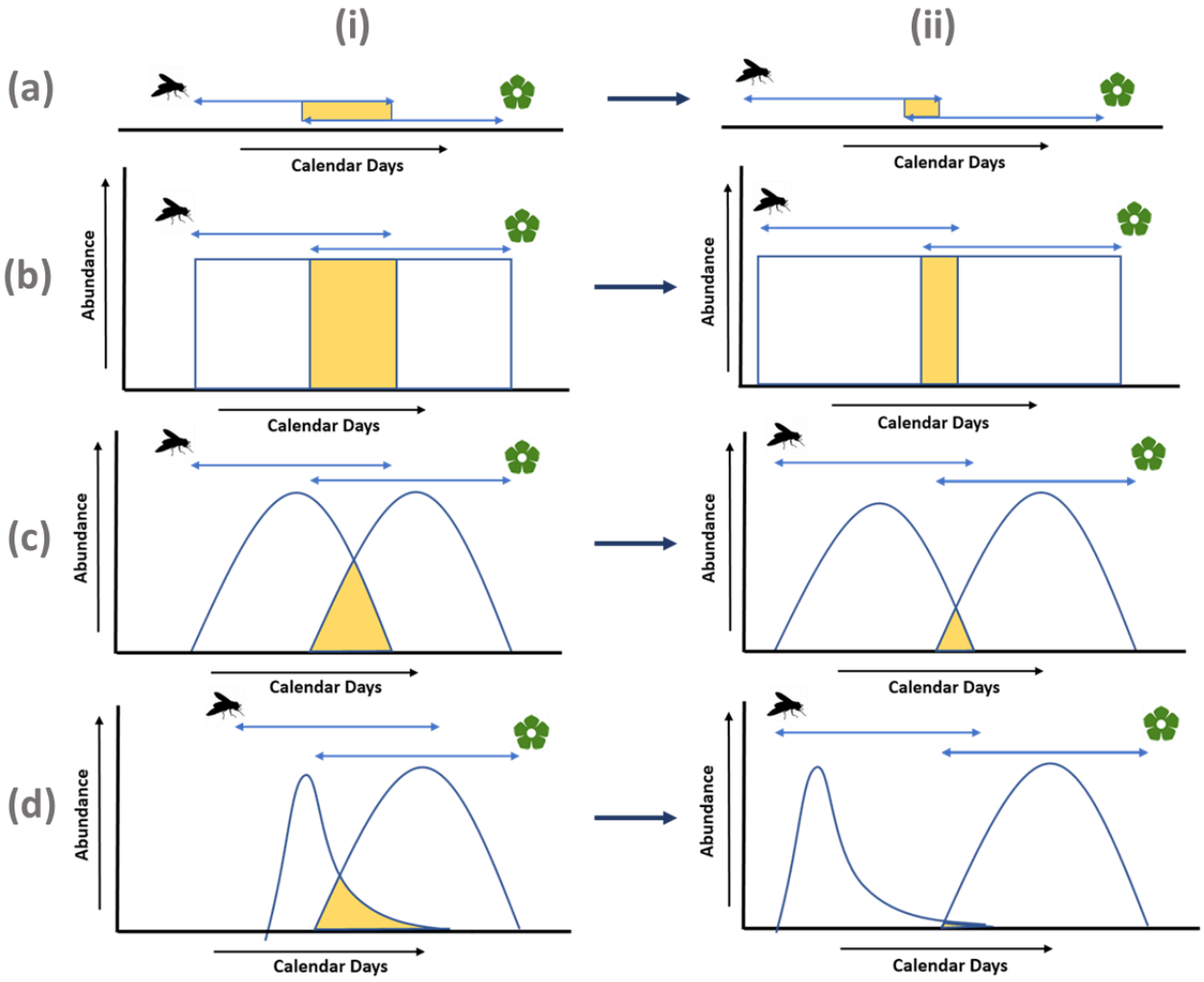

Figure 4.1: In scenario (a) the population of a pollinating insect interacts with the population of a blooming plant species on the days during which their phenologies overlap. The historic interaction shown under (i) may change over time: if the pollinator's phenology advances, then the interaction period will be reduced (ii). Interpreting these interactions purely in terms of overlapping calendar days carries the implicit assumption that species' abundance is constant over their phenology and that any loss of time overlap scales linearly to loss of interaction, as in (b). Populations, however, will not be constant in abundance over time meaning that any loss of temporal overlap will not scale linearly to loss of interaction, as in (c). This could be exacerbated (or ameliorated) if one, or both, of the interacting populations had an asymmetric abundance curve as in (d).

Modelling can be used to work out the consequences of known assumptions about how systems may change and with the appropriate structure are also able to account for the shape of phenological events. One method of accounting for species abundance in simulations of phenology shift in pollination networks is through mathematical modelling. Assuming a Gaussian distribution to species abundance during activity periods, Ramos-Jiliberto *et al.* (2018) applied different stressors to models based on 12 documented pollination networks. They found that phenology was a key factor in both species persistence and network robustness and that

changes to phenology, particularly to early or late emerging species, weakened pollination network robustness to future perturbations. These findings are consistent with other mathematical models which have found phenology key to the maintenance of network structure and connectivity (Encinas-Viso *et al.* 2012). Representing the full abundance distribution of interacting species in the manner of these mathematical models is progress in how simulations test the effects of phenological mismatch. However, these abundance distributions are an emergent property of the underlying behaviour of individual organisms (Inouye, Ehrlen & Underwood 2019). Interactions between individuals require not just temporal co-occurrence but also spatial occurrence, and the probability of spatial co-occurrence is affected by abundance. Individual-based models are one method for accounting for these complexities as they allow us to model the movement of individuals through a landscape.

To test how phenology shifts can affect species persistence once variation in abundance is accounted for, I built a spatially-explicit individual-based model to simulate changes in a pollination network within and between seasons. I described the shape of species' abundance distribution over their phenology window using a non-linear model that allowed for variance in three parameters – midpoint, rate and skew - that affected the shape of the distribution. I tied the sensitivity of these parameters to predicted changes in UK temperature over 50 years (2021-2070) and ran multiple simulations allowing different combinations of these parameters to shift. I compared simulation outcomes to those of control runs where no parameters underwent shift and assessed them in terms of species persistence and community diversity.

Methods

Model structure

I use a spatially explicit individual-based-model (IBM) to simulate the dynamics of plant-pollinator interactions within a pollination network employing both within-season and between-season dynamics (Figure 4.2). All model code was written and run using R (R Core Team 2021). At model start-up a table of temperature changes for 50 seasons is randomly sampled and saved. Temperature change for each year fluctuates around a mean average annual increase for each season. Temperature change is then used to recalculate each species' emergence phenology based on the sensitivity of their phenological parameters to temperature. From these altered phenology parameters, emergence dates for each individual recruited into the current season are calculated, the landscape is populated with plants and pollinators and the season begins. A single season is comprised of discrete timesteps (days) from 1-365, over which individuals of plant and pollinator species emerge across a timestep range derived from their species' phenology, move across the landscape, and interact. Based on these interactions, insect individuals either live until they are sexually mature or die without reproducing, while plant individuals bloom for a set lifespan and then produce a quantity of seeds proportional to the pollination service they received over that lifespan. A season ends once all individuals have emerged and died, after which both plant and pollinator offspring are recruited into the next season.

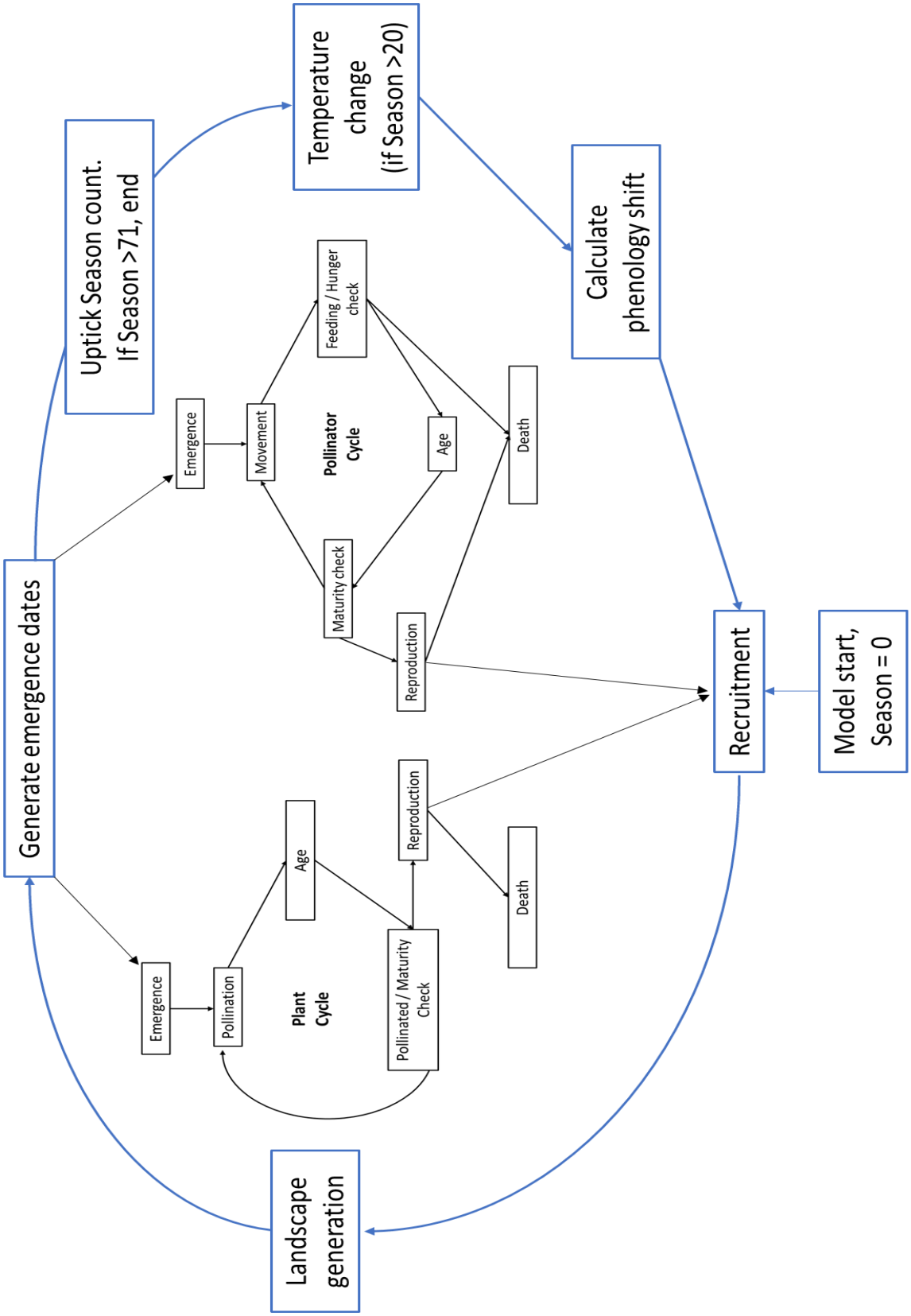

Figure 4.2: Diagram displaying structure of individual-based model. The outer loop (in blue) displays the inter-seasonal loop, which initiates on model start-up and terminates when Season > 71. The two inner loops (in black) display the intra-seasonal behaviour loops for plants and pollinators, each lasting one timestep. The intra-seasonal loops terminate when all plant and pollinator individuals are dead, at which point a new season initiates.

Phenology

I modelled phenological events, such as flower bloom, as a logistic curve, where 0 represents the start (no flowers bloomed) and 1 represents completion (all flowers bloomed) with increments in between indicating the cumulative proportion completion of the event (the proportion of total flower population which have bloomed). Each species' phenology in this model is defined by three parameters used to describe a generalised logistic equation, an adapted version of the Richards' growth curve (Richards 1959), which allows for an asymmetric response. This means that an emergence/bloom abundance distribution can be skewed in either direction, towards season start or season end (in biological terms, this can be interpreted as more synchrony in emergence than in senescence, for example, or vice-versa). A simplified version of the equation (assuming the lower asymptote is constant at 0 and upper asymptote is constant at 1) can be written as follows,

$$f(x) = \frac{1}{\sqrt[v]{1+ e^{B(M-t)}}}.$$

The parameters *v*, *B* and *M* can be interpreted in a phenological sense as, respectively, the skew, rate and midpoint of phenological events (Figure 4.3) and will be referred to in these terms henceforth. A description of these parameters follows:

The *midpoint* defines the when the peak of a phenological window will occur (in these simulations the Julian day of the peak) and, as a result, the overall annual timing of the phenological window. If the other parameters are constant then shifting the midpoint will also shift the first and last days of a phenological window by an equal amount.

The *rate* defines the length of the phenological window. An event with a high rate will complete over a short period of time, whereas an event with a low rate will

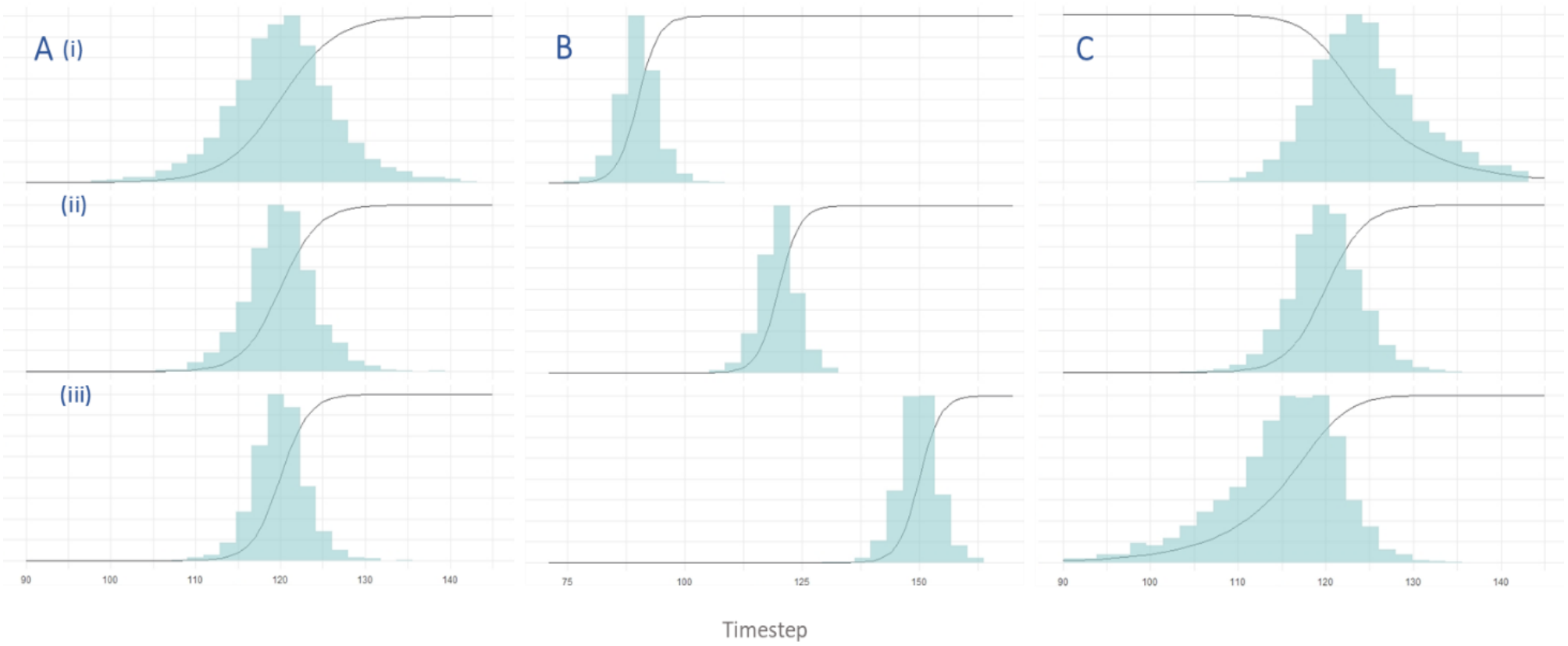

Figure 4.3: Illustration of the effects of phenology parameters on distribution of individual emergence by timestep. In each figure the black line illustrates the shape of the logistic curve derived from the Richards' growth equation while the pale blue bars are a histogram of back-transformed emergence times sampled from the logistic curve (N = 1000). Each column illustrates the effect of altering one of the three different parameters, shown at the (i) minimum, (ii) median and (iii) maximum of the start-up parameter range the IBM samples from. Parameter values not in focus are held at their median. (A) shows the effect of the scale (B) parameter at (i) 0.3, (ii) 0.45 and (iii) 0.6; (B) shows midpoint (M) at (i) 90, (ii) 120 and (iii) 150; (C) of skew (v) at (i) -3, (ii) 1 and (iii) 3.

complete over a longer period of time. The rate parameter has a symmetrical effect, so both onset and cessation of the phenological event are equally affected.

The *skew* of a phenological event exerts a similar effect to *rate* except it is applied asymmetrically. Depending on whether skew is positive or negative either the onset or the cessation of a phenological event will be affected and the greater the skew, the slower the onset or cessation will occur.

Emergence

The logistic equation can be restructured to solve for *t* as follows,

$$t = M - \frac{\log\left(\left(\frac{1}{x}\right)^{V} - 1\right)}{B}.$$

This allows us to generate timesteps for individual emergence based on the overall shape of a species' phenology, as described by the generalised logistic equation. At the start of a season, each individual in a species' population has a value for *x* assigned to it by a random uniform sample between 0 and 1. This *x* value is then fed into the transformed equation to solve for *t,* generating a timestep for the individual's emergence. Figure 3 illustrates this, with the logistic curves generated by the initial logistic equation while the histogram data are generated for 1,000 individuals using the transformed equation.

Once a bloom/emergence timestep has been generated, individuals remain inactive until the season's current timestep matches their precalculated emergence timestep, at which point they become active and may move around the landscape (if a pollinator), interact with other individuals, age, reproduce and die.

Temperature and phenology shifts

Long-term changes in the annual phenologies of plant bloom and insect emergence are currently best understood as responses to fluctuating environmental temperature (Iler *et al.* 2021). To simulate the projected effects of climate change on temperature, each year the model samples an annual temperature change from a normal distribution. This distribution is mean-centered around the average annual temperature increase projected in the UK from 2021 until 2070 under RCP (representative concentration pathway) scenario 6.0 (UKCP18 2018). The standard deviation for the sample is equal to the standard deviation of residuals from a linear model fitted to mean annual UK temperatures from 1961-2020 using the HadCRUT4 dataset (Morice *et al.* 2012). The projected temperature changes in the model are therefore aligned with a realistic model of climate change effects.

Changes in the timing of phenological events are well-studied, and I was therefore able to find literature estimates of to the sensitivity of the midpoint parameter. Duchenne *et al.* (2020) estimate phenology shifts in over 2,000 European pollinator species from 1960-2016 using a Gaussian distribution with a stated midpoint. Although Duchenne *et al.* link these midpoint shifts with temperature and climate warming, the results are not presented in terms of temperature, instead describing phenology changes in terms of days shift/year over the timeframe studied. Therefore, to convert these shifts into temperature-responses, I extracted European mean annual temperature from the time period of the study (HadCRUT4, Morice *et al.* 2012) and recalculated the presented shifts in terms of days/ degree Centigrade. Shifts in plant blooming phenology are similarly well-studied; I extracted the findings from Fitter & Fitter (2002), which estimated advances in blooming phenology for 385 UK plant species. Fitter & Fitter (2002) assess the rate of shift of first bloom phenology rather than the midpoint, and I have exercised the simplifying assumption that these shifts in first bloom date are representative of shifts in overall phenology and thus synchronous with shifts in midpoint. This approach is relatively common in phenology literature (e.g. Memmott 2007; Burkle, Marlin & Knight 2013) although it should be noted that an advance in first bloom dates could equally be

attributed to an increase in the skew parameter or a decrease in the rate parameter without the midpoint being affected.

Existing research into plant and pollinator phenologies does not include analyses that could be converted into expectations for the rate and skew parameters. To estimate reasonable sensitivities for these parameters, I visualized sample output from logistic models using the extreme limits of these parameters under maximum and minimum possible temperature samples over the 50 seasons the simulations were due to run. Within temperate European environments, plants and pollinators have a finite annual bloom or flight period – therefore any sensitivity value for these parameters which produced phenologies that extended beyond the 0-365 timestep range of a single year were not used. Any parameters below that range were considered possible.

Species trait generation

As part of the simulation, I wanted to generate species-specific traits to generate a network of species that varied in their generalism, efficacy as pollinators and in their phenological sensitivity. To create diversity in species I produced a set number of species on model-start up with traits sampled from a range of parameter values. At the beginning of a simulation, a set number of plant and pollinator species are created (N = 25 for pollinators, N = 20 for plants in the simulations reported in this chapter - for a list of all parameters used in the model refer to Table 4.1) and phenology traits are generated for each of them. The way in which phenology traits are generated are common across plant and pollinator species but there are certain species traits that are specific to each as described below.

Each pollinator species has a hunger threshold parameter (in Julian days) and a lifespan (in Julian days), both of which are sampled from a Poisson distribution with a rate parameter set to the hunger and lifespan values (see Table 4.1 for all parameter values). Each species is assigned a pollinator efficacy value sampled from a uniform distribution across a range of 0.01 – 1. This efficacy value governs how pollinators interact with plants and adds a variable degree of resource-dependent

competition among pollinators as plants will be removed from the landscape once fully pollinated (see intra-seasonal behaviour section below). Movement range, offspring dispersal range and offspring number are constant across all pollinator species (Table 4.1).

Plant species have a blooming period which is sampled from a Poisson distribution with a rate parameter set to the bloom lifespan value. Plant maximum seed count and offspring dispersal range are constant values across all plant species.

Table 4.1: Model start-up parameter values, used in all runs

Parameter	Description	Value
Global environment	*Global environment*	
Δ_T	Annual temperature increase	0.078 +- 0.9164
X_{max}	X landscape limit	40
Y_{max}	Y landscape limit	40
Phenology traits	*Phenology traits*	
M	Midpoint range	90 - 150
ΔM_{poll}	Midpoint shift (pollinator)	-3.73 +- 6.856
ΔM_{plant}	Midpoint shift (plant)	-4 +- 4
B	Rate range	0.3 - 0.6
ΔB	Rate shift	0.01 +- 0.05
V	Skew range	1.0 - 2.0
ΔV	Skew shift	0.04 +- 0.01
Shared species traits	*Shared species traits*	
N_{ind}	Initial population	150
$P_{generalism}$	Generalism probability	0.3
$\lambda_{generalist}$	Generalist lambda	12
$\lambda_{specialist}$	Specialist lambda	4
Pollinator traits	*Pollinator traits*	
N_{poll}	Species number (Pollinator)	25
E_{poll}	Pollinator efficacy	0.01 - 1
L_{poll}	Pollinator lifespan	35
H_{poll}	Pollinator hunger threshold	18
F_{poll}	Pollinator feeders maximum	2
M_{poll}	Pollinator movement	5
O_{poll}	Pollinator offspring	2
D_{poll}	Offspring dispersal range	4
Plant traits	*Plant traits*	
N_{plant}	Species number (Plant)	20
L_{plant}	Bloom lifespan	30
O_{plant}	Bloom maximum seeds	6
D_{plant}	Seed dispersal range	15

Interaction partners are then generated for plant and pollinator species as follows. Each species has a likelihood of being either a generalist or a specialist, randomly assigned from a binomial sampled set to a probability of the generalism parameter The species then has a value for number of interaction partners generated – this number is sampled from a Poisson distribution with the lambda set to either a generalist (higher) or specialist (lower) value depending on the outcome of the generalist/specialist assignation. As this designation affects the lambda from which the number of interactions partners is drawn there is a continuous scale of partners between generalists and specialists and there exists a non-zero (although low probability) chance that species designated specialist can have a higher number of partners than a generalist. This was thought to be a realistic depiction of specialism/generalism in nature, which exists as a continuum rather than a bimodal outcome. After the number of interaction partners is defined these are then randomly assigned from the species list.

Intra-seasonal plant-pollinator behaviour

Each plant and pollinator individual belongs to one of multiple species in the community, the population of which is defined either by model start-up parameters (in the first season) or by recruitment from the previous generation of that species (all subsequent seasons). At the start of each season each individual is placed on the landscape and has an emergence date assigned to it. Once the time-step equals their emergence date, individuals become active then enter the relevant behaviour loop for plant or pollinator. This behaviour loop plays out every time-step until the individual dies.

At the start of each season all individuals are assigned an X - Y location on the landscape, which in the model is a two-dimensional torus made up of square cells. Multiple pollinator individuals may co-exist on the same cell, but only a single plant individual can occupy a cell. If multiple plants are assigned the same landscape cell, competition is modelled as a lottery effect with one individual randomly sampled to survive while the other plants on that cell die, giving the plant populations density-dependent mortality. In the first season, placement is at random, with placement in

all cells equally likely, but in subsequent seasons individuals are placed on a cell sampled from a dispersal range centred on their parent's last cell location. The selection of lottery-based competition for plant survival allows for population-level competition at the expense of simulating competition-induced fitness costs over an individual's lifetime (Chesson & Warner 1981, Pigolotti & Cencini 2010). However as lottery-based competition is an established method for simulating competition and has a history of use in modelling plant competition (Kelly & Bowler 2002, Higgins et al. 2008) it was deemed sufficient for the IBM's purposes.

All individuals start the season inactive but will become active on a given timestep according to their species' phenology (see Phenology section above). When pollinators become active, each timestep they move a distance of N cells in any direction from their previous location, with N being a value uniformly sampled from a range starting at zero and capped at that species' movement parameter.

After movement, the pollinator checks whether it is on a cell occupied by an active plant. If so, it will check whether the plant is from a species that is listed as an interaction partner for the pollinator.

If the pollinator can interact with an active plant, it will feed, which resets its hunger count to 0. If it cannot interact, or if it is on an unoccupied cell, its hunger count will uptick by 1. If an individual's hunger count exceeds the hunger threshold parameter, that individual dies. If multiple pollinators occupy the same cell and are able to feed, then a single pollinator will be randomly selected to feed and all others on that cell go hungry, adding an element of resource-based competition to the pollinator populations.

If a pollinator does not die of hunger, then its maturity count upticks by 1. Its maturity count is then checked against its species' lifespan – if its maturity count equals its lifespan, then the pollinator reproduces, producing offspring equal to the pollinator offspring parameter before dying. These offspring are dormant until the next season begins.

When plant individuals become active, they remain stationary on the landscape. At each timestep, a check is performed to see whether any pollinator individuals occupy the same cell as them (post-pollinator movement) and, if so, whether those individuals are from a species listed as an interaction partner for that plant. Interaction partners for plants are determined at species-level in an identical manner to pollinators. If the plant can interact with any pollinators on its cell, then pollination occurs. When pollinated, a plant's pollination proportion is increased by the sum of the efficacy parameters of all compatible pollinators on its cell (constrained to a maximum of 1). This interaction is not necessarily symmetrical. A pollinator can feed from a plant even if that plant cannot be pollinated by that pollinator and a plant can be pollinated even if the pollinator is unable to feed from that plant. Behavioural exploits to cheat either pollinator or pollinated individual occur naturally and therefore I allowed asymmetric interactions in the model.

After checking for compatible pollinators, plant individuals age and their maturity upticks by 1. A plant will reproduce and set seed if one of two conditions is met: either the plant reaches the end of its species' blooming lifespan, or its pollination proportion equals or exceeds 1, at which point it is considered fully pollinated. When reproducing, a plant produces seeds equal to the product of its seed set parameter and its pollination proportion, rounded to the nearest whole number. Seeds produced cannot exceed the seed set parameter and cannot be below 1, as a level of selfing is assumed. After reproducing a plant dies and is removed from the landscape, no longer available to pollinators as a food source.

Model runs

Preliminary simulations indicated that the IBM exhibits high levels of instability in the plant-pollinator communities generated in early seasons due to the random sampling of interaction partners and phenologies for species start-up parameters. This can result in species not having temporal cooccurrence with any compatible

species from the first season, leading to extinctions that are due to the high levels of stochasticity in the early seasons of a run rather than the impact of in-focus phenological parameters.

To address this initial stochasticity, each run had the random number generation seed set at the beginning of the run and was allowed to run for 20 seasons without any phenology shifts. Following this burn-in, visual inspection of trends was conducted for each run to check community stability and species persistence – those with more than 5 extant species of both plants and pollinators having stable populations were selected for further use. Stable populations were determined as those which were not visually trending towards 0 by season 20. In total, 48 seeds were run for the burn-in period of 20 seasons and 12 of these seeds were selected for the final runs.

Once the random number seeds that produced stable communities were selected, five runs were performed for each seed: (1) a control run, where there were no phenology shifts; (2) a run where midpoint phenology was sensitive to temperature changes; (3) a run with midpoint + rate sensitivity; (4) a run with midpoint + skew sensitivity; and (5) a run in which all three parameters of midpoint + rate + skew were sensitive to temperature. Phenological parameters were only allowed to shift after the season count had increased beyond the burn-in period of 20, and ran to 70 seasons, for a total of 50 seasons during which phenology was allowed to shift.

By varying the phenology parameters and comparing them to the same random number seed as control runs, I was able to isolate the partial impact of each of the combinations of phenological sensitivity on species persistence and community composition. Runs with the same seed have identical species, species traits, temperature changes and exactly the same population dynamics for the burn-in period of 20 seasons. After 20 seasons, some runs will incorporate phenology shifts, which disrupts the random number seed, but the method used ensures that runs of the same seed start from exactly the same point at 20 seasons, and therefore any

differences from that point on are entirely attributable to the phenology shifts taking place.

Data collection

Each run generated five datasets. The first of these was a summary of temperature changes over the 50 seasons (seasons 21 – 70) for which temperature changes were active. As the exact temperature changes were randomly sampled, they were expected to be consistent across runs with a common random number seed. However temperature summaries were collected to confirm that expectation.

The second and third data files collected species information for each season for plants and pollinators respectively. The data included all species start-up information (see above Species generation section) and a summary of the phenology parameters both at start-up and for each season after phenology shifts had been applied.

The fourth and fifth data files collected seasonal demographic information for plants and pollinators, respectively. These included population size for each extant species in each season, 5^{th} percentile ('Start') and 95^{th} percentile (End) phenology dates in Julian days, a mean and median bloom/emergence date and accompanying standard deviations.

Data analysis

Data from the runs where phenology shifts were active were compared against data from control runs for the same random number seed. By comparing against a control run, any variance in species persistence or population demographics was attributable to the phenology parameters that were active rather than natural stochasticity between runs. Therefore differences between simulation outcomes of

the same seed should be directly caused by the phenology parameters that are active.

To draw out the differences between survival rates across runs and species classes, I fitted a binomial mixed-effect model using glmmTMB (Brooks *et al.* 2017) with run number as a random effect. To characterize the differences in final-community diversity, I calculated the Shannon index for each combination of run type and seed using the VEGAN package (Oksanen *et al.* 2020). The Shannon index is one way of assessing community diversity that accounts for both presence/absence of species and the relative abundances in which they are present in a community.

Results

Species persistence

Allowing model shape parameters to be sensitivite to temperature typically resulted in a loss of both plant and pollinator species compared with control runs (Figure 4.4). This was not true of every run and there was some unpredictability in species' response to phenological shift, with some runs showing greater species persistence than their control counterpart (notably run 2, see Figure 4.4, row 2). As a general trend, however, shift in phenological parameters resulted in increased species loss from control runs where parameters were constant.

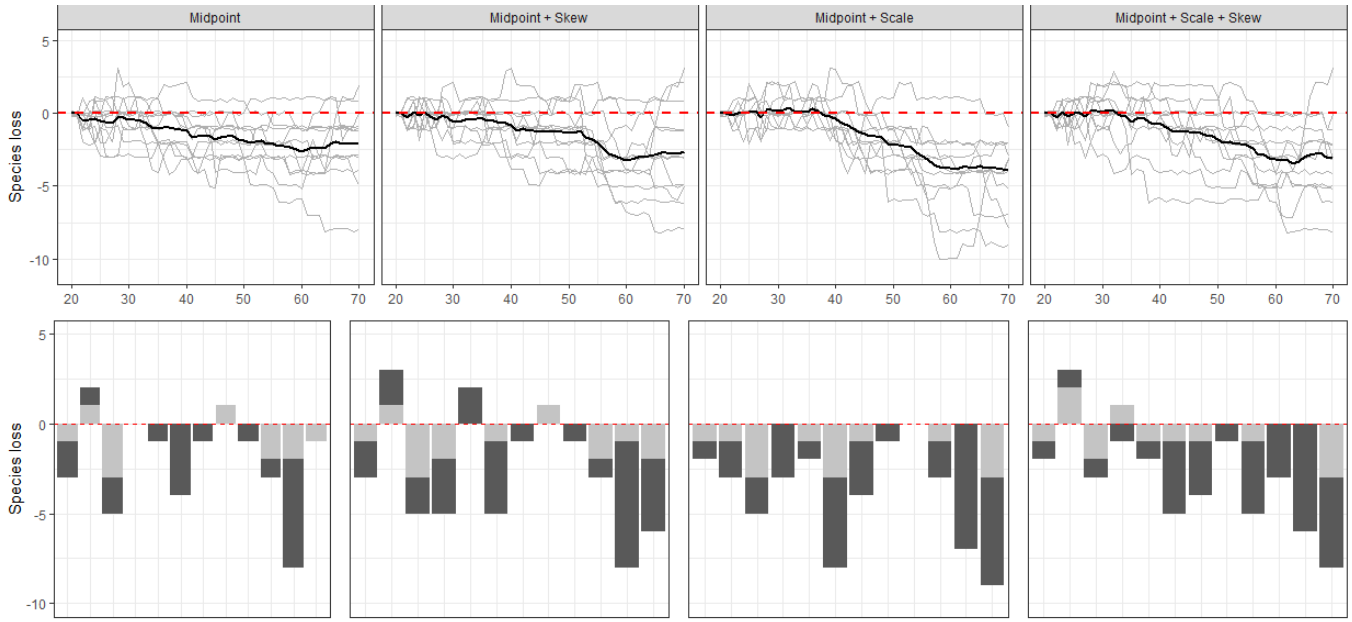

Figure 4.4: Species persistence compared to Control runs, titles at top describe which model parameters were allowed to shift in response to temperature changes. Top row charts the difference in total species count (plant and pollinator) from the Control group for each run (N = 12, in light grey) from season 20 to season 70, with the bold line showing the mean trend. Each line shows the number of species in that seed minus the number of species that were present in the Control seed. Bottom row shows the species count difference from the Control group at the end of each run (season = 70). Each bar represents a seed, with dark grey representing difference in plant species from Control run of that seed and light grey the difference in pollinator species.

Runs in which only the midpoint parameter varied exhibited the lowest decrease in species count compared to the controls (mean species loss = 2.0). Any runs in which more than one parameter varied suffered greater losses than this but parameter effects were not additive and increasing the number of parameters from two to three did not necessarily result in greater species loss. Runs with midpoint, rate and skew variance lost a mean of 3.0 species, which was greater than those with midpoint and skew variance (mean 2.6) but less than midpoint and rate runs with suffered the most severe species loss from control groups (mean 3.9).

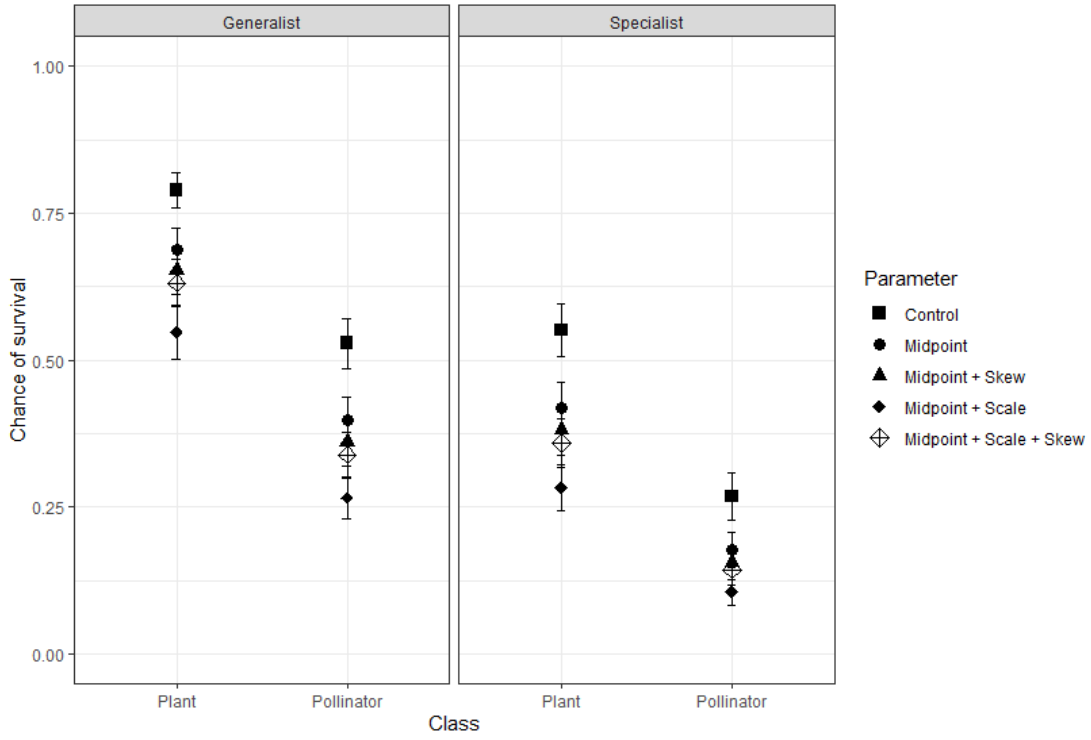

Figure 4.5: Plotted results binomial model (see Table 1) as predicted percentage chance of species survival (with standard error bars) across all 60 runs. Model structure: Persistence ~ Class + Generalist + Parameter, N = 2,309

Both in absolute and in relative terms more pollinator species were lost than plant species. Across runs with phenology shifts a mean of 5.4 pollinator species were lost across the 50 seasons from a starting mean pollinator species count of 8 (-67.5%) compared with a mean loss of 4.8 plant species from a starting species count of 10 (-48%). A binomial model fitted to species' persistence from season 20 to season 70 confirmed this (Figure 4.5, Table 4.2), with pollinator species less likely to persist than plant species (79.1% persistence chance vs 55.9%, -23.2% difference p = <0.001). Specialist species of either pollinators or plants were also significantly less likely to persist than generalists (-23.4% persistence chance for specialists, p = <0.001).

Table 4.2: Coefficient summary from binomial species survival model, fitted using glmmTMB. Model structure: Survival ~ Class + Generalist + Parameter, N = 2,309

Predictors	Likelihood of Persistence		
	Odds Ratios	*CI*	*p*
Intercept: Parameter (Control) Generalism (Generalist)	3.75	3.13 – 4.49	**<0.001**
Generalism : Specialist	0.33	0.28 – 0.38	**<0.001**
Class : Pollinator	0.30	0.26 – 0.35	**<0.001**
Parameter : Midpoint	0.59	0.48 – 0.72	**<0.001**
Parameter : Midpoint + Skew	0.50	0.41 – 0.62	**<0.001**
Parameter : Midpoint + Rate	0.32	0.26 – 0.40	**<0.001**
Parameter : Midpoint + Rate + Skew	0.46	0.37 – 0.56	**<0.001**
Model Data			
Observations	2,369		

Population demographics

Total population size (across all species) for plants remained at a consistent level across runs and phenology parameter combinations (mean = 1,595 ± 2) while the total population size of pollinators was highly variable across runs (mean = 12, 676 ± 10,258). Mean pollinator population size in runs with phenology shifts active was typically lower than that of the control group, following a similar trend to that of species loss, although midpoint runs had a slightly higher average population than control runs (Control mean = 17,573; Midpoint = 18,229; Midpoint + Skew = 10,730; Midpoint + Rate = 7, 094; Midpoint + Rate + Skew = 9,293). Despite these broader trends, however, total pollinator populations were highly variable, although as a

general rule total populations decreased when phenology shifts were present (Figure 4.6).

The total plant population had a hard upper threshold of 1,600 due to the landscape space available, with two plant individuals unable to occupy the same space. Plant populations grew to approach their upper threshold within the initial 20 seasons before temperature changes began. That plant populations operated at near-maximum capacity from the outset, coupled with the fact that plants were able to self-reproduce even in the event of mismatch with pollinators, meant that overall plant population was comparatively immune to the effects of phenological mismatch. However mismatch between plants and pollinators did affect the reproductive output of individuals and therefore ultimately affected community composition and individual species' persistence.

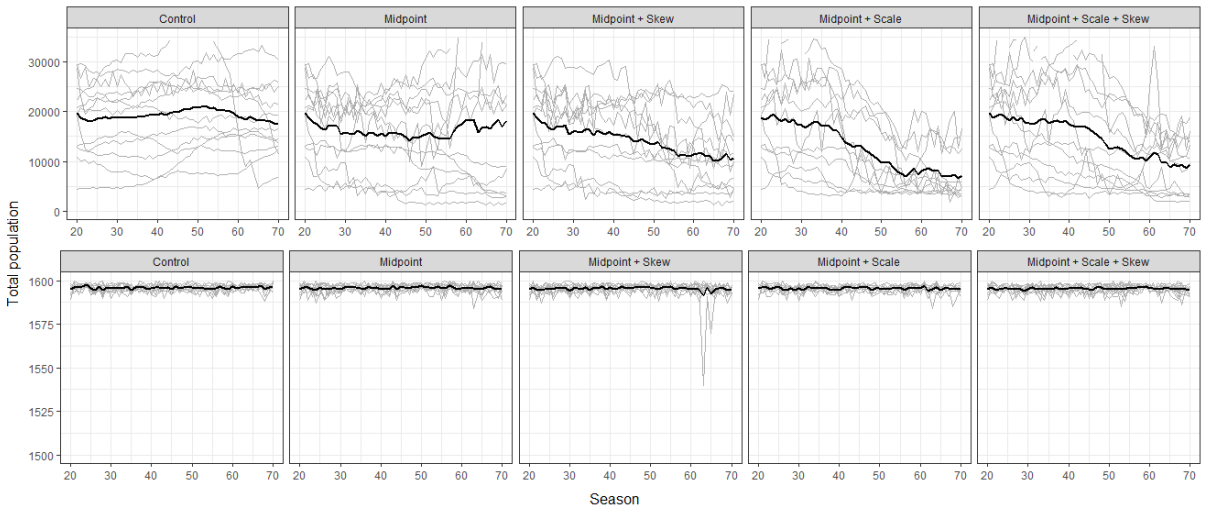

Figure 4.6: Total population of pollinators (top row) and plants (bottom row) by season for each run (grey lines, N = 12). Bold black lines show the mean population across all 12 runs for the control group and each combination of phenological parameters.

The high level of competition for space among plant species can further be seen in the population demographics of individual species (Figure 4.7). Among plant species there is a high level of stochasticity in the population trajectories of individual species even within the control group as they compete for landscape space. Conversely, pollinator species populations are highly stable in the control group but exhibit increasing levels of stochasticity in runs where phenology shifts

are present. These increasing levels of stochasticity are indicative of phenology shifts and mismatch having more immediate fitness consequences for pollinator individuals than for plants, leading to broader demographic fitness impacts.

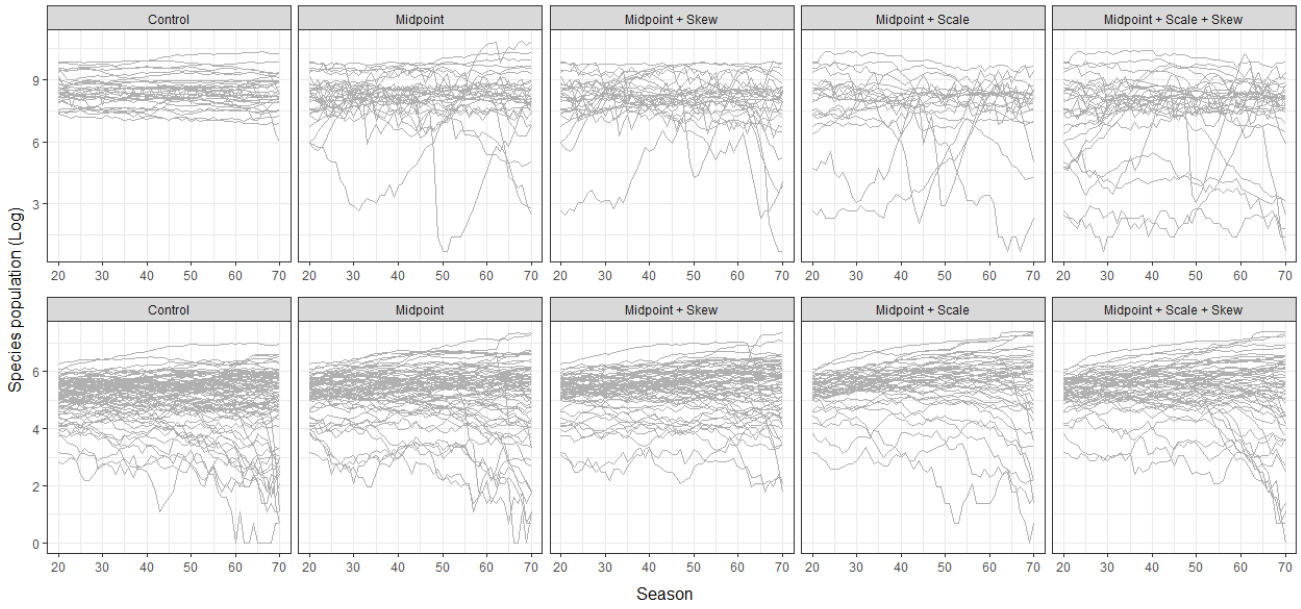

Figure 4.7: Population trajectories of species extant at season 70 across all runs, split by phenological parameters and pollinators (top row) and plants (bottom row).

Community composition

The structure of the final (season 70) communities differed between control runs and phenology shift runs in measures other than species richness. The population composition of these communities also changed, with runs incorporating phenology shift having a more highly dispersed distribution of population sizes among extant species in the final communities. Histograms of extant species' populations (Figure 4.8) illustrate that the distribution of populations exhibited a concentration of high-population species but a greater number of low-population species when phenology parameters were allowed to shift, with this effect being more pronounced in pollinator species than in plant species. The presence of several highly populous species populations is indicative of the final communities being less diverse in terms of species composition in runs with phenology shift than in control runs. In other words, even in final communities with numbers of extant species

comparable to the control runs, the communities of runs with phenology shifts present tended to be dominated by large populations of a small number of species with other species poorly represented.

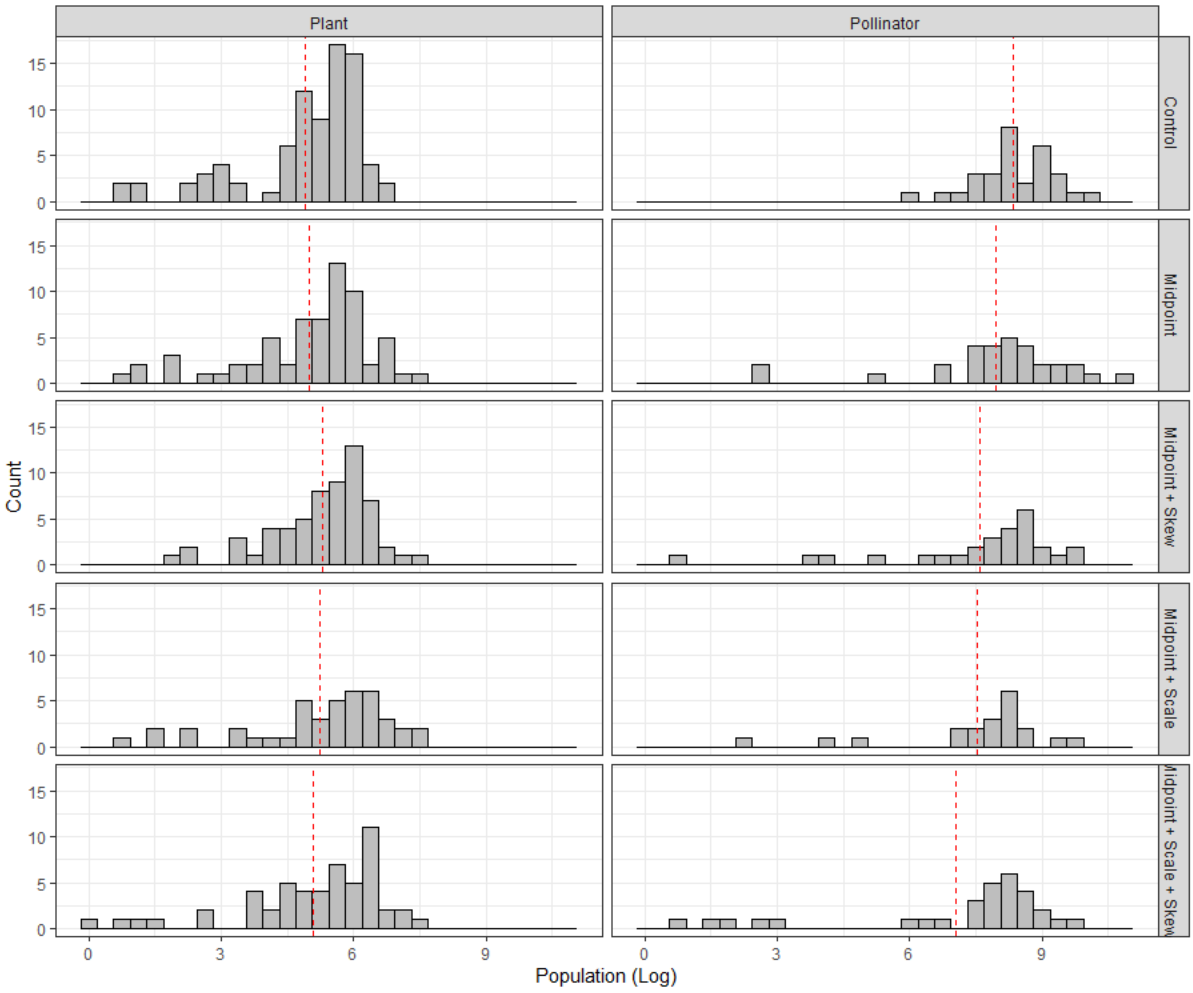

Figure 4.8: Histograms of log population for each extant species at season 70, across all 12 runs, split by plant/pollinator and phenological parameter. Red dashed line represents the mean population for each panel. (Control plant N = 82, pollinator N = 30; midpoint plant N = 65, pollinator N = 30; midpoint + skew plant N = 61, pollinator N =27; midpoint + rate plant N = 42, pollinator N = 20; midpoint + rate + skew plant N = 53, pollinator N = 30).

The lack of diversity in community composition for phenology shift runs was confirmed through computation of the Shannon index (Figure 4.9), a measure of diversity that accounts for both species diversity and relative representation of different species in a community. The mean Shannon index of all four combinations of phenology shift were lower than that of control runs (control mean = 2.24 SE ± 0.36; Midpoint = 2.01 ± 0.34; Midpoint + Skew = 1.96 ± 0.288, Midpoint + Rate = 1.67 ± 0.36, Midpoint + Rate +Skew = 1.87 ± 0.38). The trend of this loss of diversity

again followed a similar pattern to that observed for species diversity and total species population: Midpoint and Midpoint + Skew runs suffered the least relative loss of diversity and Midpoint + Rate runs the most. Again, the Shannon index was not universally lower than the control runs for all phenology combinations, and in some cases, phenologically sensitive runs outperformed their control counterparts. However, the overall impact of phenological sensitivity can be characterised as a trend towards less populous, species depauperate communities dominated by a few key species.

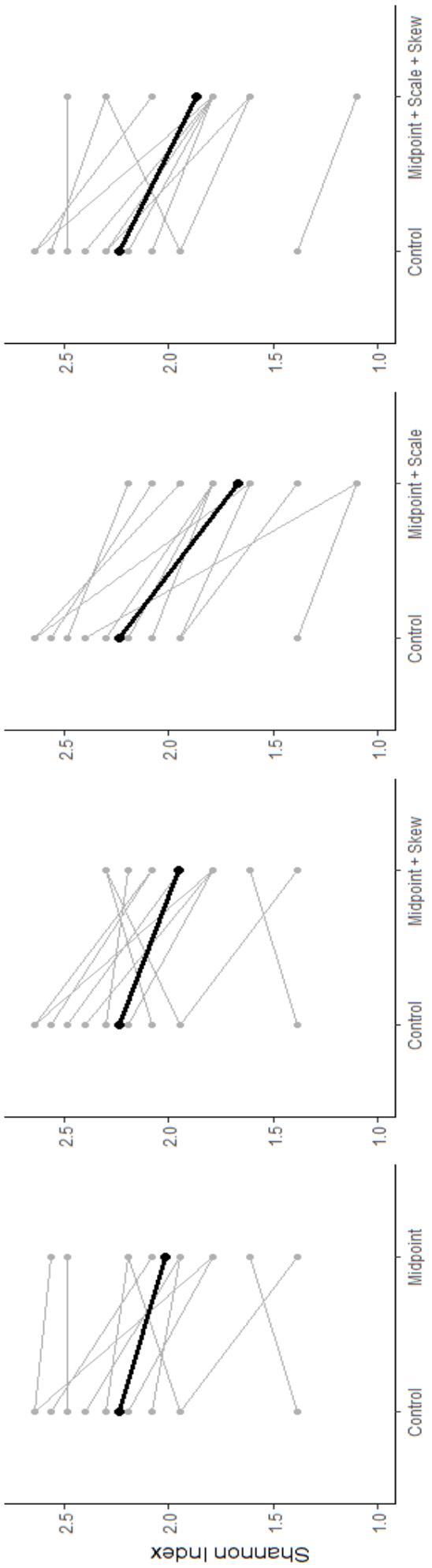

Figure 4.9: Community diversity (represented by Shannon index) at season 70 for each combination of phenological parameter, contrasted against control group. Grey lines are individual runs (N = 12 for each panel) while the bold black line is the mean across runs. Control mean = 2.24 ± 0.36; midpoint = 2.01 ± 0.34; midpoint + skew = 1.96 ± 0.288, midpoint + scale = 1.67 ± 0.36, midpoint + scale + skew = 1.87 ± 0.38

Discussion

Accounting for changes in the shape of abundance distribution across a phenology window affects predictions of mismatch and species persistence. This is highly relevant given that much of the focus of research on phenology shifts continues to be on measurement of single events (Inouye, Ehrlen & Underwood 2019) and use of single-date metrics to model species' phenology (e.g. Memmott *et al.* 2007, Bartomeus *et al.* 2011). Recent phenology studies have trended towards characterising phenological activity in terms of Gaussian distributions spanning a phenology window, with a focus on the midpoint parameter (e.g. Duchenne *et al.* 2020), but even this approach is still relatively uncommon (Edwards & Crone 2021). In my simulations variation in midpoint alone resulted in the least amount of species loss, therefore studies which focus on phenological mismatch in terms of midpoint alignment are likely to underestimate the impact of phenology shifts on mismatch – under certain scenarios the likelihood of species extinction can be underestimated by as much as 25%.

Using an individual-based model I simulated how changes to the shape of a phenological distribution affected species persistence and community composition for a generated pollination network. Three parameters that affected the shape of species' phenology were considered: midpoint, skew and rate. I found that whatever the precise parameter altered, shifts in phenology resulted in simulations losing both plant and pollinator species. Species persistence and abundance was particularly negatively affected for pollinator species, and for specialist species of either plant or pollinator. All simulations in which phenology shifts were active trended towards less speciose, less populous and therefore less diverse communities than control runs which did not have phenology shifts. Incorporating parameters describing distribution shape into the simulation did impact outcomes: runs where only the midpoint of a distribution was allowed to shift exhibited the lowest levels of species loss. Combinations of the other parameters of rate and skew resulting in higher levels of species loss over time, with up to double the number of species lost compared to runs where only midpoint was sensitive to shift.

Shape of phenological events

Our findings illustrate the need to incorporate metrics that reflect the full phenological distribution into analyses of phenology. Due to the lack of research describing the shape of phenological events one of the model's limitations is that I was not able to calibrate phenology parameters against results from the literature, with the exception of midpoint. Statistical methods that can be used to derive these parameters have already been proposed (e.g. Steer, Ramsay & Franco 2019) and these can fit distribution shapes to the kind of observational data frequently used in phenology.

It should be noted that data derived from observational recording schemes, as is often used in phenology research, can present difficulties. Variation in recorder effort can have a confounding effect on accurate representation of the underlying distribution (Chapman *et al.* 2015), but these effects can be compensated for (Pearse *et al.* 2017). Certain studies have already successfully applied fitted shapes to observational data, such as in describing the shape of butterfly phenology (Edwards & Crone 2021), but these examples are scarce. These methods still primarily assume a symmetrical shape for any fitted distribution, therefore parameters describing any element of skew would not be accounted for (although there are methods for describing asymmetrical phenological responses that have been applied to flowering phenology – see Austen, Forrest, Jackson & Weis 2014).

Species persistence

A tentative conclusion from the simulations would be that fitting symmetrical Gaussian distributions to phenologies may capture the worst-case scenario for emerging mismatches. The combined rate and midpoint parameters produced the highest rate of species extinction across simulations and both these parameters can be derived from symmetrical Gaussian distributions. These estimates could be inaccurate if asymmetry is not accounted for, however, as (for example) if a

symmetrical distribution is assumed for a distribution with high right-skew then the rate parameter for onset of the event is likely to be underestimated. Although rate and midpoint combined produced the highest extinctions rates, interaction between different parameters was not entirely predictable in the direction of its effect. Combining parameters resulted in lower species persistence than midpoint sensitivity alone and this finding was consistent across simulations. However, combining parameters did not produce an additive effect for extinction rates. Instead, combining all three parameters seemed to ameliorate negative effects and improve species persistence relative to runs with the two parameters of midpoint and rate. Given that the outcomes of these interactions are not additive, it is possible that certain values of skew could interact with other parameters in a way that would produce even lower rates of species persistence than those observed for midpoint and skew variations. That the interplay of parameters is uncertain highlights the need to embed measurements of abundance distribution in phenological studies in order to better understand how changes in shape may impact mismatches. If we do not, then we may significantly underestimate the impact of phenology shifts on species persistence.

Whichever phenology parameters were active, pollinator species had a lower chance of persistence than plant species, and specialists had a lower chance of persistence than generalists. It is a realistic assumption that plants are better able to persist in the event of mismatch with obligate partners than pollinators and this was reflected in the model assumptions. If unpollinated, plant individuals were still able to self-fertilise in the model, though pollination increased reproductive output and had demographic consequences for species' populations. Pollinator individuals, on the other hand, simply died if unable to feed sufficiently and therefore did not reproduce. There is a possibility that part of the difference between stability of plant and pollinator populations is driven by modelling choices, as the choice of lottery-based competition when modelling plant growth may have increased the stability of extant plant species populations. While lottery competition is not an inherently stable system (Chesson & Warner 1981), in the absence of competitive pressure generated by an increase in population density from other plant species, extant populations can remain stable as there are not fitness effects incurred over an individual's lifetime. In the case of pollinators there are ongoing competitive and

fitness effects throughout a pollinator's lifetime as they need to compete with other individuals for floral resources in order to survive until reproductive maturity. A plant individual's survival is instead a binary effect modelled at onset of a season and plant individuals undergo no further fitness costs over the course of their lifetime, which could lead to greater stability of species populations.

There are sound theoretical reasons why pollinators should suffer more immediate consequences from phenological mismatch with plant bloom than plants. Pollinators rely reliance on flowers as a food source and the deleterious effects of phenology shifts have been predicted to be greater for pollinators than plants, at least initially (Hegland *et al.* 2009). My findings of greater species loss in pollinators than plants therefore develops this theory even if the fitness impacts of phenology shifts on pollinators remain understudied using real-word data (Forrest 2014). While plant species were also lost under phenology shifts, this effect was less pronounced due to selfing. However, if plants are forced into higher rates of selfing due to mismatch then repeated selfing may incur costs not immediately apparent in terms of species persistence. While a majority of flowering plant species are capable of selfing, only 10-15% rely on it as a primary mode of reproduction (Wright, Kaliisz & Slotte 2013) because insect-mediated pollination results in a greater seed-set and reduced accumulation of deleterious mutations (Miller-Rushing *et al.* 2010, Wright, Kalisz & Slotte 2013). Therefore, while plants had higher species persistence in the simulations, there are likely to be negative side-effects of a raised selfing rate not accounted for in the model.

I also found that specialist species were less likely to persist than generalists. Intuitively this relationship might be expected – if the pool of interaction partners is small for a given species, if that species shifts phenology it is less likely that the species will still overlap with a partner than a species with many partners. Indeed, cases of mismatch between extreme specialists and their interaction partners have already been documented (e.g. Robbirt *et al.* 2014, Kudo & Cooper 2019). My simulation findings support the idea that specialists are at higher risk of mismatch and indicate that specialist species appear to be at greater risk of population loss and/or extinction under phenology shifts.

Community composition

I found that simulations of phenology shifts resulted in less diverse, species depauperate communities than in control groups. The disproportionately high loss of specialists compared to generalists also resulted in end communities having a reduced number of total interactions, which decreases network robustness (Song, Rohr & Saavedra 2017). Less robust networks are more highly susceptible to future stressors, such as pesticides and landscape change (Winfree *et al.* 2018). When interpreting these results, it should be noted that the simulations often did not start from a point of highly speciose networks after the burn-in period (plant species mean 9.6 ± 1.8, pollinator species mean 7.8 ± 1.0 at season 20). This relatively low species diversity was necessary from a practical perspective in order to make computation times tractable. Biologically, however, this diversity is not out of step with the diversity exhibited in arctic networks (Robinson, Losapio & Henry 2018), however higher rates of biodiversity are thought to mediate against the effects of phenology shift in pollination networks (Bartomeus *et al.* 2013). Therefore, it is possible that species persistence would be higher in networks that were more speciose at initiation, and these networks would therefore retain a more diverse community.

The observed loss of species diversity is also a potential explanation for why total pollinator populations declined alongside species loss in the simulations. It could be expected that as other pollinator species became extinct, the population of extant species would increase their populations to fill the capacity, but extant species did not fill the system's population capacity. Although the precise reason is difficult to parse from the simulation results, it has been shown that a reduction in species richness and nestedness of pollination networks increases levels of inter- and intra-specific competition between remaining species, as the pool of available interaction partners reduces (Encinas-Viso 2012). Increased levels of competition for floral resources may therefore have reduced the ability of extant species to expand their populations to compensate for the numbers lost by species trending towards

extinction. Speculatively, this could indicate that phenology shifts in pollination networks result in certain niches going unexploited, reducing the total population.

Conclusion

Our work reinforces predictions that climate-induced phenology shifts can have demographic impacts on pollination networks, ultimately resulting in species loss and less diverse communities. They predict that these impacts will be most severe for pollinator and specialist species: such taxa should be in focus when determining species that are most at risk from phenology shifts. Accounting for multiple parameters that affect the shape of phenological distributions affected simulation outcomes, with shift in multiple parameters leading to greater rates of species loss in both plants and pollinators. Considering the midpoint parameter alone, which is the state of much current phenology research, underestimates the predicted rate of species loss, with other combinations of parameter doubling the rate of species extinction in some cases. These simulations therefore highlight the need for phenology research to try to capture metrics that describe changes to the shape of phenology events as well as changes to key dates.

Chapter Five
General discussion

Phenology shifts are becoming more frequent as temperature rises increase, with considerable interspecific variation in phenological response leading to risk of temporal mismatch in pollination networks. To determine the level of this risk, and potentially identify species pairings in which it is likely to arise, we need to develop understanding of the fitness impacts of phenology shift, drivers of phenology shift and how mismatches may arise in the future.

During my thesis I examined the effects of phenology shifts in pollination networks in three ways. First, I quantified the fitness impacts of phenological variation on plant seed-set. Second, I examined phenology shifts over time in a key group of pollinators, hoverflies, and assessed how well life history traits predicted phenological response to changes in temperature. Third, I used a simulation approach to predict how phenology shifts may impact pollination networks in the future using techniques that accounted for the shape of abundance distributions within phenology windows. Here I restate the background to my chapters and the knowledge gained from them:

Chapter Two: Phenological mismatches are typically hypothesised to lead to negative fitness consequences for the interactions present in pollination networks. However, there is limited research explicitly tying phenological variance in pollination service to variance in reproductive fitness in plants. In this chapter, I induced temporal variation in the flowering time of experimental crops of *Raphanus raphanistrum subsp. sativus var. caudatus* and then measured seed set produced. I tied this to phenological variation in the abundance of pollinators present. I found that phenological variation in the relative abundance of pollinators resulted in higher seed set (and therefore reproductive fitness) in plants and that this effect was greater in certain groups of pollinators than others (notably *Bombus* species and *Apis mellifera*). I used these findings to hypothesise how the pollination service available to *Raphanus* varied across the field season.

Chapter Three: Anticipating which taxa are likely to exhibit a greater rate of phenology shift in response to climate change is important to understanding which species have the highest potential risk of future mismatch. It has been suggested that life history traits, such as diet breadth or overwintering stage, could be predictors of phenological response in certain pollinator groups, but this has only been tested in Lepidoptera. I examined phenological response to temperature in UK Syrphidae from 1980 onwards and tested whether life history traits explained variation in response. I found that the vast majority of species had advanced the start of their flight period in response to warming temperature, but this response was asymmetric across their flight period with peak flight date advancing at a slower rate and final flight date having no significant response to temperature. Overwintering stage was the only life history trait that significantly predicted phenological response, with species that overwintered as adults showing a greater sensitivity of phenological response to temperature change. Incidence of voltinism appeared to have no relationship with temperature. I concluded that it may be difficult to form general hypotheses about interspecific variation in phenological response through life history traits.

Chapter Four: Phenological events are often described in terms of the time window they encompass, but abundance distributions are not stable across phenologies. Accounting for the shape of abundance distributions and how they may change in response to rising temperatures is likely to affect expectations of phenological mismatch. I tested this hypothesis through an individual-based simulation of a pollination network, which characterised the shape of abundance distributions through three parameters (midpoint, rate and skew) and allowed these parameters to shift over 50 seasons. I found that under all simulations where phenology shifts were active, there was an increase in species loss compared to controls and communities trended towards becoming less diverse. Shift in midpoint alone, effectively the parameter much phenology research measures, severely underestimates prevalence of mismatch and, over time, also underestimates the rate of species extinction. Pollinators and specialist species were found to be more likely to become extinct than plants and generalists.

Here I reflect on what these findings mean for three core areas of phenology research and speculate on some future directions of study: (1) The measurement of mismatch, (2) Measuring and predicting phenology shifts, (3) The impacts of mismatches.

(1) Measuring mismatch

It is common to frame phenology research within the context of gaining an understanding of the risks of mismatch. However, quantifying mismatch, particularly in terms of potential fitness costs for interacting species, is challenging. One way to define mismatch is to measure historic levels of alignment of a species' abundance phenology with the phenology of its resource and then measure deviance from that alignment (Kharouba & Wolkovich 2020, see Figure 1.1). Comparison of the phenology of consumer and resource in this way is relatively common in marine research but is rarely applied to terrestrial studies of phenology (Burthe *et al.* 2012). In the context of pollination networks, effective pollinators would be the resource that flowering phenology should synchronise with and nectar/pollen availability the resource for pollinator flight phenology. Measuring phenological alignment with resources is difficult in the current pollination phenology literature for two key reasons: (1) Phenology research frequently does not describe abundance over time (see Chapter Four); (2) Variation in phenology is not always tied to variation in fitness (see Chapter Two, also Mungugia-Rosas et al. 2011). However, my work in Chapter Two indicates some methods by which we can construct continuous estimates of the phenology of pollination service against which we could compare flowering phenology.

In Chapter Two, I demonstrated that phenological variance in abundance of different pollinator taxa over a field season can be tied to variance in the seed set of plants. Therefore, it should be possible to create an estimate of pollination service available to plants over the course of the season that is based on the impact of that pollination service on seed set. If we can build a picture of phenological variation in pollination service then we can also measure to what extent flowering phenology

matches it. For example, we may hypothesise that peak flowering abundance should synchronise with peak pollination service, if this is the primary limiting factor on reproduction (Kharouba & Wolkovich 2020), and that deviance from that synchrony affects plant fitness. But to properly measure phenology mismatch and its effect on fitness we need to first measure the baseline level of synchrony, which current phenology research in pollination networks rarely seeks to define.

Within Chapter Two, I provided an estimate of pollination service using the relative abundance of pollinator groups and their impact on seed set (see figure 2.4). This is one method that could be used to build a picture of pollination service through time; certainly local pollinator abundance has been causally tied to seed set in previous research (Bommarco, Marini & Vaissiare 2012, Kehrberger & Holzschuh 2019). A potentially more accurate measure may be provided by multiplying pollinator abundance by pollinator efficacy or visitation frequency, which often show higher correlation with seed set than abundance measures (Vasquez, Morris & Jordano 2005). Using a product of efficacy and/or visitation frequency with local pollinator abundance to quantify pollination service is a common method in pollination research (e.g. Ballantyne, Baldouck & Willmer 2013, Garibaldi *et al.* 2013, Kleijn *et al.* 2015, Winfree *et al.* 2018). Therefore, employing methods of quantifying pollination service within the framework of phenology is eminently possible and would allow us to build a picture of phenological variation in pollination service that against which we could then compare flowering phenology.

It is also possible to perform the reverse, quantifying the phenology of floral resources available to pollinators. Recent research has already begun to quantify phenological variation in floral resources for pollinators through sampling nectar production (Timberlake, Vaughan & Memmott 2019, Tew *et al.* 2021). Although findings from these floral resource experiments are not interpreted in the context of phenology shifts, these techniques could easily be applied to questions of phenology mismatch. One hinderance to interpreting mismatches in the context of pollinators is relating floral resource availability to pollinator fitness, as our current capacity to anticipate the fitness costs to pollinators is limited (Forrest 2016). The extreme consequences of mismatch are clear – if a pollinator does not coincide with any compatible plants, it will die of starvation (Burkle, Marlin & Knight 2013). However, we understand very little of what fitness consequences pollinators would

suffer as a result of partial or emerging mismatches with food plants (Raffertey 2017). In social pollinators the consequences of mismatch could be examined through temporal transplants of nests, inducing them into activity at different times of the flowering season and measuring impact on colony mass and size (Goulson, Hughes & Derwent 2001, Forrest 2010).

If we are able to define the fitness impacts of floral resources on pollinators or pollinator efficacy on seed set, then we can start to build a picture of how expected fitness fluctuates through time and compare whether flight phenology or flowering phenology synchronises with that (Yang 2020). However, when interpreting mismatch through the phenology of activity periods and their level of synchrony with resources, it should be noted that there are myriad reasons why we would not expect variation in resources to map perfectly to fitness consequences in pollination networks. Although there are some morphological traits that seem to govern pollinator efficacy (Stavert *et al.* 2016, Phillips *et al.* 2018, Goulnik *et al.* 2020) there is also considerable interspecific variation in efficacy apparently unrelated to these traits (Bartomeus *et al.* 2017). Nectar production varies intraspecifically across flowering seasons (Parachnowitsch, Manson & Sletvold 2019). Confounding factors such as pest abundance or variation in plant investment in reproduction may counteract the response of seed-set to pollination service received (Jesson & Burd 2010, Parsche, Frund & Tscharntke 2011). Therefore resolving the phenology of the interacting species in a pollination network in fitness terms will be difficult, however it is certainly something to aspire towards and it is possible to resolve on a local scale. One way to reduce the complexity of this task would be to initially focus on interactions where one or both interaction partners have a high level of specialism. Identifying appropriate interactions has accompanying difficulties – interactions with a high level of specialism are rare – but there is evidence that phenology is a trait subject to strong selection in specialists (Ollerton & Diaz 1999) and shifts in phenology can lead to high fitness consequences for specialists (Robbirt *et al.* 2014). My work in Chapter Four indicates that specialist species are most at risk from mismatch, so concentrating on these species should also hypothetically be most important in understanding which species are most threatened.

Framing expectations of phenology match and mismatch in terms of synchrony with fitness peaks is a challenging task, but it will provide a more rigorous framework for

posing questions about phenological mismatch and therefore is a goal future research should strive to move towards. To understand in which systems and between which species these mismatches are likely to emerge, phenology research needs to move towards a more predictive understanding of phenology shifts.

(2) Measuring and predicting phenology shifts

Research into phenology shifts often takes a descriptive approach to characterising shifts (e.g Duchenne *et al.* 2020, Buntgen *et al.* 2022). Within studies of phenology shift the described changes are typically taken to be indicative of future shifts, but one of the challenges in phenology research is assessing whether shifts are predictable. In order to accurately describe species' phenology and, hopefully, anticipate the trajectory of phenology shifts in the future, we need to both understand the drivers of shift and produce more predictive models of phenology.

When looking to understand how and why species exhibit specific phenology responses, the emphasis is generally placed on gaining understanding of the processes and cues generating them (Chmura *et al.* 2019). In Chapter Three I found that species traits which have been successfully tied to phenological responses in butterflies (e.g. Diamond *et al.* 2011) are generally poor predictors of response in hoverflies. The traits tested in Chapter Three are typically not described as taxa-specific and could be generalisable to other arthropod groups. Therefore, it is telling that I found limited evidence for life traits explaining phenology response within hoverflies. This may in part be due to the rather patchy trait information that exists for some hoverfly species compared with butterflies. However, understanding of the natural history of hoverflies is comparable to that of other pollinator taxa such as solitary bees, beetles and other Diptera. Therefore challenges around natural history knowledge are likely to be found when attempting to generalise predictions about phenology response to other taxa.

If one of the goals of phenology research is the ability to predict phenology shifts then there are benefits to more explicitly reflecting this in phenology studies. As evidenced by the Hoverfly Recording Scheme data used in Chapter Three, there is a wealth of long-term phenology data already existing in the form of monitoring schemes (in particular across Europe, Asia and Northern America, but see Bush *et al.* 2018) and museum collections (e.g. Olsen *et al.* 2020). Much of these phenology data are already being consolidated in publicly-accessible databases (Lane & Edwards 2007, Templ *et al.* 2018, Buntgen *et al.* 2022) along with finely-resolved meteorological data (Hollis *et al.* 2019). There has been considerable research in the last two decades taking a primarily descriptive approach to phenology shifts (e.g Duchenne *et al.* 2020, Buntgen *et al.* 2022) or hypothesis-testing drivers of shift (e.g. Kharouba *et al.* 2014). With access to large databases considerable opportunities exist for predictive modelling approaches such as machine learning. Machine learning techniques have successfully been employed to form predictions of phenology shift in plants (Czernecki, Nowosad & Jabłońska 2018, Dai *et al.* 2019), but are not commonly applied to questions of animal phenology and constitute a potential route forward to more predictive models of shift.

Collated phenology databases also provide opportunities to employ novel methods of describing the shape of phenology events, which my findings in Chapter Four indicated as being of high importance to understanding the long-term consequences of phenology shift on species interaction. Despite calls for the shape of abundance distributions to be accounted for in phenology analysis (Inouye, Ehrlen & Underwood 2019, Visser & Gienapp 2019) methods describing distributions, particularly ones with asymmetry, are yet to be routinely integrated into research (but see Austen, Forrest, Jackson & Weis 2014). However, my findings in Chapter Four indicate that understanding how distributions may change along with phenology is key to determining the potential impacts of phenology shifts. One way to build iteratively on my thesis work would be to apply these methods to the HRS data used in Chapter Three as my findings in this chapter already indicate an asymmetrical phenology response to temperature, with first date of flight more highly responsive than date of peak flight or final flight date.

The proliferation of compiled data sets and the possible research gains to be made from them do not occlude the need for collecting phenology data on a more refined

local scale, such as long-term monitoring of specific sites (Denny *et al.* 2014) or manipulative field experiments (Morton & Rafferty 2017). Wadgymar *et al.* (2018) demonstrate how these approaches can be combined to generate insights into how environmental cues influence phenology by combining observation records over 43 years with field manipulations of 6 species of forb. From analysing trends in the long-term data Wadgymar *et al.* (2018) hypothesised which species would be more responsive to heat cues for flowering phenology and which were more responsive to diurnal cues. Wadgymar *et al.* (2018) then tested and supported these hypotheses in the field by manipulating the temperatures that the different species were exposed to (through snow removal or addition) at different times of the year. Long-term observational data can therefore be used to yield hypotheses for testing in manipulative experiments, which can in turn be used to inform the analysis of large, collated phenology data sets.

(3) The impact of phenology mismatches

In Chapter Two, I demonstrated how plant reproductive fitness can be affected by variance in pollinator phenology, and in Chapter Four I simulated how phenology shifts can lead to severe fitness impacts, and ultimately species extinction, in plants and pollinators over time. Predictions of high rates of species loss from phenology shifts are in line with those from existing research (Memmott *et al.* 2007, Burkle, Marlin & Knight 2013). Therefore, the potential exists for phenology shifts to severely reduce biodiversity in pollination networks even in the absence of aggravating effects from other stressors (Ramos–Jiliberto *et al.* 2018).

One factor that is of prime importance to understanding whether scenarios of species loss from phenology shift will occur is understanding what the capacity is for plants and pollinators to adaptively respond to phenological mismatch. One way in which species can respond is behaviourally. Simulations of shifts in pollination networks do not always account for changes in interaction pairings (Memmott *et al.* 2007, Ramos-Jiliberto *et al.* 2018), however turnover of interactions is widespread in pollination networks (Carstensen, Sabatino & Morellato 2016, CaraDonna *et al.*

2017). If species have a high degree of generalism, then they may simply shift their interaction partners to match whichever species they co-occur with – pollinator species may well be less restricted in this regard than plant species as they are frequently more generalist in their pairings (Willmer 2012). However, it should be noted that both my work in Chapter Four and Burkle, Marlin & Knight (2013) accounted for the possibility of novel interactions emerging with phenology shifts and still predicted high levels of species extinction. However, it may be that the turnover of interactions in these simulations was lower than that which may occur naturally, which would alleviate species loss.

A second way in which species may respond is adaptively, either by altering their phenological response to track historic interaction partners or by adapting to new interaction partners. Evidence for both kinds of adaptation have been found in insect species. For example, warming temperatures have reduced the abundance of long-corolla flower species that co-occur with two species of specialist bumblebee. Previously adapted to these deep flowers, the bumblebees have adapted by becoming more generalist. Over 40 years the bumblebee populations have shifted from having long tongues specialised to their previous interaction partners to short tongues which allow them to be more effective generalist foragers (Miller-Struttmann *et al.* 2015). Whether the bumblebees' adaptation represents a plastic shift in phenotype expression or underlying selection on genotypes is unclear in this example. However, another example seems to show genotype selection in action as an adaptation to phenology shift. The specialist moth *Operophtera brumata* relies on bud burst from a specific oak species for larval food, but synchrony between egg hatch and bud burst has been disrupted by rising temperatures. Selection to maintain synchrony with oak bud burst has caused egg temperature response to change genetically, favouring genotypes with a slower development time so that hatching date remains constant even in the face of rising temperatures (Van Asch *et al.* 2013). Understanding the extent of these adaptive responses and the limits of them is key to understanding the long-term risks of phenology shift. It is telling that these examples come from specialist insect species. My results from Chapter Four, in line with existing research, indicate that specialists are most likely to be under threat from phenology shifts and therefore should have the strongest selection pressures acting on them. Also in line with existing literature, my work from Chapter Three shows that species with earlier emergence or flowering dates exhibit higher

rates of shift. Therefore early-emerging specialists should both be under the highest selective pressure and be at the greatest risk of emerging mismatch. Future research into potential adaptive responses of species should look to species that fit these criteria as study systems.

If phenology shifts are expected to negatively impact on species persistence in pollination networks it is worth asking what this means for the stability of these networks in the face of a variety of other stressors such as habitat loss, disease and pesticide use (Goulson *et al.* 2015). Set alongside tangible threats that operate within a much shorter timescale, the seemingly inexorable effect of long-term climate change may seem a more intractable problem. Happily, many of the measures that could reduce the impact of long-term phenology shifts are in line with contemporary conservation efforts. Fundamentally, greater biodiversity increases pollination network robustness and reduced susceptibility to future disruption, and this seems as true for phenological stressors as for any other (Bartomeus *et al.* 2013, Bartomeus *et al.* 2021, Senapathi *et al.* 2021). Habitat corridors for plants and pollinators, such as roadside verges (Phillips *et al.* 2020), can increase genetic diversity in linked populations, which may help adaptive responses to phenology shift (Visser 2022). Phenology can also be considered more explicitly in conservation efforts – if we can understand the phenology of floral and pollinator resources, as described in the first section 'Measuring mismatch', then this will aid in understanding of resource availability throughout the season. For example, Memmott *et al.* (2010) predicted future reduction in floral resource availability in the early and late periods of pollinator flight seasons due to phenology shift and recommended incentivising farmers to plant wildflowers with flowering phenologies that would fill these deficits (see also Timberlake *et al.* 2019). Improving understanding of which species are susceptible to shift will allow us to anticipate these emerging resource gaps in the future.

The impact of phenology shifts on pollination networks will therefore be defined by how well species within those networks can adapt. But there are conservation efforts that can be made to increase current biodiversity that may help to protect networks from future impacts. Phenology research can help to bolster conservation efforts by identifying and predicting resource gaps over time.

Conclusion

The field of phenology research has undergone considerable growth over the last three decades, but considerable knowledge gaps remain. To understand the consequences of phenology shifts, we need to build stronger definitions of mismatch and be better able to predict where these might emerge. I have argued above that we should understand mismatch in terms of fitness consequences for interacting species. Understanding fitness consequences necessitates first measuring direct fitness impacts from phenology mismatch and then mapping these onto a model of phenology that accurately reflects how phenology affects abundance through time. If the research field can move towards accurately determining mismatch, then predictive models of phenology can be used to more accurately pinpoint which species and networks may be more susceptible to future impacts of phenology shifts.

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