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The Burnt-Tip Orchid (*Neotinea ustulata*), future restoration in Kent

A thesis submitted to the University of Kent for the degree of

MSc Biodiversity Management

2021

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School of Anthropology and Conservation

Declaration

I declare that this thesis has been composed solely by myself and that it has not been submitted for any other degree or professional qualification except for MSc Biodiversity Management (by-research). Except where stated otherwise by reference or acknowledgement, the work presented is completely my own.

Jacob Coles

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Abstract

The Orchid species *Neotinea ustulata* is a small terrestrial species native across Europe, the populations found in the British Isles have been decline since the 1930's. As part of this decline the populations once found in the county of Kent have reduced to just one site in the past decade. The decline of this species is little understood and the drivers of it have only been speculated about in recent literature.

This research aimed to assess two areas of concern for the species long-term viability, the effects of anthropogenic climate change on it's pollination phenology and the potential for pollinator decoupling and the public perception of the species to develop an understanding of the species within the funding landscape.

The Best-Worst Scaling Type 3 questionnaire constructed and distributed indicated the species as being competitive from within the funding landscape within the context of other species found it its immediate habitat, calcareous grasslands. Scoring the highest proportion of the votes in five of the six choice experiments conducted among both relevant orchid and flora species found on calcareous grasslands.

The pollination ecology of *N. ustulata* is little known in the British Isles with pollinating species only known from Germany. The known pollen vector for the subspecies var. *ustulata* (*Leptura* livida) is known from the British Isles whereas the pollen vector for var. *aestivalis* is not regularly reported from the British Isles. Potential pollen vectors for var. *aestivalis* in the British Isles were identified as *Tachina grossa¸T. fera, T. ursina,* and *T. lurida*. All pollen vectors showed a response to historic increases in springtime temperature of between 1.9 and 11.9 days earlier per °C increase in mean springtime temperature for peak flight dates. *N. ustulata* also showed the same response with a change of 4 days earlier per °C increase in mean springtime temperature.

The findings of this thesis indicate the potential for positive community engagement with the conservation of *N. ustulata* as well as the potential risks to the pollution ecology of *N. ustulata* with advancing climate change.

Contents

Chapter 1: Introduction

The Kent Flora

Located on the most south-easterly part of the British mainland, Kent is considered by many to hold the greatest diversity of flora species found throughout the British Isles (Philp, 1982). Whilst many species are found exclusively in other regions of the country (Bowmer, 2008, Harrap and Harrap, 2005), the geography and geology of the county assist in creating an area hospitable to many different flora species (Philp, 1982). The location of the county has multiple impacts on the species to which the area is habitable.

Kent sees mean temperature higher than many other parts of the country throughout the year (Ogley, 1991), whilst high summer mean temperatures and low precipitation rates can make areas inhospitable, higher mean winter temperatures make the area more hospitable to more plant taxa (Tullock, 2005). Similar milder climates are found elsewhere in the country, such as Devon (Met Office, 2021), making them suitable to hardy/semi hardy non-native species grown for domestic horticulture (Candlin, 2021). However, the location of these other areas are more isolated from seed vectors, whereas the southern coast of Kent is the part of the British mainland closest to the European mainland, providing a wide range of species that seed vectors could introduce to the county. With its proximity to other European countries making it the most likely land area for species from mainland Europe to be introduced by natural seed vectors. This was seen when in 2020 the orchid *Serapias vomeracea* was found for the first time in the UK at a site in Kent, the plants have been recorded again in June 2021 although the site has not been disclosed (Anon. Pers. comm. 2021). The discovery of new species to the British Isles in Kent and other nearby areas is likely to increase in the coming years, where anthropogenic climate change makes habitats at increasingly higher latitudes suitable for more species as its effects develop (Lenoir and Svenning, 2013). It has been hypothesised that the Giant Orchid (*Himantoglossum robertianum*) could be the species to next make the jump across the English Channel and could already be growing at sites in Kent although no flowers have been recorded as of summer 2021 (Anon. Pers. comm. 2021).

Although the natural migration of flora species across the English Channel is possible for many species whose seed vectors can easily cross the distance (i.e. wind, migratory birds) (Gouyon *et al.*, 1987), others may need to be assisted in their latitudinal shifts if their seed vector is unable to move their population to the new land masses (Vitt *et al.*, 2010).

Calcareous Grasslands

One of the habitats that characterises the county is that of calcareous grasslands (Kent Wildlife Habitat Survey Partnership, 1995). These are areas of grasslands often found along chalk-rich geological areas, characterised by an alkaline substrate, and often pieces of chalk and similar rock found throughout the substrate . The conditions provided by the substrate often makes the sites habitable to a unique group of species that can tolerate the basic soil types (Wallis De Vries, 2002). Species with specific substrate pH requirements are potentially at greater risk than those of a more variable soil pH (Singh *et al.*, 2011). Many species have historically been able to co-exist with agricultural practices undertaken on calcareous grasslands (Ridding *et al.*, 2020, Poschold, *et al.*, 1998), however as agricultural practices have developed methods for increasing the agricultural yield of land areas through the use of artificial compounds designed to maximise productivity (Storkey, Moss and Cussans, 2010). These practices have now made them unsuitable for many of the species that evolved to the conditions, leading to many of the calcareous grassland habitats suitable for specialist flora species (Mitchley and Xofis, 2005) becoming increasingly isolated from other plant populations and potentially becoming prone to genetic inbreeding (Leblois, Estoup and Streiff, 2006). One of the taxonomic groups with the highest proportion of species found on calcareous grasslands is the family Orchidaceae, with 22 of the 52 British native species being found on calcareous grasslands within the British Isles (Harrap and Harrap, 2005).. All of these species are found within Kent, making it the most orchid diverse county in the British Isles (Johnson, 2019).

Orchid Conservation

Due to the specific conditions required for orchid seed germination, restorations or reintroduction initiatives often establish methods of *ex-situ* cultivation before the initiation of any restoration (Ramsey and Stewart, 1998, Decruse *et al.*, 2003). Orchid seed germination rates *ex-situ* are relatively low when compared to other plant taxa grown *ex-situ*. The specific ecology that surrounds orchid seed germination in the wild (Koopowitz, 2001) see the frequent use of micropropagation techniques to increase the likelihood of orchid seed germination (Arditti, 2008). Whilst the use of micropropagation techniques is not mandatory for the germination of orchid seeds, seeds of some temperate orchid species are known for sporadically germinate when set in the pot of their seed parent when grown in cultivation (Yam and Arditti, 2009), this method cannot be used to germinate and establish plants reliably.

A 2016 literature review assessed the success of 75 studies involved in the translocation/reintroduction of Australian native Orchidaceae species between 2007 and 2014 and their reported success rates (Reiter *et al.*, 2016). Whilst the species included in this review are ecologically unrelated to the species concerned in the British Isles, the effect of methodology and translocation successes will translate to plant restorations/reintroductions globally. This study outlined a set of success criteria for perineal flora reintroduction/restorations, defining how successful reintroductions/restorations should be classified through the development of the restored/reintroduced population and its potential recruitment. Reiter *et al.* (2016) define these into 4 discrete stages for long-term success. Years 1-3: a large enough number of plants should have survived the initial translocation for the transplanted population to be considered a viable population, which represent a spread of genotypes of the source population, an emergence rate is present in the new population comparable or above that seen in wild populations and the rate is stable after the initial deaths related to translocation shock. Years 3-7: Plants should be established, flowering and there should be evidence of successful fruit set, rates of emergence, flowering and fruiting should not be significantly below benchmark populations. Years 8-14: Emergence rates stable, evidence of recruitment in recipient site, flowering and fruit set rates should not be below benchmark population. Years 15+ Population on recipient site should be self-sustaining, and its area of occupancy should be stable or increasing, number of new recruits should exceed the mortality rate, population growth should be greater than or equal to 1. Whilst the timings involved are just guidelines and could be amended to suit the temporal aspects of relevant species the outlined criteria should be aimed towards and adhered to for any existing reintroduction/restoration programmes.

Other reported statistics in the paper include the success rates of both local and global translocations, whilst every reported translocation resulted in some re-emergence of plants in successive flowering seasons, re-emergence rates varied considerably, one case study reported that on site 'C' translocated plants of *Caladenia clavescens* re-emergence rates were 19%, whereas the related species *C. cruciformis* on site 'D' was 97%, however the mean re-emergence rate for the Australian review was 61.63%, a high enough re-emergence rate to allow for a positive succession into long-term success. Other important considerations include ecological knowledge of species prior to translocation, with only one species translocated to a site where the pollinator is known prior to translocation. Other species were translocated without prior knowledge of pollinator presence/absence Decreasing the likelihood of the translocated individuals transitioning into a long-term sustainable population through a lack of reproductive success and low seed set, only 2.8% of the species reviewed globally recorded recruitment in the translocated sites. Only 12% of the reviewed translocations also involved translocating enough plants to constitute a population large enough to be considered sustainable for long-term survival, only one of the translocated populations showed a 100% mortality rate.

Orchids of the British Isles

Compared with many similar sized tropical nations the United Kingdom has relatively few native Orchidaceae species, with 52 species known to have sustainable populations in the country (). Of those, all 52 are terrestrial species, found in many different habitats and often having a greater dependence on their mycorrhizae than tropical epiphytes (Koopowitz, 2001). Two of the native species are known mycoheterotrophs (Merckx, 2013), parasitising their associated fungus for their entire life cycle, and producing no chlorophyll (Bowmer, 2008). Of those 52 species, a number are known to be particularly rare nationally, *Ophrys fuciflora*, the Late Spider Orchid, is known only from the Kent Downs in the UK (Harrap and Harrap, 2005), while *O. sphegodes*, the Early Spider Orchid, is known only from coastal sites in Kent Dorset and Essex. The sole member of the slipper orchid genus, *Cypripedium* native to the UK is *Cypripedium calceolus*, was almost extinct in the wild (Harrap and Harrap, 2005) until recent work cultivating the species *in-vitro* and successfully reintroducing the species to two sites (Ramsey and Stewart, 1998), the locations of which are not regularly divulged.

Most species are considerably more common, with several species known in the vernacular with the title ‘common’, of these the most recorded is *Dactylorhiza fuchsii,* the Common Spotted Orchid, this species is regularly reported nationally from all areas of the British Isles (Harrap and Harrap, 2005). Most of the native species are not nationally common (Bowmer, 2008), rather many are locally common being confined to suitable habitats with an uneven national distribution (Harrap and Harrap, 2005). The most common species alongside *D. fuchsii* are *Neottia ovata*, the Common Twayblade, *Anacamptis pyramidalis*, the Pyramidal Orchid and *Orchis mascula*, the Early Purple Orchid (Harrap and Harrap, 2005).

The list of orchids found in the British Isles is regularly being updated, as the local climate is becoming more suitable for species from mainland Europe (Lord and Whitlash, 2015). It is hypothesised that with climate change, species are starting to move across the English Channel and are becoming established along the southern coast, with Kent being a hypothesised first land for many of the species. Over recent years species of Tongued Orchids, *Serapias*, have been recorded in the British Isles for the first time (Anon, Pers. comm. June 2021) as their suitable ranges has moved northwards, due to anthropogenic climate change . Whilst this may benefit some species (), the latitudinal shifts of population ranges could potentially see the UK become climatically unsuitable for other currently extant species in the future (Charitondou *et al.*, 2021).

Most species native to the British isles are currently facing some form of threat from human activities (Harrap and Harrap, 2005). Many species with more restrictive habitat requirements are under threat through the advancement of land use change to meet the demands of modern society, such as the mycoheterotroph *Neottia nidus-avis*, a species which requires pristine woodland to successfully sustain its mycorrhiza and relationships with the tree species its often associated with (Bowmer, 2008). Some of the more common species can be regularly seen along roadsides in many parts of the country. For example, Wiltshire roadsides can often be seen with large numbers of *A. pyramidalis* growing at densities sometimes exceeding 10 plants per square metre (Harrap and Harrap, 2005, pers. obs.). Whilst species that find these habitats favourable are in the minority, some of the rarer species are starting to find these habitats favourable. *Himantoglossum hircinum* is one of the largest orchids native to the British isles (Harrap and Harrap, 2005) and is most famously found through the village of Sandwich Bay and its nearby golf course. In the area it is common, being found on roadside verges, domestic gardens and sandy roadside laybys (pers. obs., June 2021). Nationally the species is listed as Vulnerable, having undergone a rapid decline after 1934 (Harrap and Harrap, 2005). Local reports have identified a new colony growing along a major roadside, near Maidstone, in the past few years (Anon, Pers. comm., June 2021). These sites, whilst not suitable for every species, can provide important new habitats, in many cases with large distances of semi-continuous sites. These sites also benefit from a lower footfall than many of the isolated grassland sites that are the primary habitat for many of the endangered orchids of the British Isles (Bowmer, 2008). Continuous roadside habitats provide an opportunity for species to colonise across relatively long distances (Vermeulen and Opdam, 1995) if populations start to expand from historic declines many species saw through the 20th century (Fay, 2015).

British Orchid Conservation

Despite the recent declines of multiple species nationally, there have been relatively few initiatives engaging with the conservation of declining orchid species as there have been with large, charismatic tropical species. Most existing initiatives involve simple protective measures designed to protecting extant populations against grazing and anthropogenic damage to the plants to maximise their chances of producing offspring and maintain if not expanding the local population (pers. obs. June 2021). The precedent for population restoration within the British Isles is the successful restoration of *Cypripedium calceolus* (Ramsey and Stewart, 1998). The sole Cypripedoideae species native to the British Isles, Jakubska-Busse *et al.* (2021) identified the threatening factors for the species within Europe as illegal wild collection for domestic gardens and grazing.

The threat from wild collection of plants, either through the removal of individual flowers or inflorescences, or the removal of plants to enter the international orchid trade, have been well reported from the tropics (Hinsley, *et al.*, 2018), with collection for both the international horticultural trade and traditional medicine trade being major drivers. Although illegal wild collection is known to be a factor that has influenced declines of British native orchids in the past (Jakubska-Busse *et al.*, 2021), little is known about the effect it may have on the wider taxa native to the British Isles.

In the UK the 'Monkey orchid' (*Orchis simia*) is known from only three sites, Park Gate Down, Kent and a site near Henley-on-Thames, Oxfordshire (Johnson, 2019). The plants now known from Park Gate Down were the result of human intervention following the discovery of a few flowering plants near Faversham in 1965 (Johnson, 2019). Seed from these plants was taken and scattered across different, potentially suitable sites across Kent (Dave Roberts, Pers. comm.) This resulted in a handful of plants flowering in successive years at Park Gate Down, as plants were successfully pollinated over the successive flowering seasons the population has expanded to where now a few hundred plants flower annually (Johnson, 2019) Although personal observation suggests this number is more in the region of 100-150 in 2021. Considering this method has resulted in the successful establishment of a long-term viable population through the establishment of the Kent population of *O. simia* (Johnson, 2019) as well as being used in the restoration of *C. calceolus* (Ramsey and Stewart, 2008), it can potentially be considered as a primary method for future conservation actions. However, the success rate of this conservation action is low and established populations may be vulnerable to human interference (Reuter *et al.*, 2016), especially as these sites are now often garnering much greater footfall than in previous decades (Johnson, 2019). Plants produced by modern micropropagation methods would also provide more control over plant placement and potentially generate a greater percentage of successful translocations (Reuter *et al.*, 2016). These more labour-intensive methods will be more resource heavy and as such the risk of translocation failure would need to be minimised. Although the two could be used in tandem to attempt to restore a population with a heterogenous age structure, rather than the homogenous age structure provided by either seed scattering or plant translocations independently.

Pollination asynchrony

As the effects of anthropogenic climate change develop, one of the most widespread and significant changes is likely to be the increases in mean temperatures due to human development (Alexiadis, 2007). Increases in mean monthly temperatures change the environmental cues that species use to time biotic events (Molnar *et al.*, 2012). Potential pollinator species for many orchids and other flora use temperature-based cues to time their emergence from dormancy/pupation once temperatures have increase sufficiently for the conditions to be hospitable to many insect species (Forrest, 2016). As temperatures reach the hospitable range for potential pollinator species earlier in the year as the expected trend continues to emerge, insect emergences will continue to advance earlier in the year (Forrest, 2016). Many orchid and flora species use similar climatic cues to initiate growth from their winter dormancy. However, some native species such as *Ophrys* spp. and *Neotinea ustulata* are wintergreen and as such use photoperiod as their flowering trigger (Tali *et al.*, 2004, Jacquemyn and Hutchings, 2015). Using photoperiod as a biotic trigger maintains a consistent timing of biotic actions with the constant nature of photoperiod changes (Ettinger *et al.*, 2021). The required interactions for successful pollination of many pollinator-specific species requires interactions to be consistently timed with the flora species flowering being timed with the pollinator emergence (Nilsson, 1992). In pollination interactions between a pollinator and a flora species utilising a deceit pollination style, early emergence is a more important factor, pollinator learning causes pollination success to decrease over a plants' flowering period when using a deceit pollination style (Nilsson, 1992). Continued changes to emergence dates in response to climatic factors could have a significant negative impact on future pollination rates if pollinator emergence dates continue to advance leading to a pollination asynchrony for future generations (Sun *et al.,* 2009), reducing the species' fecundity. Although changes in springtime temperatures likely have a small impact on first flowering times in species with a photoperiod flowering trigger, with increases in temperature accelerating the biotic actions involved in the production of the inflorescence and flowers (Lopez and Runkle, 2005), although this is not a causal relationship.

Whilst the issues described above are present in many species' ecology, this thesis will use the orchid species *Neotinea ustulata* as its case study. Threats of damage to habitats due to the advancement of human development and agriculture are present to most if not all extant species through different socioeconomic causes and mechanisms (Wilson, 1989). The risk of pollination asynchrony affects all angiosperms that utilise a different flowering trigger to their prospective pollinator species (Wilcock and Neiland, 2002). Wild collection for both medicinal purposes and for international wildlife trades affects many extant species in countries, where indigenous practices, foods and cultures dictate their collection and use (Hinsley *et al.,* 2018). Although individuals or groups who are collectors for their own personal use often have a desire to safeguard the species utilised for future harvestings through sustainable collection (Rajasekaran, Warren and Babu, 1991), individuals or groups who collect plants for economic gain often do not have the same concerns over future crop safeguarding so exploit species more unsustainably than many indigenous users (Iizuka and Katz, 2011). *N. ustulata* presents a case study of a species undergoing a significant recent decline through factors related to the advancements of agricultural practices (Tali *et al.,* 2004) that is not currently under any relevant protection or action plan to restore declining and extinct populations.

The species' decline is thought to be the greatest decline undergone by and orchid species for 150 years (Tali *et al*., 2004), since the historic decline of *Orchis simia*, which became virtually extinct in the whole of the British Isles during the 19th century (Johnson, 2019). In the case of *O. simia* the decline was so great that it was not included in the 1865 publication 'Illustrated Handbook of the British Flora', with the only reference to the species being in the description of the similar species *Orchis militaris*; mentioned as the synonym *O.* tephrosanthos (Bentham, 1865). *O. simia* declined to 2 confirmed populations and has since been established on another site (Johnson, 2019). The source population has remained stable since the establishment of the Kent population (Johnson, 2019), but the low numbers and highly isolated populations could potentially lead to long-term population viability issues as the effects of multi-generational inbreeding could impact the species, reproductive fitness, resilience to external factors or other stochastic events (Broquet *et al.*, 2010).

Neotinea ustulata (L.) R.M.Bateman, Pridgeon & M.W.Chase

Neotinea ustulata is a small terrestrial orchid species, found across Europe, named in both its specific and vernacular names with reference to the deep red floral 'hood' and end of the short (10-15cm) inflorescence (*u*stulatus meaning burnt in Latin) (Tali *et al.*, 2004). Known as either the 'Burnt' or 'Burnt-tip' in the common vernacular, it was once widespread across England, but is now restricted to a very small range, with the largest populations in the United Kingdom located in Wiltshire (Johnson, 2019). Parsonage Down, near Shrewton is one of the largest populations in Northern Europe, with population estimates of at least 30,000 plants (Tali *et al.*, 2004). The species has seen a dramatic decline in recent years, with only 75 of the 435 populations having known to have survived (Tali *et al.*, 2004). Only 10 of these 75 populations are known to regularly comprise 200 plants (Foley, 1987, 1990). Although more recent population change may have seen that number reduce, literature on the species in the past 15 years is limited, especially UK focused.

Historic publications differ slightly on the morphological description of the species. Bentham 1865 lists the 'Dwarf Orchis' (*Orchis ustulata* syn. *N. ustulata*) as a species 'seldom above 6-8 inches high' (15-20cm) consistent with contemporary descriptions however the 'spike length' of 1-2 inches (2.5-5cm) is considerably shorter than contemporary plants and descriptions of the species (Tali *et al.*, 2004). Whilst small individuals can still be found on extant sites with this diminutive size, they are often young plants that may be flowering for the first time. This change in descriptive morphometrics could be the result of habitat changes. Decreased grazing on sites consistent with rabbit population fluctuations due to disease and other factors would increase mean grass length on suitable sites for *N. ustulata* and as such plants with longer inflorescences could present more accessible flowers to potential pollen vectors increasing reproductive success of that phenotype. Other descriptors of the species' range in the British Isles, include 'occurs in many parts of England, but neither in Scotland or Ireland' as well as its habitats being simply described as 'dry, hilly, open pastures' (Bentham, 1865). While the species still persists within this habitat, its current extant range is considerably reduced than its range through the 19th century.

Flowering occurs between May and June for most populations, although the variety *aestivalis* is known to flower later, starting in June and still being present into July and August (Kümpel and Mrkvicka, 1990). Plants of the early flowering form (var. *ustulata*) are known to be wintergreen (Tali *et al.*, 2004), while it has been suggested that plants of var. *aestivalis* begin to emerge March-April (Kümpel and Mrkvicka, 1990). Fruiting then occurs in July-August with plants producing small seed capsules containing between two and four-thousand seeds (Tali *et al.*, 2004), with the small size of the seeds allows for efficient wind-dispersal (Jersakova and Malinova, 2007). Tali *et al.* (2004) calculated a fruiting percentage of 20.9% of plants across five Estonian populations, however they note that seed set in Great Britain is 'relatively infrequent and is not likely to exceed this figure'. Whilst *ex-situ* seed germination is currently not understood for *N. ustulata* it is hypothesised that the techniques would be similar to those developed for germination of *C. calceolus*, due to the potential existence of seed dormancy (Ramsey and Stewart, 2008).

Ecology and habitat of *Neotinea ustulata*

In the British Isles *N. ustulata* is most often regarded as a chalk grassland species, with many of the best sites being protected sites or areas owned by the Ministry of Defence (Tali *et al*., 2004). Parsonage Down and the neighbouring Yarnbury Castle (an Iron Age Fortification) are designated Sites of Special Scientific Interest (SSSI), both holding healthy populations of *N. ustulata*. It is classified as a European temperate species (Preston and Hill, 1997), favouring areas with humid and warm conditions during the summer, and able to tolerate cold conditions, but probably not suited to repeated freezing and thawing

(Tali *et al.*, 2004). Favoured sites are short, lightly grazed calcareous grasslands, and tend to occur in localised colonies in sunnier areas, although plants have been known from sites with different substratum (Davies *et al.*, 1983). Calcareous grasslands comprise the primary habitat within the British Isles. Sites tend to have a well aerated substrate with a calcareous based and humus rich. Suitable pH levels have been recorded as 6.0 to 8.5 (Procházka and Velísek, 1983) and 5.2 to 7.3 (Arditti, 1992). The colony at Yarnbury Castle is only found on the southernmost part of several, concentric ramparts, with 65 inflorescences recorded in June 2020 within close proximity to each other, forming three semi-distinct clusters (pers. obs. June 2020). Grazing also appeared to be minimal on the site, with the SSSI designation livestock grazing cannot be performed on the site, grazing is therefore carried out by naturally occurring herbivorous mammals. Rabbit (*Oryctolagus cuniculus*) droppings were easily found on the site, even amongst the *N. ustulata* inflorescences, while a scapula likely from a roe deer (*Capreolus capreolus*) was also found upon visiting the site (pers. obs.).

N. ustulata sites in the British Isles are also sites which have never likely been treated with artificial herbicides, pesticides or fertilizers (Tali *et al.*, 2004). Due to the increase and intensification of agriculture in the UK (Robinson and Sutherland, 2002) sites which fulfil these criteria are becoming increasingly rare (Mitchley and Xofis, 2005). Sites where the species has been recently extant in Kent have seen changes which have made them unsuitable. The site at Wye known as the 'Crown Field' due to the large, crown shaped image excavated into the hillside to commemorate the coronation of King Edward VII in 1902, where *N. ustulata* was known, has not seen plants since 2006 (Johnson, 2019). A visit to the site in April 2021 showed that the site's current ecological state would make it unsuitable for the species under the above criteria (pers. obs.). The major issue present on the site was the intense grazing by cattle and sheep and possible evidence of early season grass cutting. With *N. ustulata* being a known wintergreen species (Kümpel and Mrkvicka, 1990), grazing through the winter or a hay cut being taken before flowering will severely damage the plants, threatening their long-term survival through a possible reduction of energy restoration.

These factors also bring doubt into any chances of the species being benefitted by ‘rewilding’ projects in the UK. These projects often utilise and repurpose land often from agricultural practices (Jepson and Blythe, 2020), land which is highly likely to have had artificial pesticides, herbicides or fertilisers used upon them. One formerly extant site in Wiltshire, Clearbury Rings, an iron age fortification, is an area surrounded by land both grazed and cultivated (pers. obs. June 2020). This site is part of the slope surrounding the ancient fortification and whilst set aside by the landowner seems to have followed with the national decline. The site itself probably has not seen any artificial chemicals applied onto it, but possibly has been subject to chemicals diffusing across the hillside. A visit in June 2020 recorded no individuals, but many associated species were extant, species which possibly are not as susceptible to these compounds (pers. obs. June 2020).

***Neotinea ustulata* in Cultivation**

N. ustulata is a species that is poorly known in cultivation, with micropropagation of the species being described as ‘virtually impossible’ (Tali *et al.*, 2004), with micropropagation being the main cause of pessimism in the outlook. Aseptic micropropagation of many British native orchids is known to be difficult (Fay, 1988), with *in-situ* plants seeming to have a greater reliance on their mycorrhizae than many tropical epiphytic species grown in aseptic micropropagation (Phil Seaton, Pers. Comm.). Prior work has failed to extract a fungal sample from root cuttings taken from Parsonage Down in the past (Dave Roberts, Pers. comm.). Collecting a fungal sample would allow for research into micropropagation techniques using a species-specific fungal sample (Arditti, 2008). Without successful *ex-situ* cultivation any future assisted restoration of the Kent meta-population would be limited to plant translocations and seed scattering. Plant translocations would allow for an increase in local genetic diversity, but would at best maintain the national genetic diversity, with a probable reduction due to likely translocation failures (Abeli and Dixon, 2016). At present, in the United Kingdom only the population of *N. ustulata* at Parsonage Down is large enough to provide plants for future plant translocations.

An internet search reveals two commercial listings for the species, one from a large commercial nursery, Jacques Amand Intl (Accessed 18/06/2021) and an unrelated eBay listing (Accessed 18/06/2021). No further information can be found about the number of plants in ex-situ cultivation, whilst these listings may be evidence of some wild collection, it is unlikely to be the main driver of the decline in the British Isles.

Neotinea ustulata **in Kent**

N. ustulata was first recorded in Kent in 1732 near Gravesend in a translation *of Tournefort's History of Plants growing about Paris,* by John Martyn, a professor of botany in Cambridge (Johnson, 2019). Records increased throughout the 18th and 19th century, being known in the vernacular at the time as 'Dwarf Orchid' referring to the relatively small size of the inflorescence. The 1898 publication of 'British Orchids' (Webster, 1898) stated: 'On some of the green sloping Kentish Hills this little orchid is very abundant, and during summer quite enlivens the landscape with its quaintly conspicuous flowers'. Other historic reports describe the species as being relatively abundant across its Kentish range. Although never described as common, Anne Pratt stated 'it is not one of our common *Orchises*; though in some parts of the neighbourhood of Dover it is so plentiful that we have gathered from a single bank as many as twenty specimens, and still left an abundance for other lovers of wildflowers' (Johnson, 2019), it was seen as one of the more novel species regularly encountered. It was often noted at the time on its similarity to *Orchis purpurea*, a trait which influenced the vernacular name 'Dwarf Orchid' and *N. ustulata*'s initial description as *Orchis ustulata.* Records of *N. ustulata* seem to have remained stable throughout the 19th century, although no centralised records exist.

Entering the 21st century *N. ustulata* seemed to have entered a decline and may have suffered the most severe decline of all British Orchid species during the past 60 years (Foley, 1994). Possibly also experiencing the most severe decline (80%), having been lost from 2010 of its historic 265 tetrads (10x10km squares) (Preston *et al.*, 2002). After 1945 it seems that the species was only known in Kent from Queensdown Warren, Wye Downs and two downland sites near Lydden (Johnson, 2019). Johnson

(2019) last saw *N.* ustulata at Queensdown Warren in 1988, and said: ‘this was probably one of the last times it appeared on this traditional site’. Tali *et al.* (2004) listed the main causes of loss as: the increase in destructive agricultural techniques, such as ploughing, during World War Two and The reduction in rabbit based grazing following the widespread impact of myxomatosis on the rabbit population and damage related to ‘man-made incursions’.

In the 21st century the species has continued to decline, becoming sparsely recorded at Wye, with the last reliable record being in 2006 (Johnson, 2019). The Lydden sites also recording individuals until 2006. In 2013 when two small plants flowered, and have flowered sporadically until 2019 (Alfred Gay, Pers. comm.). No inflorescences were recorded at the site in 2020-21. With the species known to be relatively short-lived, as reported in Tali (2002), with most of the 464 recorded plants flowering between one and four years, before dying or entering a vegetative state. Plants are capable to entering a vegetative state for between one and three years, as such the Lydden plants may still be present. Another possible situation with the Lydden plants may be an absence of inflorescence due to a reduction in resource levels; in 2019 the inflorescences were pollinated, in an attempt to have a successful seed set on the site (Dave Roberts, Pers. comm.). The low rate of seed set in the UK will reduce the likelihood of this being successful, but the plants may still be present and could produce inflorescences in the future. If any of the seed was viable and has successfully germinated, then plants may be recorded on the site in greater numbers in the coming years. However, for the population to be viable over time outbreeding methods would need to be considered to maintain genetic diversity within the local population (Broquet *et al.*, 2010). Given the very low number of individuals recorded since 2006 the Kent meta-population of *N. ustulata* is possibly functionally extinct due to a very low, potentially highly fragmented population (Broquet *et al.*, 2010).

Threats to Extinction

Internationally, the species is listed as one of 'Least Concern' based on the IUCN Red List criteria (Kew Plants and Fungi, 2021). However on local and national levels the species is listed by different classifications. In England and Great Britain, the 2014 publication of 'A Vascular Plant Red List for England' (Stroh *et al.*, 2014) lists the species as 'Endangered' under the IUCN threat criteria 'A2c' with the species undergoing a >50% reduction in its area of occupancy in the past 30 years, and multiple risk factors having been identified in literature. Tali *et al.* (2004) list the major factors contributing to the decline of the species as overgrazing, and the use of artificial pesticides, herbicides and fertilisers on or near the species'. Grazing as a factor can be easily controlled on sites of conservation relevance, either through changes in agricultural practices on the site to exclude livestock or appropriate fencing to exclude livestock (Watkinson and Ormerod, 2001). Agricultural practices are generally factors which have a negative impact on *N. ustulata (*Tali *et al.*, 2004), however many orchids on the British Isles are found on agricultural sites which undergo hay cutting through the Autumn to produce silage for feeding cattle through the winter months (Djordjevic *et al.*, 2016). This style of site management is suitable for species such as *Ophrys apifera*, *Anacamptis pyramidalis* and *Dactylorhiza fuchsii* which all enter a dormant state after fruiting/flowering (Harrap and Harrap, 2005), allowing them to survive a hay cut in September/October. In comparison *N. ustulata* produces its first leaves after its flowering/fruiting (Tali *et al.*, 2004) which would make them susceptible to major if not fatal damage if hay cutting were to be undergone throughout the year.

The potential sensitivity of the species to artificial compounds used in agriculture (Tali *et al.*, 2004) may be the defining factor regarding the species future conservation. No data can be found on lethal thresholds or specific compounds that are negatively impacting the species populations. However, if the species is sensitive to any artificial herbicides, pesticides and fertilisers in residual trace amounts then it may render potentially suitable sites unsuitable for future restoration. This information is not forthcoming at present and would need to be understood to assess site suitability for any future restoration efforts.

Other potentially significant factors that may have an impact as to *N. ustulata*'s long term viability could be any changes to its pollination phenology (Robbirt, *et al.*, 2011) and/or changes in suitable habitats with the increase in local temperatures associated with anthropogenic climate change (Charitondou, *et al.*, 2021). The pollinating species identified by Tali *et al.* (2004) are all species that overwinter underground emerging once temperatures have increased enough to be hospitable (Stireman *et al.*, 2006). As local climates change and the temperatures trend towards earlier emergences due to higher mean temperatures there could be some future disparity surrounding the flight dates for viable pollinating species and flowering dates for populations of *N. ustulata* (Adedoja, 2020). The pollinating species in the British Isles is currently unknown, with the known pollinating species for *N. ustulata* being recorded from Germany (Voth, 1984, Mrkvicka, 1991) and no continuous records of *Tachina magnicornis* are present in the British Isles (NBN Atlas, 2021). Identification of viable pollinating species within the British Isles and predicting any potential phenological changes with regards to *N. ustulata*'s pollination would be an important step in understanding the risks imposed by anthropogenic climate changes on the long-term viability of *N. ustulata* populations in the British Isles. Also informing of any potential human intervention needed to ensure the maintenance of genetic diversity within local populations (Broquet *et al.*, 2010). Future climatic changes could also change the latitudinal range for *N. ustulata* leading to a shift in the species range (Lenoir and Svenning, 2013), an issue compounded by its criteria for viable habitat being highly specific and uncommon (Tali *et al.*, 2004), this habitat isolation could see issues surrounding a natural translocation of the species in relation to climatic changes with the species potentially requiring assisted migration to new habitats due to the lack of continuous habitats (Vitt *et al.*, 2010).

A lack of habitat connectivity and genetic outbreeding between populations could also be an issue for the species going forward (Broquet *et al.*, 2010). The relatively small population sizes seen by the species as it has undergone its decline, with just 10 populations over 200 plants in 2004 (Foley, 2004) although this number is likely much lower as its decline has probably continued in recent years. The small number of populations nationally combined with the isolation of those populations will likely see an increase in genetic inbreeding and deleterious mutations arising in smaller populations increasing their susceptibility to other extinction pressures (Broquet *et al.*, 2004). This is an easy risk to alleviate, pollen can be moved between populations and plants of distinct populations can be used to artificially pollinate flowers and then allowed to fruit and set seed. If the crosses produce viable seed, genetic diversity should be maintained across the local and meta-populations, although seed setting rates are thought to be very low in the British Isles (Tali *et al.*, 2004). This would be a simple but labour-intensive method of attempting to maintain genetic diversity within the species as travelling large distances would often be needed to ensure pollen transfers between distinct, isolated populations.

If the current decline continues unabated, then intervention will be required to maintain national or local populations. Understanding the threats directly will create better guidelines as to future safeguarding or restoration work In accordance to the IUCN reintroduction criteria (IUCN/SSC, 2013). A more contemporary understanding of the species threats would also allow for a more targeted and efficient approach to population safeguarding. It would be recommended that research into contemporary threats be undertaken before the initiation of any conservation action plans.

Aims

The aims of this thesis is to review the situation of *N. ustulata* surrounding *N, ustulata* within Kent, and to review the potential for future restoration of the Kentish meta-population. As well as develop areas of knowledge that are currently not known that may affect the outcomes of future conservation of the species.

Due to potential changes in local and national COVID-19 restrictions during the time in which this work was undertaken data chapters had to be designed which would be minimally impacted by potential changes to travel restrictions or an imposition of self-isolation which would interrupt potential research.

In order to carry out an assessment of future conservation measures of *N. ustulata* two studies were selected and designed to assess different areas of the species’ conservation; chapter 2 is a study assessing the potentially of *N. ustulata* to be competitive amongst species in its immediate environment for access to funding, using a Best-Worst Scaling (BWS) type 3 questionnaire (Louviere *et al.*, 2015). The development of the questionnaire and relation to this wider study was interpretating the results and assessing the placement of *N. ustulata* amongst other species from the same habitat, the results from the questionnaire can be used in relation to any of the species included in the analysis for the public preference for involvement in potential future conservation actions against the other species included.

Chapter 3 aimed to assess potential changes in the pollination phenology of *N. ustulata* through the analysis of the changes of *N. ustulata* and selected potential pollinator species in response to temperature. Predicting future changes and long-term suitability for the potential pollinator species to be viable pollinator species for *N. ustulata* throughout projected anthropogenic climate changes. Projecting future phenology changes in response to anthropogenic climate change allows for a projection of future risks to successful naturally occurring cross-pollination provided by the potential pollinator species. Originally this study aimed to directly observe pollination to confirm UK based pollinator species, something that does not currently appear in literature, but this was deemed too great a risk with travel and accommodation. Alongside the recording of a UK pollinator species other aims of a pollination study included observing the success of male and female reproductive success through the observation of plants throughout their flowering and fruiting period. The repeated observations and travelling that a study of this nature would require contributed to the greater risk involved with the completion of this chapter, both health-based risks and the chances of travelling being stopped part way through the observation for various reasons. This chapter aims to assess the potential changes to the plant-pollinator interaction from the perspective of *N. ustulata*, considerations are made to the pollinators ecology and works under the assumption that the species identified in the study are viable (van der Cingel, 1995) the later-flowering form not known from Kent, the pollinator vectors of var. *ustulata* are not known in the British Isles so assumptions had to be made as to their identities based on taxa morphologically similar to the known pollen vector in Germany, a species with no consistent record base for the Mainland British Isles. Due to COVID-19 restrictions at the time of undergoing the research no pollen vectors could be identified in order to more reliably select potential pollen vectors for the Kent native var. *ustulata*.

Chapter 2: Public preference towards *Neotinea ustulata* (L.) R.M.Bateman, Pridgeon & M.W.Chase, within the context of chalk grassland orchids and flora

Introduction

As the need for species conservation develops through the negative impacts that human development and modernisation have on the wider environment, an increasing number of species require funding and public presence that may not increase proportionately with the number of species requiring human intervention to secure the long-term population viability (Walsh *et al.*, 2012). Whilst species that have undergone the greatest declines due to human impacts have the greatest need for intervention they may not necessarily be in receipt of any available time or funding as rarity often forms only one part of the criteria that determine potential species for conservation actions (Cofre and Marquet, 1999). Many species secure funding to engage conservation actions but success rates can often have other factors which influence them (Larson *et al.*, 2016), many species require community engagement alongside funded conservation actions to ensure the effectiveness of any conservation actions (Roberts and Jones, 2013). Species conservation status often requires additional benefits to secure potential funding streams to initiate conservation action, in both short and long-term time scales

One of the most prevalent habitat types in Kent is the large areas of calcareous grassland that characterises areas of its coastline and the Kent Downs (Blackwood and Tubbs, 1970). The chalk-rich substrate that characterises this habitat is often viable habitat for a disproportionate number of species endemic to the habitat (Kruckeberg and Rabinowitz, 1985). As local agricultural operations have intensified over the 19th and 20th centuries increasingly large areas of land have been utilised for the growth of crops (Firbank *et al.*, 2008). The intensification of agricultural practices and the artificial chemicals utilised to maximise crop yields have led to soil degradation for other sites within the watershed (Firbank *et al.*, 2008). Whilst these compounds have successfully increased crop yields, they have generated negative impacts of neighbouring land areas by introducing artificial compounds which impact the growth of many flora species (Firbank *et al.*, 2008), alongside pesticide leeching decreasing insect populations affecting pollination of both crop and wild flora species.

Of the species found in this habitat, one of the groups that rely on it the most are the Orchidaceae species native to the British Isles with 22 of the 52 known native species found on calcareous grasslands (Harrap and Harrap, 2005), with 20 being primarily found on calcareous grasslands, including some of the species with the most restricted ranges and greatest declines (Johnson, 2019).With the advancement in human development and agriculture many of the sites viable for many flora species are now heavily isolated further restricting ranges and isolating populations (Tscharntke *et al.*, 2005). *Orchis simia* has been known from 'Very few' sites nationally since their decline through the 19th Century (Johnson, 2019) with their population being restricted to two sites nationally, Park Gate Down, Kent and a site near Henley-on-Thames. The Kent site being the result of seed scattering around the county after two plants were discovered flowering at a site near Faversham in 1965 (Johnson, 2019). The translocated seed established and now a few hundred plants flower annually. Whilst the plants on the site have continued to reproduce, no plants have been recorded nearby indicating an expansion of the population.

Extant species of conservation priority which are often novel in form, function or are seen as objectively desirable are often given a 'Flagship Species' status (Caro, 2010). These species are used to represent their ecosystem, habitat or taxonomic group in the wider public consciousness and are often used as a method to increase the funding provided towards the conservation of a species, or its relevant habitat (Caro, 2010). Whilst this method may not be suitable for every species, The utilisation of a flagship species can better engage third party stakeholders, often allowing for a greater uptake of conservation actions relevant to the 'flagship species' and other species in its immediate surroundings (Caro, 2010). If species of conservation importance are more easily funded, then the projects that may concern them in the future may be more heavily or consistently funded (Home *et al.*, 2009). This allows for a greater range of activities to safeguard the species, or more long term funding allowing for a greater level of protection over time (Caro, 2010), with the required duration to assess any potential impacts that the project may have, allowing for further refinement of practices to better utilise funds.

For a flagship species to be successful it needs to be marketable (Hunter and Gibbs, 2009). Some of the most successful flagship species are those that have been marketed to the public through the actions of large NGOs, marketed in such a way as to draw attention to the facts of their conservation issues, heightened by the often-desirable form or novelty of the species (Home *et al.*, 2009). The efficacy of the 'Flagship Species' model is debated (Caro *et al.*, 2004), with arguments over a possible over-reliance on it as a model for wider conservation aims. For *N. ustulata* it seems an appropriate model to use; flora species are under-represented in the 'Flagship Species' model (Pany and Heidinger, 2017), and therefore at a relative disadvantage for funding allocation and public engagement with the majority of species utilised within public engagement for conservation purposes being large charismatic mammals (Hunter and Gibbs, 2009).

Assessing this species for a flagship species status should also evaluate any negative externalities that extra protection, funding or public awareness may have (Jordan, 1995). Potential negative externalities such as those reported regarding the European Otter (*Lutra lutra*), where their predation has applied pressures to local fish farms (Sales-Lui *et al.*, 2009) and commercial fisheries (pers. obs.), threatening livelihoods as a result of damage to fish stocks. Enhanced legal protection in the UK prevents fishery owners from encountering the individual once on their property (Gov.uk, 2021). There are also concerns over predation on species with a greater conservation priority than *L. lutra*, the 'Critically Endangered' European Eel (*Anguilla anguilla*) (Jacoby and Gollock, 2014) has been shown to form 20% of *L. lutra*'s diet despite forming only 5% of the aquatic biomass (Britton *et al.*, 2017). Due to *N. ustulata*'s biology and ecology it is unlikely that issues like this may occur, but any negative externalities should be considered before a recommendation is made.

The flagship species designation and the potential marketability of species given that designation make those species more likely to gain funding for conservation actions (Caro, 2010), either funding for species-specific programmes, or wider scale projects acting on issues on an ecosystem or habitat level (Caro, 2010). Creating a net benefit for multiple species with similar ecology or habitat requirements through positive externalities and indirect beneficial actions (Jordan, 1995). These potential positive externalities should be considered in balance with the potential negative externalities of any potential species conservation actions, rather than positive and negative externalities being assessed in isolation (Jordan, 1995).

The possibility of using marketing to influence the access to conservation funding for species of conservation importance can be dependent on factors relevant to its potential conservation actions (Home *et al.*, 2009). Conservation based descriptive factors can be inconsistent when given as descriptive measures used in species profiling. Conversely, species of future conservation concern may not be listed to the same severity in the same databases but current population trends which may be causing the potential concern (Cardoso *et al.*, 2012), but their marketability may be impacted by the reduced severity of population trends by the reduction in the severity of the species classification on publicly cited databases.

Assessing the marketability of *N. ustulata* was assessed using a Best-Worst Scaling Type 3 methodology (Louviere *et al.*, 2015). This methodology allows for the easy assessment of different ‘cases’. Most commonly this methodology is used in medicinal scenarios assessing the preference of patients in the possible administration of treatment plans concerning long-term, chronic conditions which require continuous treatment that may have detracting side effects (Flynn *et al.*, 2007). This allows medical researchers to select possible future treatments based on the possible conglomeration of negative effects that treatments may incur (Cheung *et al.*, 2016). Studies using this methodology are often able to include measures of objectivity into the selection of criteria or the use of choice set including choices that are objectively better or worse, allowing for the rationality of the respondents to be confirmed (Brown, *et al.*, 2011). The ‘real-world’ nature of the species selected negated the possibility of testing for objectivity would not be feasible, the subjectivity that the study set out to interpret, specifically in relation to *N. ustulata*, over the interpretation of conservation relevant descriptive factors would impact the construction of choice sets with designed objectivity.

Methods

Ethics

This study received ethics approval from the Research and Ethics Committee of the School of Anthropology and Conservation, University of Kent.

Questionnaire

The Best-Worst Scaling (BWS) type 3 based questionnaire comprised 5 sections, prior informed consent, demographic questions and 3 BWS experiments on two species sets. The questionnaire used for the experiment can be found in the appendix of this thesis.

Following a page providing information about the purpose of the study and the participants rights and expectations, the questionnaire started with a series of demographics questions, assessing, gender, age, academic discipline, role and location.

Following the demographics questions, there were three sections comprising BWS experiments. Species group 1 were orchid species found primarily on Kent's chalk grassland habitats as outlined in Johnson 2019. *Dactylorhiza incarnata* and *D. maculata* where excluded as they are primarily found on marshes and heathland respectively (Johnson, 2019). This resulted in a list of 20 orchid species.

All species were assessed from records collected from the NBN atlas from the earliest dated records through to 31st December 2019. Records were accessed from nbnaltlas.org

BWS choice sets were created from species group 1 through ranking according to a rarity index. The rarity index used was derived from the total record count between 1990-2019 from the NBN atlas.

The counts were converted into an index score using the following formula:

$$I_{rarity} = -Log_{10}\left(\frac{1}{N_{1990-2019}}\right)$$

Once ranked the species were separated into their relevant pentiles based on their rarity index score to form five Rarity Ranked Groups (RRG). This was carried out for both orchids and flora species (Species sets 1 and 2 respectively)

In order to compare *N. ustulata* to the Species Set 2 the rarity pentile containing *N. ustulata* (Species set 1, P_2) was added to the choice sets from Species Set 2, limiting any effect rarity may have in the attribute-based question. The RRG's were assigned a number and sent through a random number generator to assign their choice set, each choice set therefore contained one species from each Rarity pentile. Choice sets derived from Species Set 2 also contained one species from Species set 1 from the rarity pentile containing *N. ustulata*, providing five species per choice set.

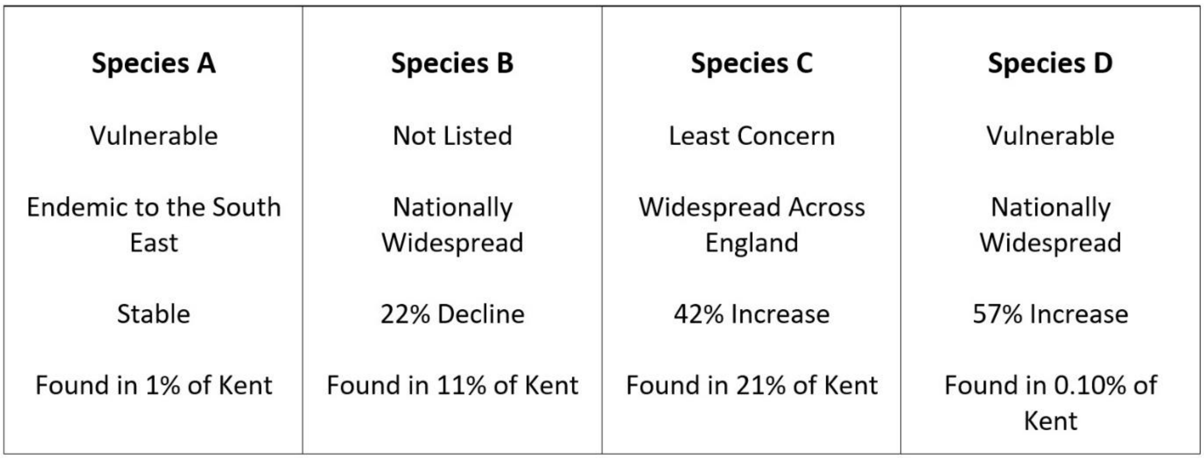
The first choice experiment used four attributes, with a focus on their spatial distribution through their Land Coverage and National Distribution, as well as their British Red list classification (Stroh *et al.*, 2014) and percentage change in records. This spread of descriptors provided two discrete and two continuous variables for each species presented for each species in each choice set.

A percentage change of records was derived from the records provided by the NBN atlas for each species, using the decade 1990-99 as the benchmark to assess any change against the 2010-19 decade. Species with a <10% change in records were described as 'Stable', all others were described as 'Decreasing' or 'Increasing' along with the percentage change.

British Red List classifications were extracted from the 2014 Vascular Plants publication (Stroh *et al.*, 2014), species described as 'Data deficient', 'Waiting' or species not listed were described as 'Not Listed'. These were coded onto a scale 1-5, with 1=Critically Endangered, 2=Endangered, 3= Vulnerable, 4=Near Threatened and 5=Least Concern, species listed as 'Data Deficient', 'Waiting' or species not listed on the British Red List of Vascular plants were excluded from analysis due to having too small a sample size within the species selected for analysis.

An estimate of the Kent land coverage of each species was derived via a count of tetrads of BSBI (Botanic Society of Britain and Ireland) species distribution maps accessed from BSBI.org/maps. These were then compared with the total number of tetrads within the Kent County boundary, only landlocked tetrads with ≥50% of their total area with the county boundary were counted. Tetrads along the county's coastline were counted regardless of the proportion of land mass within them.

The national spatial distribution of each species was estimated as one of six categories for the ease of interpretation by participants. The selected descriptors were: ‘Kent Endemic’, ‘South-East Endemic’, ‘Southern Endemic’, ‘Mostly found in Southern England’, ‘Widespread across England’ and ‘Nationally widespread’ The choice sets were expressed as a series of choice cards, as can be seen in Fig. 1.



Species A	**Species B**	**Species C**	**Species D**
Vulnerable	Not Listed	Least Concern	Vulnerable
Endemic to the South East	Nationally Widespread	Widespread Across England	Nationally Widespread
Stable	22% Decline	42% Increase	57% Increase
Found in 1% of Kent	Found in 11% of Kent	Found in 21% of Kent	Found in 0.10% of Kent

Fig. 1 Species set 1, Choice set 1, *Orchis simia, Gymnadenia conopsia, Ophrys apifera* and *Dactylorhiza viridis* respectively

BWS experiments two and three, differed from experiment one with the addition of a photograph if the species in question. Wherever possible images covered by a creative commons license was used, however in some cases this was not possible and therefore images were used with credit provided to the owner. Experiment two used photographs that provide a wider context of the inflorescence, plant (wherever possible) and its habitat. Experiment three focused on the inflorescence, removing the wider context, focusing more on the species form.

All choice sets maintained the same species and the same order throughout the questionnaire. The two image-based experiments were separated for respondents using a non-imaged-based experiment, aiming to minimise any bias that may be created by having them answered concurrently.

The questionnaire was distributed through the School of Anthropology and Conservation, University of Kent

A total of 60 questionnaire responses were collected over a 28 day period and results inputted into a spreadsheet where the responses could be screened for anomalous responses (i.e. species selected for both best and worst); none were detected. The responses were assigned a value of ±1, the operator denoting the best/worst selected by the respondent. These values were used to generate a 'Best-Worst' score based on the attribute and photograph-based questions following Casini, Corsi, and Goodman (2009). For this case, the BWS score is simply calculated as the sum of best and worst rankings for a species *i*, as shown in the equation below.

$$BWS = \sum_{i}^{B} - \sum_{i}^{W}$$

The discrete descriptors, National Distribution and British Redlist classification, were analysed through One-Way ANOVAs and a Post-Hoc Dunnetts T3 test. Descriptor categories that only contained one case were removed for the ANOVA, as they are not suitable for pairwise comparison through the Post-Hoc test.

Species set 2, pentile 2, would also be compared to the corresponding pentile that was introduced to include orchid species of similar rarity index scores to assess compatibility of this method to include *N. ustulata* within the flora-based choice sets through the analysis of the corresponding pentiles' Best-Worst Scores through One-Way ANOVAs.

Results

Demographics

Of the 61 respondents, over half were in the age group 18-24 (51%; n=31), with the remaining four categories being relatively evenly spread (25-34, n=9; 35-44, n=7; 45-54, n=9; 55-64, n=5). This skew towards a younger demographic was also seen through the respondents' 'role' with almost half (48%, n=29) stating that they were an 'undergraduate'; 21% (n=13) were taught postgraduates, 12% (n=7) were research postgraduates, 16% (n=10) were academic staff and one respondent indicated their role as 'Other'. The modal postcode for respondents was CT2, with 26 respondents (43%) and 10 respondents (16%) gave CT1; the remaining 31% of respondents were from surrounding Kent postcodes. The disciplines of the respondents represented a wide spectrum of the disciplines within the school, with 29 (48%) and 22 (36%) respondents from conservation and anthropology respectively. Respondents engaged in 'Environmental Social Science and Human Geography' comprised a minority of 6 respondents (10%), while 4 (7%) indicated their discipline as 'other'

Best-Worst Scaling – attributes

Linear regressions between Kent land coverage and the attribute-based score returned a significant relationship ($R=0.724$, $p<0.001$) for the Orchidaceae based choice cards. Linear regression run for the flora based choice sets' Kent Land Coverage and the attribute BWS was not significant ($R=0.126$, $p=0.549$). Linear regressions were also performed on the Percentage Change in the National Records (1990-2019); against the different Best-Worst Scores Kent Land Coverage: Aggregate BWS (Orchids: $R=0.159$, $p=0.504$, Flora: $R=0.126$, $p=0.548$) National Records Percentage Change: BWS_{Attr} (Orchids: $R=0.128$, $p=0.592$ Flora: 0.126, $p=0.549$) National Records Percentage Change: BWS_{Agg} (Orchids: $R=0.285$, $p=0.223$, Flora: $R=0.051$, $p=0.808$), using the Bonferroni corrected significance threshold of $p<0.0125$.

Best-Worst Scaling – species

Within the Orchidaceae choice sets (Table 1)*N. ustulata* ranked highest of the aggregate BWS, an aggregate BWS of 49 (ranked highest), Wide-Photo BWS of 15 and Close-Photo BWS of 52 (ranked highest) comprised the aggregate BWS of 101. Scoring above the in the three choice experiments indicates the species as 'desirable' when compared against orchid species extant in the same habitat type. Compared with the other flora species (including Species set 1, P_2), *N. ustulata* ranked 2nd in the aggregate BWS, behind *Euphrasia pseudokerneri*, a species from the same choice set, representing Species set 2, Q_1, in direct competition for the same BWS scoring points. The aggregate score breakdown shows *N. ustulata* scored considerably lower than *E. pseudokerneri* in the attribute question with scores of 40 and 9 respectively, (*N. ustulata* ranked 2nd in the choice set), with *N. ustulata* scoring higher in the image based experiments (leading the points scored in the choice sets, comprising 52% and 45% of the responses for the wide and close photographs respectively).

Table 1, The Best-Worst Scores (BWS) Collected for the 20 orchid species (species set 1) across the three different choice experiments

Species	**Attribute BWS**	**Close-Photo BWS**	**Wide-Photo BWS**	**Image BWS**	**Image BWS Ranking**	**Attribute BWS Ranking**	**Aggregate BWS**	**Aggregate BWS Ranking**
Orchis simia	38	9	12	21	8	4	88	7
Gymnadenia conopsia	-2	5	2	7	12	14	48	13
Ophrys apifera	-44	25	35	60	2	16	90	6
Dactylorhiza viridis	8	-39	-49	-88	19	7	18	17
Ophrys fuciflora	47	18	12	30	7	2	115	2
Anacamptis morio	3	33	17	50	4	10	109	3
Neottia ovata	-50	-47	-54	-101	20	19	-50	20
Ophrys insectifera	0	-4	25	21	8	13	70	11
Spiranthes spiralis	4	-36	10	-26	15	9	36	14
Orchis anthrpophora	42	7	-17	-10	13	3	88	7
Dactylorhiza fuschii	-48	39	27	66	1	18	94	5
Herminium monorchis	2	-10	-20	-30	16	11	26	15

Platanthera bifolia	5	17	20	37	6	8	95	4
Himantoglossum hircinum	34	-12	-11	-23	14	5	72	10
Pseudorchis albida	13	-34	-26	-60	17	6	23	16
Orchis mascula	-52	29	17	46	5	20	54	12
Neotinea ustulata	49	15	37	52	3	1	159	1
Anacamptis pyramidalis	-47	4	5	9	11	17	-3	19
Platanthera chlorantha	1	-36	-44	-80	18	12	9	18
Ophrys sphegodes	-3	17	2	19	10	15	79	9

Comparison between Photo BWS and the primary colour of the flowers of all species included in the questionnaire species showed no significant differences in pairwise comparisons (Tables 2 and 3). The Analysis of Variance between groups of the flora primary colours was significant (p=0.009, df=6).

Table 2, Pairwise comparisons between primary colours Photo BWS in Species Set 1 (Orchids), overall p=0.115

Species set 1		
Colour	Colour	sig.
Green	Pink	0.340
	Purple	0.235
	White	0.966
	Yellow	0.938
Pink	Green	0.340
	Purple	0.922
	White	0.615
	Yellow	0.769
Purple	Green	0.235
	Pink	0.922
	White	0.272
	Yellow	0.598
White	Green	0.966
	Pink	0.615
	Purple	0.272
	Yellow	1.000
Yellow	Green	0.938
	Pink	0.769
	Purple	0.598
	White	1.000

Table 3, Pairwise comparisons between primary colours Photo BWS in Species Set 2 (Flora), overall p=0.009

Species set 2		
Colour	Colour	sig.
Brown	Green	0.234
	Indigo	0.061
	Pink	0.123
	Purple	0.112
	White	0.16
	Yellow	0.902
Green	Brown	0.902
	Indigo	0.702
	Pink	0.732
	Purple	0.899
	White	1.000
	Yellow	1.000
Indigo	Brown	0.061
	Green	0.702
	Pink	0.996
	Purple	1.000
	White	0.565
	Yellow	0.992
Pink	Brown	0.123
	Green	0.723
	Indigo	0.996
	Purple	1.000
	White	0.285

	Yellow	0.998
Purple	Brown	0.112
	Green	0.899
	Indigo	1.000
	Pink	1.000
	White	0.858
	Yellow	0.997
White	Brown	0.160
	Green	1.000
	Indigo	0.565
	Pink	0.285
	Purple	0.858
	Yellow	1.000
Yellow	Brown	0.902
	Green	1.000
	Indigo	0.992
	Pink	0.998
	Purple	0.997
	White	1.000

Comparisons between the P_2 of both species sets that were included in the flora based BWS experiments showed no significant differences in the scoring between the two pentiles for both the Attribute BWS (df=9, p=0.546) and aggregate BWS (df=9, p=0.660). The two Pentiles were equivalent within the flora based BWS experiments.

Table 4, Pairwise comparisons between the attribute BWS score of species set 1 (Orchids) when grouped by their approximate national distributions, overall p=0.030 (One-way ANOVA)

Distribution 1	Distribution 2	sig.
South-East Endemic	Southern Endemic	1.000
	Mostly Found in Southern England	0.382
	Widespread Across England	0.567
	Nationally widespread	0.05
Southern Endemic	South-East Endemic	1.000
	Mostly Found in Southern England	0.913
	Widespread Across England	0.857
	Nationally widespread	0.595
Mostly Found in Southern England	South-East Endemic	0.382
	Southern Endemic	0.913
	Widespread Across England	0.985
	Nationally widespread	0.225
Widespread Across England	South-East Endemic	0.576
	Southern Endemic	0.857
	Mostly Found in Southern England	0.985
	Nationally widespread	0.981
Nationally Widespread	South-East Endemic	0.05
	Southern Endemic	0.595
	Mostly Found in Southern England	0.225
	Widespread Across England	0.981

Table 5, Pairwise comparisons between the attribute BWS score of species set 2 (flora) when grouped by their approximate national distributions, overall p=0.036 (One-way ANOVA)

Distribution 1	Distribution 2	sig.
Southern Endemic	Mostly Southern England	1.000
	Widespread across England	1.000
	Nationally Widespread	0.098
Mostly Southern England	Southern Endemic	1.000
	Widespread across England	1.000
	Nationally Widespread	0.121
Widespread across England	Southern Endemic	1.000
	Mostly Southern England	1.000
	Nationally Widespread	0.011
Nationally Widespread	Southern Endemic	0.098
	Mostly Southern England	0.121
	Widespread across England	0.011

Analysis of the responses to the flora choice experiments for species set 2 (Table 5) returned significant post-Hoc tests for the National distribution between only the two categories ‘Widespread across England’ (n=5) and ‘Nationally Widespread’ (n=6) (p=0.011), *Orchis anthropophora* was removed from this analysis as the sole species within the category ‘South-East Endemic’. No other categorical comparison between national distribution categories provided significant comparison between either specie set (Tables 4 and 5).

Table 6, Pairwise comparisons of the different attribute BWS Scores of species in species set 1 (Orchids) when grouped by their British red list classification (Stroh *et al.*, 2014), overall p<0.001 (One Way ANOVA)

Red List 1	Red List 2	sig.
Endangered	Vulnerable	0.026*
	Near Threatened	0.299
	Least Concern	0.003*
	Not Listed	0.45
Vulnerable	Endangered	0.026*
	Near Threatened	1.000
	Least Concern	0.012*
	Not Listed	0.717
Near Threatened	Endangered	0.299
	Vulnerable	1.000
	Least Concern	0.077
	Not Listed	0.742
Least Concern	Endangered	0.003*
	Vulnerable	0.012*
	Near Threatened	0.077
	Not Listed	0.993
Not Listed	Endangered	0.45
	Vulnerable	0.717
	Least Concern	0.742
	Near Threatened	0.993

*Significant at p<0.05

Table 7, Pairwise comparisons of the different attribute BWS Scores of species in species set 2 (Flora) when grouped by their British red list classification (Stroh *et al.,* 2014), overall p<0.001 (One Way ANOVA)

Red List 1	Red List 2	sig.
Endangered	Vulnerable	0.306
	Near Threatened	0.352
	Least Concern	0.028*
	Not Listed	0.033
Vulnerable	Endangered	0.306
	Near Threatened	1.000
	Least Concern	0.016*
	Not Listed	0.136
Near Threatened	Endangered	0.352
	Vulnerable	1.000
	Least Concern	0.146
	Not Listed	0.282
Least Concern	Endangered	0.028*
	Vulnerable	0.016*
	Near Threatened	0.146
	Not Listed	0.997
Not Listed	Endangered	0.033*
	Vulnerable	0.136*
	Least Concern	0.282
	Near Threatened	0.997

*Significant at p<0.05

When grouped by their British red list classification (Stroh *et al.,* 2014) the attribute BWS Scores showed significant differences between the following groups in species set 1 (Orchids): Endangered-Vulnerable (p=0.026), Endangered-Least Concern (p=0.003) and Vulnerable-Least Concern (p=0.012) (Table 6). In the pairwise comparison between red list classifications in species et 2 (Flora) (Table 7) the following groups showed significant differences between Attribute BWS Scores: Endangered-Least Concern (p=0.028), Endangered-Not Listed (p=0.033) and Vulnerable-Least Concern (p=0.016) Both species sets returned one-way ANOVA's with a significance of p<0.001.

Discussion

Across the two species sets *N. ustulata* scored significantly above the mean. Ranking highest in species set 1 through the BWS_{agg}, BWS_{attr} and ranking third in BWS_{phot}. Comparatively this indicates that *N. ustulata* is more desirable for involvement in future conservation actions than many other orchid species found in the same habitats (Home *et al.*, 2009), often on the same sites (Johnson, 2019, Harrap and Harrap, 2005). This comparative test also shows that species with terms associated with rarer species scored higher comparative BWS_{attr} than species of higher rarity pentiles. The top four BWS_{attr} scoring species (the species that dominated the best scores in the attribute BWS experiment) all included 'Endemic' in their distribution descriptors and showed a negative national percentage change. The other two species described as endemic (*Herminium monorchis, Ophrys sphegodes*) both showed positive national percentage changes and their scoring did not significantly deviate from the mean, ranking 11th and 15th respectively from 20 species. Although there was not a significant correlation between national percentage change and the BWS_{attr} scoring, it likely formed a function in the decision-making process for the respondents. The significant correlation between the Kent land coverage and BWS_{attr} indicated that this local focus played a significant role in the decision-making process of the respondents, with species with a greater degree of local rarity being a more desirable option for future conservation measures. This was not stated as an aim for the outcome of the questionnaire, but with the respondents being linked with the University of Kent, answers relating to species with a greater local rarity could imply that locally targeted information regarding species involved in potential conservation actions may be an important step in securing better local engagement with potential stakeholders and third parties whose compliance may be needed to safeguard species.

The data collected for species set 2 indicates a similar result compared with species set 1, although the Attribute BWS and the Aggregate BWS did not correlate with either continuous variable presented in the attribute-based choice experiment. Significant differences were present in the British red list classification categories relative scoring, with the differences being between values either side of the category 'Near Threatened' with this category forming a median category, no significant differences were present between it and any other classifications. The differences present were present only between species of classifications more severe than ‘Near Threatened’ and those less severe. This would indicate the species with red list classifications less severe than ‘Near Threatened’ are perceived to be species of little conservation relevance, while those more severe are those of significant conservation relevance. This is a trend also shown in the species set 1 response further supporting this interpretation. As was seen in species set 1 primary flower colour had no significant difference on the outcome on the Photo BWS score, indicating a more complex reasoning behind desirability of flowers (or the functional equivalent) through objective means. Although among species set 1 the species with smaller, less colour diverse flowers scored lower, the questionnaire design omits any objective measure of flower form or other factors influencing floral desirability.

Questionnaire design could have been improved by the inclusion of a multiple choice, self-assessment of the descriptive factor that most influence both their ‘best’ and ‘worst’ selection. This would have provided a subjective measure that could be used to improve the analysis of the questionnaire responses. As such, the results and their interpretation of responses of the BWS survey are limited by the current knowledge of the interpretation and driving factors behind the ‘desirability’ of species for future conservation programmes. At present there is little understanding around the subjective drivers of public perception of species involved in conservation projects. This topic could be an important one as the public exploits of movements such as ‘Extinction Rebellion’ forcing environmental issues into the public consciousness (Shah, 2019). This potential increased interaction between the public and conservation professionals increases the opportunities for conservation to engage with the general public with issues surrounding biodiversity loss and means to help prevent it (Kobori, *et al.*, 2016). Utilising ‘flagship species’ and other species which are marketable to the general public provides an opportunity to further engage the public with conservation issues and progress conservation actions (Caro, 2010) with an enhanced ‘desirability’ of the species targeted by single species conservation actions. Understanding the factors which influence public desirability of these species would allow for a better level of engagement from the public as potential flagship species could be better selected for potential public engagement. This study used a comparative method to advise the suitability of *N. ustulata* for a ‘flagship species’ designation with regards to the flora of calcareous grasslands within

Kent and the United Kingdom. A better understanding of these factors would allow for a more objective view on species desirability without the need for a preliminary comparative study.

The BWS Type 3 methodology also limits the interpretation of the comparative information derived from the choice experiments. The results collected for species set 1 can be streamed into four distinct quartiles, The four highest scoring species form the first quartile as their responses formed the greatest proportion of the 'Best' species. Equally the four lowest scoring species of each experiment formed the greatest proportion of the 'worst' votes. The quartiles either side of the mean were represented as minority votes in each BWS experiment. This limits the comparative data presented through this questionnaire design, with species only ever being directly compared to those in their selected choice set. Greater definition would be achieved through the construction of a second BWS Type 3 experiment with species being streamed by their relative placement from the first round of choice experiments. The Species set 2 data is distributed in a similar way, with five pentiles represented through the inclusion of species set 1 P_2 to introduce orchid species equivalent to *N. ustulata* into the species set. The 'worst' scores were distributed across more species than in species set 1, with one species representing 67% of the possible vote then a further nine species between 43% and 25% of the possible 'worst' vote. However, in this case *N. ustulata* scored in the highest scoring quartile in five of the six choice experiments, only being comparatively worse in the species set 2 attribute choice experiment against *Euphrasia pseudokerneri*, which was described with a greater national percentage change of -72%, compared to -10% of *N. ustulata*. British red list classification and national distribution of both species was listed as the same, while the Kent land coverage was only slightly different between the two, 7% and 2% respectively.

Alongside the potential benefits to the conservation of *N. ustulata* that may result from its use as a 'flagship species' there are also potential indirect benefits to many other flora species extant on the same habitats. The potentially sensitive nature of *N. ustulata* to artificial pesticides, herbicides (Tali *et al.,* 2004) and fertilisers would indicate the most effective conservation measures for the species would revolve around the protection of extant sites over the potential reintroduction to extinct sites which may be rendered unsuitable due to the historic use of these chemicals. These measures would prove beneficial to other species that may be threatened by similar chemicals or species subject to land use change found on the same contemporary sites. This effect would not be limited to flora species and include many insect species subject to population reduction due to the use of artificial pesticides (Goulson *et al.*, 2015).

Potentially promoting the conservation of *N. ustulata* through its use as a flagship species for wider chalk grassland flora could have negative externalities on other taxa, these negative externalities may threaten extant species more severely and potentially negatively offset any positive impacts to *N. ustulata* populations (Buckley and Crone, 2008). Positive outcomes from conservation actions that may be possible as a result of a potential increase in *N. ustulata* conservation action may lead to an increase in population sizes. Due to the ecological placement of *N. ustulata* (Tali *et al.*, 2004) no other species would be likely displaced or negatively impacted by larger population sizes. An increase in third party awareness of *N. ustulata* and its potential promotion as part of a flagship species conservation model may see an increase in footfall on its extant sites. This could lead to an increase in the trampling of plants on the site, this would not just affect *N. ustulata* but many other extant species. Increased footfall does pose a substantial risk to smaller species which may not be the most visible to many walkers, *N. ustulata* is a species at particularly prone to trampling due to its small size (Johnson, 2019). However, measures can be put in place to mitigate as much of this potential negative externality as possible, better signage as well as direct protection of the plants could be employed to limit the impacts of increased footfall.

Despite the potential for negative externalities impacting *N. ustulata* and other associated species, it would be beneficial to utilise *N. ustulata* as a flagship species for chalk grassland orchids and/or chalk grasslands. The choice experiments indicated it as one scoring above average, in the highest scoring quartile in five of the six BWS type 3 experiments, indicating it as a species that is desirable for it to be involved in future conservation efforts. Its ecology makes it likely to involved multiple other species indirectly through its conservation through the likely need to increase measures to safeguard extant sites. Although measures may need to be put in place to mitigate any potential trampling that may occur to its inflorescences or other associated species which may be prone to potential damage.

Chapter 3: Phenological response of *Neotinea ustulata* (L.) R.M.Bateman, Pridgeon & M.W.Chase and its putative pollinators to changes in temperature

Introduction

The current trends of anthropogenic climate change are seeing the development of many different regional, national and global changes to climates (Hansen and Stone, 2016). The apparent increase in frequency of abnormal weather events (De Sario *et al.*, 2013), increased local precipitation (Trenberth, 2011) and the global trend towards higher mean temperatures (Hansen and Stone, 2016) all pose different threats to species globally. Understanding how these changes, both current and predicted, can allow for conservation to determine possible changes and issues that may derive from anthropogenic climate change (Watson *et al.*, 2013).

Whilst most species globally face some threat to their continued survival from anthropogenic climate change (Summers *et al.*, 2012), plant species have decreased adaptive capacity due to their spatially static growth habits. Many ambulatory species can migrate in response to the gradual shift in their suitable climatic envelope, either altitudinally or latitudinally (Charitondou, *et al.*, 2021), the static growth habits of plant species make individuals unable to change their location in response to local climatic factors. Populations can shift their ranges both altitudinally and latitudinally over successive generations through the migration of future generations (Charitondou *et al.*, 2021). However, this effect is not suitable for all species. Taxa with specific habitat requirements may not be able to shift their spatial ranges due to habitat isolation (Vitt *et al.*, 2010), with seed vectors being required to provide the spatial migration of successive generations. Naturally occurring seed vectors may not be present for many species, as such human involvement may be required to effect spatial changes in populations ranges through methods such as 'assisted colonisation' (Vitt, *et al.*, 2010). For species that populations can remain stationary, either through local climates remaining suitable or through natural adaptation, the increase in local temperatures could alter climatic cues needed to time the annual biotic actions involved in sexual reproduction or the interruption of dormancy of many species (Adedoja, *et al.*, 2020).

Many insect species have a similar relationship with temperature in temperate climates, with many species entering a winter dormancy to persist through inhospitable temperatures (Danks, 2002). With many species requiring temperatures overcoming a threshold in order to break their dormancy (Danks, 2002). The increase in local mean temperature associated with anthropogenic climate change. Whilst species can persist through increasing early emergence dates, potential interactions with other species may be altered if species enter the required life stages at different times.

Unlike many flora species, pollination within the family Orchidaceae is relatively pollinator sensitive (Tremblay, 1992), it is not uncommon for species to be reliant predominately on a single species for their pollination (Tremblay, 1992). Within the family, known pollination mechanisms form three distinct groups: pseudocopulation (Schiestl, 2005), deceit (Schiestl, 2005) and pollinator rewarding (Nilsson, 1992), Pseudocopulation relies on imitating the form of specific insect species, and often genders, to enough of a degree that the pollinator species attempts to use structures of the flower as a means to sexual reproduction (Schiestl, 2005), flowers of this method tend to show very low visitation rates. Deceit pollination can include pseudocopulation as a pollination mechanism, but generally refers to deception through the replication of visual for fragrance cues to imitate flowers that produce some reward to visiting insects (Schiestl, 2005). Visitation rates of deceit pollinated species are often low (Tremblay, 1992). Pollinator rewarding species provide some substance to the pollinator species in order to attract them towards the inflorescence and flowers (Nilsson, 1992). Providing direct rewards to pollinating insects often sees higher visitation rates than pseudocopulation or deceit mechanisms (Nilsson, 1992). Rewards to pollinating species can take multiple forms, species in the genus *Angraecum,* among others, produce quantities of nectar at the end of the ‘spur’, a structure formed of the labellum (Wasserthal, 2014). Species of the genera *Lycaste* and *Stanhopea* produce fragrant oils, attracting male Euglossine bees, these flowers enhance the attractiveness of the males to females of the same species increasing the reproduction rates of visiting males (Dressler, 1968).

Floral structures within the Orchidaceae also enhance the specificity of their pollination mechanisms. The geometric relationship being specific to morphometrics of the potential pollinator species (Tremblay, 1992), providing a consistent placement of the pollinia onto the visiting insect. These pollination mechanisms can be simple height-based geometries, limiting pollinator sizes that successfully make contact with the viscidium (Tremblay, 1992), or more elaborate structures, such as those seen in the Cypripedioideae subfamily, involve temporary entrapment within a structure formed by the labellum (Pemberton, 2013). This morphometric relationship between flower and pollinator allows for consistent pollinia placement and retrieval between plants of the same species (Tremblay, 1992). Further, it reduces the chance of pollen exchange between different species, while increasing the likelihood of cross-pollination between plants of the same species, and thus maintaining genetic diversity within a population (Tremblay, 1992).

The risk posed to plant-pollinator interactions through anthropogenic climate change is that of pollination asynchrony (Wilcock and Neiland, 2002), the specific nature of orchid pollination ecology makes them particularly prone. Other flora species with less specific pollination mechanisms can rely on a variable list of pollinating species during their flowering period, allowing for a greater degree of resilience to potential changes in visiting insect species (Oliver *et al.*, 2015). The high degree of pollinator specificity within the Orchidaceae therefore requires pollinator flight dates to be in synchrony with the flowering time of the orchid (Tremblay, 1992). Such synchronous interactions can be achieved by both parties not responding to the local climate (i.e. flight and flowering times could respond to the photoperiod), or the parties respond in a similar manner to changes in climate conditions (i.e. the rate of response of the pollinator and orchid to increasing temperature is the same). Flowering times synchronous with pollinator flights will allow for consistent fecundity across a population and consistent rates of generational succession (Wilcock and Neiland, 2002), potentially maintaining extant populations. Species involved in plant-pollinator interactions that use differing variables to time their annual cycles could potentially develop pollination asynchronies if the timing variables changed over time (Adedoja, *et al.,* 2020).

The decline that *N. ustulata* has experienced through the 20th century and probably has continued through to the present (Tali *et al.*, 2004) has reduced local populations and the national meta-population to levels where possible population restoration, may be necessary to ensure long-term survival. As such any potential future restorations would require consideration of the IUCN ‘Guidelines for

Reintroductions and Other Conservation Translocations’ (IUCN/SSC, 2013). These guidelines include statements relevant to *N. ustulata* and its future conservation. Annex 3.2. states: ‘There should generally be strong evidence that the threat(s) that caused any previous extinction have been correctly identified and removed or sufficiently reduced’ (IUCN/SSC, 2013). This statement is currently not met by the knowledge base relating to *N. ustulata* and its current decline in the British Isles. Whilst factors have been previously attributed to its decline, information on the specific nature of the factors effecting the species survival is not sufficient to provide the information required to adhere to Annex 3.2.. Annex 3.3. states: ‘Where a high degree of uncertainty remains or it is not possible to assess reliably that a conservation introduction presents low risks, it should not proceed, and alternative conservation solutions should be sought’ (IUCN/SSC, 2013). Whilst it is implicit from Annex 3.2. the lack of information surrounding the identified threats on the species and their mechanisms contributing to the wider decline would indicate a higher uncertainty for any translocation actions (Abeli *et al.*, 2020). Other areas where reduced knowledge contributes a greater risk of failure to any translocations include uncertainties surrounding UK specific pollinators, with no taxa successfully identified as a pollinator within the British isles for *N. ustulata* var. *aestivalis*. Given the current state of knowledge surrounding *N. ustulata* pollination biology and wider ecology (Tali *et al.*, 2004), any restoration efforts are at risk of failure. As a result, further research is required to better understand the factors influencing the species’ decline and the factors that may underpin successful future restoration efforts.

One factor that is relevant to Annexes 3.2. and 3.3. is the current lack of understanding relating to the species pollination in the British Isles (Tali *et al.*, 2004). Whilst seed set rates in the UK are thought to be low (Tali *et al.*, 2004) the species is able to successfully maintain populations where other factors may allow for populations to remain. Whilst the specific taxa are not currently known, species morphometrically similar to the identified pollinators in Germany (Voth 1984, Mrkiva, 1991, van der Cingel 1995) are present on the mainland British Isles and as such provide a list of species with potential to act as viable pollen vectors for *N. ustulata*. This study will use these putative pollen vectors to determine the extent to which plant-pollinator asynchrony any impact the long-term survival of the species.

Whilst the threats outlined above are applicable to a large number of species globally, this study will use *Neotinea ustulata*, the Burnt tip Orchid, as a case study to investigate potential pollination asynchronies that may develop as a result of local climatic changes driven by anthropogenic climate change.

The pollination of *N. ustulata* has been poorly studied. The monograph of the species by Tali *et al.* (2004) lists two different pollinator species, one for each recognised variety, with the data on pollen vectors for the species described as 'scarce'. The variety var. *ustulata* has been recorded being pollinated by the beetle *Leptura livida*, while the pollination of var. *aestivalis* has been recorded in two separate studies (Voth 1984, Mrkvicka 1991), being pollinated by the Tachinid fly, *Tachina magnicornis* Zett (then listed as *Echinomyia magnicornis*). The evidence outlined in Voth (1984) is substantial, 9 approaches and 49 visits recorded for a small population of 11 plants in a 4 hour period, with 7 of the 13 individuals carrying 26 pollinia. However, evidence of *T. magnicornis* on the UK mainland is lacking. Only one distribution map for *Tachina magnicornis* records the species on more than the Channel Islands (NBN Atlas, 2021). With the consensus being a lack of reliable evidence for the species across the British Isles for *T. magnicornis*. This may not negatively impact the pollination of *N. ustulata* as other species of *Tachina* are known to have very similar morphology, with molecular analysis being the only reliable identification method.

Tachina biology is relatively complex. As a parasitoid wasp species they utilise other taxa to form part of their reproductive ecology (Stireman *et al.*, 2006). Tachina species parasitise multiple taxa, undergoing an indirect incubation, with the parasitised species ingesting the eggs and incubating the fly larvae until they consume the host insect (Stireman *et al.*, 2006). The non-specific nature of the genus' parasitism makes them more resilient to biodiversity loss than other taxa-specific Tachinidae taxa.

The dormancy undergone by the *Tachinid* species identified due to their subterranean state is likely broken by temperature-based cues as the conditions become hospitable for their adult stages (Stireman *et al.*, 2006). The difference in the use of different variables to influence the initiation of different life stages could potentially contribute to the establishment of a pollination asynchrony as the effects of anthropogenic climate change develops (Adedoja *et al.*, 2020).

Methods

Records for potential pollinator species were downloaded from the NBN Atlas (https://www.nbnatlas.org/). Recorded dates were transcribed into a continuous scale of days from March 1st that the species was recorded. Records were then filtered to remove any potentially anomalous or duplicated data points. Records with incomplete dates, either just the year or year and month recorded, were removed. Multiple records in some data sets listed records for 1st January, these records were excluded as cases of inaccurate dating. All such records were from the 19th century and likely represent records that lack the day and month. Records were also assessed for possible duplicates. For this data duplicates were defined as records for the same taxa, from the same site, recorded on the same day. Multiple records from the same individual on the same day could remain in the dataset if they were from notably different sites. Site locality was assessed using the georeferenced information attached to the records downloaded. Excluding select records based on date and site locality also prevented individual records being present in multiple databases entering the analysis and therefore issues of non-independence.

The species identified for use in this study as potential pollinator species are: *Tachina fera*, *T. grossa, T. ursina, T. lurida,* and *L. livida*. All share a similar morphology to *T. magnicornis*, have flight times that overlap with the flowering period of *N. ustulata*, and have substantial numbers of records from the UK.

Records for *N. ustulata* were obtained from Dr Karen Robbirt, based on observations of preserved herbarium specimens that were accurately dated. This yielded 92 records at the correct flowering state, dated 1823-1955 (Robbirt 2012). These herbarium records were unable to separate the two described subspecies of *N. ustulata* so records were assessed for comparisons for known and potential pollinators for *N. ustulata* rather than separately for the two varieties.

The species-specific records were tested for normality and outliers, before proceeding with any further analysis.

UK mean monthly temperature data for the years 1800-2020 was obtained from the UK Meteorological Office website: (http://www.metoffice.gov.uk/research/hadleycentre/CR_data/Monthly/HadEWP_act.tx). Temperatures for each of the nine months preceding the flowering of *N. ustulata* were collected from this dataset and associated with the records with regards to the 'Start Date' supplied with the records. The months January-May inclusive were temperatures from the year of the record date, with the months September-December inclusive having their temperatures allocated from the year preceding the record date. The three seasons, Autumn, Winter and Spring, used in the analysis were calculated as the mean of the three inclusive months in the sets, September-November, December-February and March-May respectively.

Analysis was carried out on the records with Pearson Correlations and Analysis of Covariance (ANCOVA) tests on SPSS 25. Linear regressions were carried out for the nine months preceding the beginning of flowering of *N. ustulata* September-May, regressions were also carried out on the seasonal mean temperatures. ANCOVA and the appropriate Post Hoc tests were done on the March-May interval and the month with the greatest significant regression coefficient from within that interval. Significance thresholds were altered to conform with Bonferroni corrections in the case of multiple experiments with the same datasets. Pearson Correlations and linear regressions used the Bonferroni corrected 95% significance threshold of $p<0.004$.

A predictive model was used to validate the linear regressions, predicting the mean flight date given the mean springtime temperature recorded in 2020 using the regression line comparing it with the mean days since March 1st recorded from the accessed NBN records. This was used to predict the future patterns in flight dates of these potential pollinating species up to a mean springtime temperature of 12℃, a 22% increase on the 2020 mean March-May temperature of 9.87℃

Results

The filtered records resulted in 67 to 81% complete and independent records for the pollinator species (Table 4). Herbarium records for *N. ustulata* provided a much lower yield of 53%, (Table 5) however this was due to the lack of complete dates on the observed specimens.

Table 8: Availability of UK data for five putative pollinators of *Neotinea ustulata*

Pollinator taxa	Total records sourced	Incomplete dates	Duplicated dates	Total viable records (n)	Percentage (%)
Leptura livida	1216	165	230	821	67.5
Tachina lurida	133	18	9	106	79.7
Tachina ursina	931	38	179	714	76.7
Tachina fera	5738	143	953	4643	80.9
Tachina grossa	2088	161	414	1513	72.5

Table 9: Availability of UK data for the orchid *Neotinea ustulata*

Orchid taxon	Total records sourced	Incomplete dates	Duplicated dates	Total viable records (n)	Percentage (%)
Neotinea ustulata	172	74	0	92	53.5

Pearson correlations between the selected temperature intervals and flowering times of *N. ustulata*, identified only a significant correlation that being the Spring (March-May) mean temperatures (Table 6). Flight time of all potential pollinator species, with the exception of *Tachina fera*, had a significant correlation with Spring (March-May) temperature. Correlation between flight date and specific months varied between the potential pollinators, however there was a significant correlation with April temperature for all potential pollinators and all but *T. grossa* had a significant correlation with February temperatures.

Table 10, Pearson correlation (r value) between flowering or flight date expressed as days since March 1st, and observed temperatures for the preceding nine months and three season, p values expressed in parentheses

	***Neotinea ustulata* (n=92)**	***Leptura livida* (n=821)**	***Tachina lurida* (n=106)**	***Tachina ursina* (n=714)**	***Tachina fera* (n=4643)**	***Tachina grossa* (n=1513)**
Jan	0.156 (0.138)	-0.187* (<0.001)	-0.236* (<0.001)	-0.086 (0.021)	-0.026 (0.072)	0.005 (0.835)
Feb	-0.253 (0.015)	-0.132* (0.002)	-0.150* (<0.001)	-0.174* (<0.001)	-0.080* (<0.001)	-0.060 (0.019)
Mar	-0.168 (0.110)	-0.088 (0.040)	-0.088 (0.120)	-0.196* (<0.001)	-0.064* (<0.001)	0.019 (0.470)
Apr	-0.240 (0.021)	-0.159* (<0.001)	-0.246* (<0.001)	-0.144* (<0.001)	-0.052* (<0.001)	-0.150* (<0.001)
May	-0.215 (0.039)	-0.168* (<0.001)	-0.220* (<0.001)	-0.067 (0.073)	-0.006 (0.706)	-0.142* (<0.001)
Sep	0.173 (0.100)	-0.074 (0.085)	-0.800 (0.021)	-0.045 (0.232)	-0.007 (0.646)	-0.002 (0.951)

Oct	-0.054 (0.608)	0.012 (0.776)	0.024 (0.497)	0.092 (0.014)	0.037 (0.031)	0.054 (0.036)
Nov	-0.025 (0.810)	0.097 (0.024)	0.162* (<0.001)	0.033 (0.378)	0.026 (0.073)	0.127* (<0.001)
Dec	-0.112 (0.289)	0.046 (0.0289)	0.017 (0.625)	0.044 (0.243)	0.024 (0.109)	0.022 (0.388)
Spring	-0.298* (0.004)	-0.194* (<0.001)	-0.260* <0.001)	-0.108* (0.004)	-0.034 (0.022)	-0.127* (<0.001)
Autumn	0.041 (0.699)	-0.005 (0.910)	0.051 (0.144)	0.046 (0.223)	0.029 (0.047)	0.100 (<0.001)
Winter	-0.104 (0.324)	-0.116 (0.007)	-0.158* (<0.001)	-0.076 (0.043)	-0.030 (0.038)	-0.009 (0.715)

* Bonferroni corrected 95% significance threshold p<0.004

For those potential pollinators that showed a significant correlation between flight date and mean Spring temperature, the phenological shifts based on a linear regression ranged from -3.2 to -11.9 days °C^{-1}, whereas *N. ustulata* showed a flowering response of was -4.0 days °C^{-1}. As noted previously, while *N. ustulata* showed no significant correlation between flowering time and specific mean monthly temperatures, all five potential pollinators showed a significant correlation between flight time and mean April temperature. Based on the mean April temperatures, the phenological shift of potential pollinators based on a linear regression ranged from -2.1 to -6.4 days °C^{-1}.

Table 11: Phenological shift in response to a 1°C based on a linear regression between flowering or flight date expressed as days since March 1st and mean monthly and seasonal temperatures alongside r^2 values expressed in parentheses

	Neotinea ustulata	***Leptura livida***	***Tachina lurida***	***Tachina ursina***	***Tachina fera***	***Tachina grossa***

	n=92	**n=821**	**n=106**	**n=714**	**n=4643**	**n=1513**
Jan	1.038 (0.024)	-2.548* (0.056)	0.029 (<0.001)	-1.572 (0.007)	-0.835 (0.001)	0.078 (<0.001)
Feb	-1.631 (0.064)	1.486* (0.023)	-2.925* (0.021)	-2.705* (0.030)	-2.327* (0.006	-0.788 (0.004)
Mar	-1.317 (0.028)	-1.124 (0.008)	-6.605 (0.065)	-3.768* (0.038)	-2.345* (0.004)	0.287 (<0.001)
Apr	-2.566 (0.580)	-3.613* (0.061)	-6.396* (0.077)	-2.739* (0.021)	-2.131* (0.003)	-2.408* (0.022)
May	-2.119 (0.460)	-3.816* (0.048)	-5.767 (0.030)	-1.868 (0.005)	-0.275 (<0.001)	-3.034* (0.200)
Sep	1.95 (0.030)	-1.383 (0.006)	-1.626 (0.002)	-1.179 (0.002)	-0.348 (<0.001)	-0.032 (<0.001)
Oct	-0.574 (0.003)	0.335 (0.001)	3.927 (0.019)	2.135 (0.009)	1.264 (0.001)	0.930 (0.003)
Nov	-0212 (0.0.001)	2.239* (0.81)	-0.952 (0.001)	0.575 (0.001)	0.952 (0.001)	1.936* (0.016)
Dec	-0.830 (0.120)	0.161 (<0.001)	-0.251 (<0.001)	0.475 (0.002	0.506 (0.001)	0.204 (<0.001)
Spring	-4.046* (0.890)	-5.692* (0.068)	-11.924* (0.102)	-3.309* (0.012)	-1.935 (0.001)	-3.240* (0.016)
Autumn	0.700 (0.002)	-2.125 (0.003)	-1.916 (<0.001)	-1.530 (0.002)	-1.134 (0.001)	-0.155* (0.100)
Winter	-1.054 (0.011)	-2.125* (0.0025)	-1.916 (0.005)	-1.530 (0.006)	-1.134 (0.001)	-0.155 (<0.001)

* Bonferroni corrected 95% significance threshold p<0.004

Analysis of covariance between the pollinator species and *N. ustulata* indicated significant differences between the reaction between both *N. ustulata* and the potential pollinators: *L. lurida* (df: 911, p <0.001), *T. fera* (df: 4732, p <0.001), *T. grossa* (df: 1603, p <0.001), and *T. ursina* (df: 802, p <0.001) in response to mean springtime temperature. *T. lurida* showed no significant difference to its reaction to mean springtime temperature to *N. ustulata* (df: 197, p=0.062). Reaction between Plant and pollinators across all single months with the most significant linear regression between March-May inclusive (April: *L. livida, T. lurida, T. ursina, T. grossa*, March: *T. fera*) indicated Significant differences between all pollinator species and *N. ustulata*: *L. livida* (df: 911, p <0.001), *T. fera* (df: 4732, p <0.001), *T. grossa* (df: 1603, p <0.001), *T. lurida* (df: 197, p <0.001) and *T. ursina* (df: 802, p <0.001).

A predictive model for pollinator reactions to future temperature increases (Fig.2) indicates similarity between the predicted mean record dates and the observed mean record dates from the accessed records, validating the use of linear regressions to predict future phenology changes for most of the selected potential pollinator species. *Tachina lurida* exhibited significant differences between the predicted and observed flight dates, making the linear regression model invalid to predict its future temporal habits. The predicative model also indicates that the potential pollinator species are influenced more by future springtime temperature increases than *N. ustulata*, with their emergences being primarily driven by temperature, whereas *N. ustulata*, being a wintergreen species, most likely uses photoperiod as the primary timing factor for its annual flowering, with temperature acting to accelerate or decelerate biotic actions. The predictive model also indicates that *N. ustulata* has the median dated record count of all the species analysed, a mean of days 89 since March 1st. The future distribution of *N. ustulata* amongst the flight dates of potential pollinator species indicates that the species selected could act as pollinating species for the foreseeable future, fitting within the range of predicted flowering dates for *N. ustulata*.

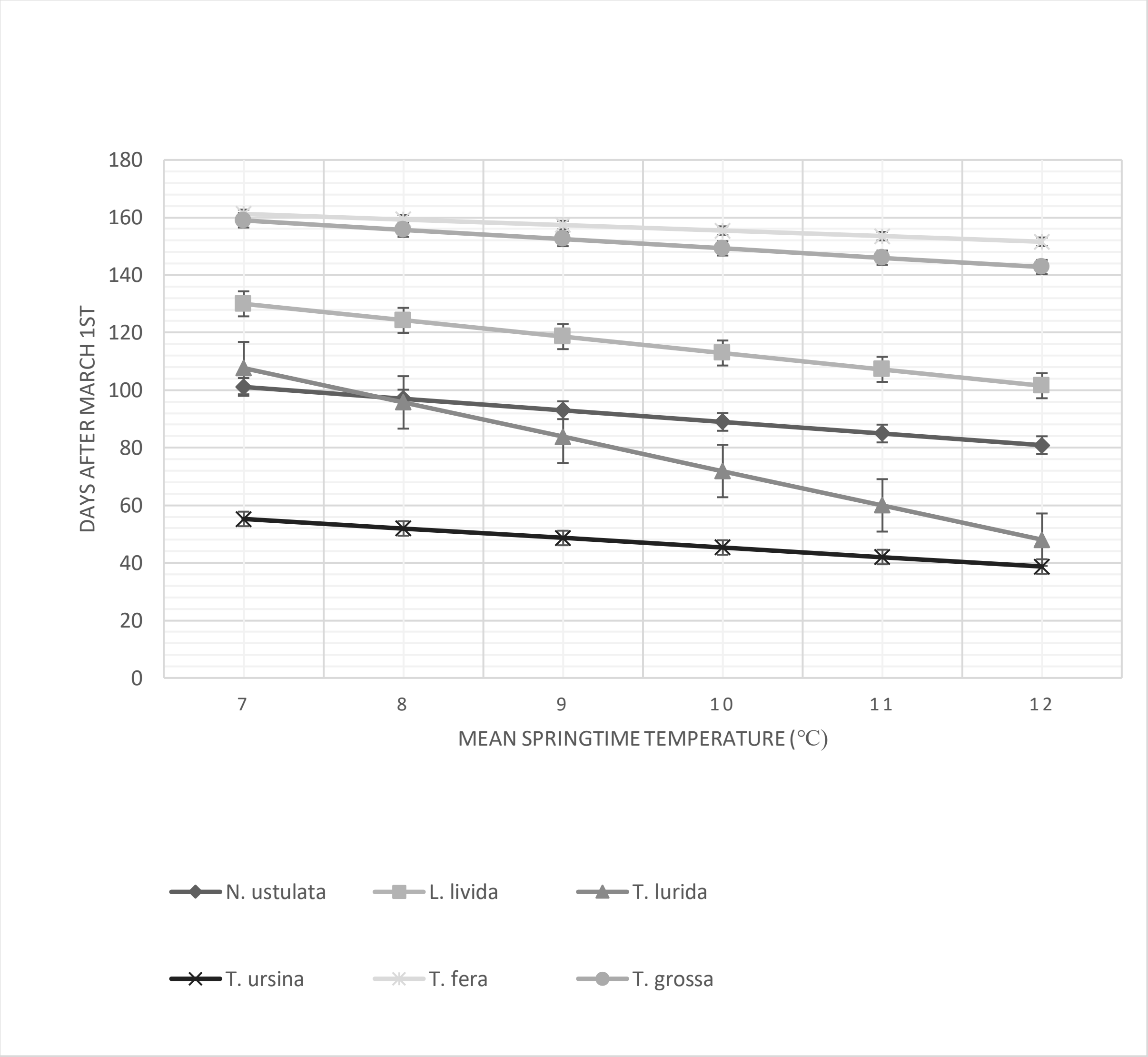


Fig. 2, Peak flowering/flight dates changes in response to mean Springtime Temperature (T)(℃), expressed as days since March 1st (D), Based on the following Linear regression equations for both *N. ustulata* and putative Pollinators: *Neotinea ustulata* (D= -4.046T + 129.435), *Leptura livida* (D= -5.692T + 169.830), *Tachina Lurida* (D= -11.924T + 191.128), *Tachina ursina* (D= -3.309T + 78.414) *Tachina fera* (D= -1.935T + 174.787), *Tachina grossa* (D= -3.240T + 181.655)

Discussion

Based on the results presented, all of the potential pollinator species selected would be suitable species for successful cross-pollination of *N. ustulata*. The beetle, *Leptura livida,* was included in the dataset to represent the pollination of var. *ustulata*, as a known pollinator (Tali *et al.,* 2004). var. *aestivalis* does not have a known pollinator that is present on the UK mainland, this would be recommended to be the subject of future research into the species pollination ecology. Without pollinator species confirmed through comprehensive field observations, knowledge of the potential phenological changes that may happen to the pollination ecology of *N. ustulata*, and the ecology of the pollinator species will be limited to a theoretical model for future changes. The results here would suggest that if the species selected would be viable species for successful pollination of *N. ustulata*, a multi sampled observation period be designed to see if the visiting insect species are constant or transient with species specific flight numbers, making the species reproduction more resilient to future climatic changes.

Whilst individual pollinator species may show significant differences in their response to both mean springtime temperature and singular month mean temperature changes, which may see potential divergences in flight/flowering dates between *N. ustulata* and its pollinator species in the British Isles, as the species undergoes potential pollination disentanglement. If the species does act as a pollinator generalist (Tali *et al.*, 2004) in the British Isles then the existence of similar species which may potentially act as viable pollen vectors for *N. ustulata* would allow for an adaptability to changes in mean springtime temperatures with other species' phenological events bringing them more into line with mean flowering dates for *N. ustulata* allowing them to act as modal pollen vectors as other species mean flight dates reduce their frequency within *N. ustulata*'s immediate habitat around its peak flowering times.

Based on the data collected however it appears unlikely that a potential pollination asynchrony could establish between *N. ustulata* and the potential pollinator species selected. This conclusion is based upon the assumption that the species selected are involved in the pollination of *N. ustulata* across its range in the British Isles and that *T. magnicornis* is not present on the British mainland. The latter can be assessed primarily through the review third party records as has been done here, but it would be limited to the extent of the records completeness and the wider knowledge about *Tachinid* identification (Stireman *et al.*, 2006). The former is an important knowledge base to develop to better inform any future restoration efforts, with the pollination of the species being an underpinning factor in long-term population maintenance for both extant and restored populations (Wilcock and Neiland, 2002). With the species involved in the plant-pollinator interactions of *N. ustulata* currently uncertain, any future restorations would have an increased chance of long-term failure due to lack of sexual reproduction within the restored population, decreasing the chances of producing a more contiguous national meta-population to promote exchanges in genetic diversity.

The *Tachina* species selected likely have shortcomings in their knowledge due to their status as relatively unknown taxa to most third-party observers, their life cycle also makes them relatively unmarketable (Stireman, *et al.*, 2006), with parasitism being seen as an 'unpleasant' method of reproduction. If *N. ustulata* is to be utilised as a flagship species (Caro, 2010), and the ecological relationship between *N. ustulata* and the selected potential pollinator species confirmed, it would be possible to utilise a multi-species conservation approach and use *N. ustulata* as an 'umbrella species' (Caro, 2010) and extend funding to as many species as possible to assist in the conservation of the species in the future. Protecting the potential pollinator species of *N. ustulata* would be an important part of any future conservation actions to ensure successful sexual reproduction of the species allowing for a sustainable generational succession.

Anthropogenic climate change may pose potential threats to the long-term survival of *N. ustulata*. Tali, et at. (2004) reported that one of the major factors influencing the growth of the species being water, with plants being susceptible to being waterlogged, this was implied through field observations in June 2020, with plants being located solely on areas with localised elevation, among species consistent with drier substrates (pers. obs., June 2020). A regularly reported potential effect of anthropogenic climate change is an increase in precipitation (Gleick, 1989), potential effects of this precipitation increase could be the increased chance of plants being waterlogged, with the potential of increasing mortality rates within populations. Placing pressure of sexual reproduction to replace lost numbers. If the fecundity of populations, both extant and restored is impacted by issues concerning pollinator numbers alongside any present or future threats to extinction of the species, both locally and nationally, then population sizes and numbers could reduce over time as sexual reproduction rates cannot regenerate the numbers of plants lost (Wilcock and Neiland, 2006), leading to a gradual decline towards localised extinctions as has been seen in Kent over the past 50 years (Johnson, 2019).

Whilst the information presented does not categorically aid in possible future restoration efforts, this information and any future pollination studies will assist in reducing uncertainty over potential future reproductive success of *N. ustulata* in both extant and any future populations to be established, assisting the criteria outlined in the IUCN Reintroduction guidelines (IUCN/SSC, 2013)

Information on the long-term survival of the pollinator species identified with the effects of continued anthropogenic climate changes cannot be confirmed. Although the generalist nature of the *Tachina* species' parasitism allows for a more reliable adaptive capacity than species specific parasitism (Stireman, 2006) and may create greater resilience in the species response to both climatic events and other events that could pose greater risks of extinction. Although research on the *Tachina* species is not regularly produced, any future work concerning *N. ustulata* would be advised and investigate the pollinators more. Identification of a pollinating species of *N. ustulata* within the British Isles would be a significant development in any potential future conservation efforts of the species within the British Isles. This study worked under the assumption that one/all of the species identified can function as pollen vectors for *N. ustulata* an assumption that could not be tested. The findings of this study would require complete revision if this assumption can be identified as false.

As the potential plant-pollinator interactions of *N. ustulata* would be an underpinning factor to the fecundity of both extant and potentially restored populations (Reiter *et al.*, 2016), it would be a recommendation of this study to be an important part of the ecology of *N. ustulata* to be understood in the British Isles for any future conservation. Identification of pollinator species would provide a minimum indicator as to potentially viable sites for future restorations through the basic knowledge of viable pollinators ecology (Reiter *et al.*, 2016), with trapping studies confirming the presence of suitable pollinating species before the reintroduction of any plants. The presence of suitable pollinating insects alone cannot deem a site suitable for plant reintroductions, but it can advise as to further investigation without damaging the site, with trapping being a much less invasive study than other factors required for long-term plant survival.

Chapter 4: Discussion

Introduction

Neotinea ustulata is a small terrestrial orchid species found across Europe, primarily on calcareous grasslands, it produces short (10-20cm) inflorescences between May and June. In the UK the species is primarily found in small, isolated populations with only ten known populations regularly producing 200 inflorescences (Tali *et al.,* 2004). After senescence the plant produces a small rosette of leaves in August-September (Tali *et al.*, 2004) and maintains its leaves over winter until the initiation of flowering the following year. The species has known to have been in decline since the 1930's (Johnson, 2019, Tali *et al.*, 2004), becoming limited to protected sites (i.e. SSSIs) and Ministry of Defence owned sites (Tali *et al*, 2004).

The decline that *N. ustulata* is currently experiencing nationally, is a cause for concern for the long-term viability of the species in the United Kingdom and as such conservation action should be taken in order to reverse its recent population decline. Whilst never a common species (Johnson, 2019) the recent decline has severely reduced the Kent population, to where only two plants are currently known in the county (Johnson, 2019, Alfred Gay, Pers. comm.). Large populations do exist nationally, with the population at Parsonage Down, Wiltshire, known to comprise 3000 Plants (Tali *et al.*, 2004), many smaller populations have become extinct with only 75 populations known of 2004 (Tali *et al.*, 2004).

Listed as Endangered on the 'British Red List for Vascular Plants' under the criteria A2c, having undergone a >50% population reduction in the past 30 years (Stroh *et al*., 2014). The species has continued to decline through the 21st century with populations not being recorded in recent years (Bersweden, 2018, pers. obs.). No studies of the species population size and distribution have been undertaken since 2004, therefore exact knowledge as to the state of the decline since 2004 is poorly known. Factors threatening the continued persistence of the species have been identified as overgrazing and the use of artificial compounds associated with intensified agriculture (Tali *et al.* 2004), although this has not been thoroughly investigated.

Little is currently known about many areas of the species ecology, with no information about the pollination ecology of the species within the British Isles, other than speculation of ‘very low’ seed set rates (Tali *et al.*, 2004). Pollination studies have been carried out in some European countries, including the identification of a viable pollen vector for var. *aestivalis* in Germany, the Tachinid wasp *Tachina magnicornis*. As well as a viable pollen vector for var. *ustulata*, *Leptura livida*.

Calcareous grasslands, as with many habitat types are subject to degradation and habitat loss due to land use change (Watkinson and Ormerod, 2001). Many species found on calcareous grasslands are species of conservation importance (Wallis de Vries, 2002), with many species’ endemic to the habitat. It is also an important habitat for British native orchid species, with 20 of the 52 native species being found on calcareous grasslands (Harrap and Harrap, 2005, Bowmer, 2008), many of which are also threatened with extinction by anthropogenic activities. Whilst many species native to calcareous grasslands are declining, species have been successfully conserved. The orchid *Ophrys fuciflora* is a species found only within the British Isles on the calcareous grasslands of East Kent. Restricted to only a few sites, populations have been preserved and restored through protection from grazing as well as habitat management (pers. obs., June 2021).

Through the two research chapters the potential limitations for future conservation action regarding *N. ustulata* has been assessed to better facilitate any future actions. Whilst there is much to be learnt about the species’ ecology (Tali *et al.* 2004), these two chapters aim to provide an initial knowledge of the species in relation to its conservation. Specifically, the chapters focus on the species’ competitiveness in relation to potential species-based funding, and the response of *N. ustulata* and its putative pollinators to climate change.

In order for *N. ustulata* to be subject to future conservation action the species will need to be competitive with other species in order to compete for limited funding opportunities (Caro 2010). Competition for funding will require the species to be one that is subjectively desirable to be preserved. If the species is desirable enough for involvement for future conservation the species may be suitable to be utilised as a ‘flagship’ species for the wider ecosystem of calcareous grasslands in Kent (Caro, 2010), and thus potentially assisting with public engagement with conservation of the habitat.

The phenological interaction between the putative pollinator of *N. ustulata* and the plants inflorescences is an interaction based on responses to climatic events for the taxa involved (Robbirt *et al.*, 2011). *N. ustulata* var. *ustulata* is a wintergreen species (Tali *et al*., 2004), with the annual production of an inflorescence likely being in response to changes in photoperiod, a factor with no annual variation, although temperature can affect the speed of inflorescence growth (Robbirt *et al.*, 2011). The putative pollinator species' emergence from their sub-terranean over-winter pupation is in response to increasing temperatures throughout the spring (Stireman *et al.*, 2006), a factor that can have large annual variation, changing the peak flight dates for Tachinid species potentially changes the pollination ecology of *N. ustulata* (Wilcock and Neiland, 2002). If the plant-pollinator interaction changes significantly enough due to changes in springtime temperature caused by anthropogenic climate change, pollinator species may not be present during the flowering period of *N. ustulata* leading to reproductive failure of the species (Wilcock and Neiland, 2002).

The species has been known in Kent since the 18th century, with regular reports through the 19th century (Johnson, 2019), reports declined in proportion to the national decline through the early 20th century. Since 1945 the species has only been recorded from four locations, Plants have not been recorded from Queensdown Warren since 1988 (Johnson, 2019). The other three sites persisted into the 21st century, until 2006 when no inflorescences were recorded on any of the known sites (Johnson, 2019). Two inflorescences were recorded on one site near Lydden in 2013 and have flowered sporadically until 2019 when they were pollinated by hand (Alfred Gay, Pers. comm., Johnson, 2019), no inflorescences have been recorded since (as of July 2021).

Contribution to the Field

Neotinea ustulata and the Funding Landscape

Any potential conservation actions undertaken to attempt to reverse the recent decline of the species will require the funding required to undertake any potential actions. As such the species will be competing either directly or indirectly with other species for any given funding opportunities (Caro, 2010). The Best-Worst Scaling methodology utilised in the research chapter attempted to indicate any preference towards or against *N. ustulata*. Any preference towards the species would have no direct impact on the conservation measures required for the species long-term survival, but may have influences on multiple stakeholders that may make engagement with potential conservation of the species more likely (Caro, 2010). The results collected from 61 respondents over a 28-day period indicated a preference towards *N. ustulata* in 5 of the 6 choice experiments undertaken. With all 4 of the image-based experiments showing a preference towards *N. ustulata*. The choice experiment that did not show a preference towards *N. ustulata* showed little deviation from the mean, indicating a potential indifference when compared with the other species in its choice set.

Whilst species for which ‘preference’ is shown using this methodology is not directly indicative of a species that directly receives a greater amount of funding, the species may be competitive within the funding landscape when compared to other species from its direct environment (Caro *et al.*, 2004). The preference indicated by the Best-Worst Type 3 Methodology (Casini *et al.*, 2009) used may indicate a better public engagement with the species and any future conservation measures undertaken. An increase in conservation action focusing on *N. ustulata* is not of any direct benefit to species in its direct environment (Caro, 2010), however any potential measure put in place to safeguard extant populations would have wider positive impacts to many other species, suggesting that *N. ustulata* could be used as a ‘flagship’ species for chalk grassland flora (Caro *et al.*, 2004).

As with any species that may require conservation action to secure its populations long term viability, there is potential for conservation measures or an increase in population to have a negative impact on other species within its direct habitat (Jordan, 1995). However increased public awareness that may result from any conservation actions can be speculated to have a negative impact through an increased risk of plants being damaged by visitors to extant sites. Although the risk posed by trampling is typically low, especially for species with prominent inflorescences.

Pollination phenology of *Neotinea ustulata*

The pollination ecology of *N. ustulata* in the British Isles is poorly understood (Tali *et al.*, 2004). No pollinator has been identified for the Later flowering form, var. *aestivalis*, present on the mainland

British Isles. The pollinator of the early flowering form, var. *ustulata (*Tali *et al.*, 2004), is known from the mainland British Isles (*Leptura livida*), although this variety has not been recorded in Kent (Johnson, 2019). Five species have been identified as potential pollinators for *N. ustulata* var. *ustulata* in the British Isles: *Tachina grossa, T. fera, T. lurida, T. ursina* and *Leptura livida*. The *Tachina* species identified are all morphologically similar to the known pollinator of var. *aestivalis* on mainland Europe, *Tachina magnicornis* (Tali *et al*¸ 2004). In this chapter it assumed that all of the species selected for analysis were potential pollen vectors for *N. ustulata* during their flight dates, although future studies would be required to identify the pollinator.

The response of all species potentially involved in the plant-pollinator relationship to changing mean springtime temperatures due to anthropogenic climate change were assessed. All potential pollinator species showed changes to flight dates in response to changing springtime temperatures, with flight dates moving earlier with warming temperatures. *N. ustulata* also changed its peak flowering dates earlier in response to increasing springtime temperatures (4.0 days°C^{-1}). Whilst individual pollinators rate of change in response to temperature differed from the rate of change shown by peak flowering of *N. ustulata*, (*Leptura livida*: 5.7 days°C^{-1} , *Tachina lurida*: 11.9 days°C^{-1}, *T. ursina*: 3.3 days°C^{-1}, *T. fera*: 19.4 days°C^{-1} and *T. grossa*: 3.2 days°C^{-1}) pollination asynchrony may be unlikely as the modal pollen vector could potentially change in response to increasing temperature over time.

Future Research and recommendations

One of the original aims of this research was to carry out a detailed pollination study, however due to COVID-19 restrictions at the time of research, the potential risks of requiring a period of self-isolation during the study period and stopping the collection of data through the flowering period of *N. ustulata.* This aspect of research was abandoned. Research into the species pollination ecology is therefore recommended as a priority for future research. Whilst there is knowledge of the species' pollination ecology on mainland Europe (Tali *et al*., 2004), there is little knowledge of the species' pollination ecology in the British Isles; although Tali *et al.* (2004) speculated the seed set rate of *N. ustulata* in the British Isles as being 'very low'. Knowledge of aspects of the species' pollination ecology are required in order to generate a profile of extant populations. The identification of viable pollen vectors for *N.*

ustulata within the British Isles would allow potential restoration efforts to identify site viability with the presence of suitable pollinator(s) to allow species to naturally reproduce (Reiter *et al.*, 2016). Other factors that may influence pollination success should also be assessed, proximity to other plants of the same species as well as other flowering taxa during the flowering period of *N. ustulata* would provide an understanding of the community composition that influences reproductive success of *N. ustulata*. Other factors such as both male and female reproductive success and fruiting success should be carried out, as well as viability testing of seed collected to generate a viability rate

Alongside the understanding of the pollination ecology of *N. ustulata,* the fungal ecology of the species should also be researched. The role fungi play in orchid seed germination is readily understood (Arditti, 1992, Arditti, 2008), with the formation of fungal pelotons within cells of the testa, providing the energy requirements of the seed for successful germination (Arditti, 1992). This process continues until the protocorm develops its first leaves and can meet its biotic energy requirements through photosynthesis (Arditti, 1992). Knowledge about the fungal species involved in the mycorrhizae of *N. ustulata* is lacking, with prior attempts to extract fungal pelotons failing (Dave Roberts, Pers. comm.). Developing an understanding into the mycology of extant sites, as well as the fungal species involved with germination of *N. ustulata* seed would allow for a greater understanding of the requirements needed for the survival of the fungal species required for the germination of seeds to supplant the natural losses from any *N. ustulata* population for long-term population maintenance.

The effects of anthropogenic climate change should also be considered on the fungal ecology of *N ustulata*. Both Increasing mean temperatures and changes to precipitation patterns may have impacts as to the growth of fungal bodies in calcareous grasslands due to changes to the hydrology of the sites. Tali *et al.* (2004) also observed that plants of *N. ustulata* were most often found in drier areas on extant sites, although no research on this was cited. This trend can also be observed on sites of differing elevations, such as the ancient fortifications at Yarnbury Castle and Pewsey Downs, Wiltshire (pers. obs. June 2020)

Once the pollination and fungal ecology of the species is understood, steps can be taken towards creating micropropagation protocols for the species to germinate and grow plants *in-vitro*. Germination of the seeds *ex-situ* is believed to be challenging due to the lack of understanding of the mycorrhizal association and seed behaviour with respect to a dormancy or maturation period. The germination of seeds *in-vitro* is the first stage towards being able to produce plants *ex-situ* with the eventual aim of producing plants for future restorations of the species *in-situ* as was done in Ramset and Stewart (1998). Although further knowledge of species cultivation is required to grow plants onto a size to minimise translocation failures (Reiter *et al*., 2016). Any future micropropagation will require an understanding of the fungal ecology of *N. ustulata* in order to advise the techniques and protocols to generate the highest germination rates (Arditti, 2008). Fungal samples collected from the roots or *N. ustulata* may also be required for higher germination rates, although the use of septic micropropagation requires successful extraction of fungal pelotons (Arditti, 2008).

Developing the understanding of the causes of the current decline of the species should also be a priority for future research. Ensuring compliance of the IUCN reintroduction guidelines (IUCN/SSC, 2013), any causes of the decline should be understood, and the effects reversed or mitigated before the initiation of any future restorations. The speculated causes of the decline, over-grazing, and the use of artificial compounds in agriculture (Tali *et al*., 2004), could be issues that remain and would need the potential threat to be reversed if future conservation of the species through plant translocations can be successful. Research into the causes of the species’ decline is therefore required.

Conclusions

Overall, the outlook for the future of *N. ustulata* in Kent is bleak. Whilst populations persist nationally, potentially only the population at Parsonage Down, Wiltshire (Tali *et al*., 2004), is large enough to sustain the populations long-term genetic viability. The population at Parsonage Down is potentially the most important population of the species in the British Isles for future conservation. As the largest population nationally, if not in Northern Europe (Tali *et al*., 2004), it would provide the greatest genetic diversity of any population nationally, for any future restoration in Kent.

The Kent population has reduced to no more than two known plants as of 2019 (Johnson, 2019; Alfred Gay, Pers. comm.), however, whilst the species may not be extinct within the county, it may be functionally extinct requiring human intervention. Similar species have been recovered from population contractions in recent decades (Johnson, 2019), but they have not recovered without human intervention (Ramsey and Stewart, 1998, Johnson, 2019). *Orchis simia*, the monkey orchid, required human intervention through seed scattering across various potentially viable sites across the county following the discovery of two flowering plants near Faversham in 1965 (Johnson, 2019). This action established the population known today from Park Gate Down, where 100+ plants flower annually (Johnson, 2019, pers. obs., June 2021). *Cypripedium calceolus* has been recovered through more intensive interventions, with the production of plants from seed collected *in-situ* and plants translocated to new sites (Ramsery and Stewart, 1998). Both methods may be viable methodologies to potentially restore the Kent meta-population. Although they both require fundamental information about the ecology of *N. ustulata* that is currently unknown (Tali *et al.*, 2004).

One of the factors influencing the long-term conservation prospects of *N. ustulata,* is the lack of ecological knowledge surrounding the species (Tali *et al*., 2004), knowledge that will need to be developed if the species is to be conserved in the future. Basic understanding of the causes of the decline the species is currently undergoing will hamper any future restoration efforts (IUCN/SSC, 2013). The potential causes of the decline have been speculated to be under-grazing by herbivorous mammals, over-grazing by livestock and the use of artificial pesticides, herbicides and fertilisers (Tali *et al.*, 2004), although no follow up work was carried out to confirm these speculations. Defining the exact causes of the decline will need to be carried out prior to any future restoration efforts in accordance with the IUCN guidelines for reintroductions (IUCN/SSC, 2013), with the eventual aim of removing or mitigating the factor(s) causing the contemporary decline.

Other major areas of the understanding of the species and its ecology will need to be addressed. Understanding the mechanisms around seed germination *in-situ* will be required to develop understanding around the early life stages of *N. ustulata*. Whilst understanding the seed ecology of the species is important for the selection of potential new sites for *N. ustulata*, the understanding of germination requirements can be translated into *ex-situ* cultivation of the species from seed (Ramsey and Stewart, 1998). *Ex-situ* cultivation of the species from seed will be a large step in any future restoration of the species, without the methods to produce plant in *ex-situ* cultivation any future restoration will be limited to seed scattering and plant translocation from extant populations (Reiter *et al.*, 2016). At present, only the population at Parsonage Down is currently large enough to support translocations.

The information identified within the data chapters can suggest some potential positivity in the species outlook. Many species of conservation priority are often restricted by the funding that can be accessed (Caro *et al.*, 2004) and potential community engagement (Caro, 2010). The identification in Chapter 2 of the species as one of preference towards future conservation within the context of both chalk grassland orchids and flora. Whilst this is no guarantee of a better future outcome (Caro, 2010, Caro *et al.*, 2004), the species can be said to have a greater chance of conservation success with the better community engagement that the conservation preference that was indicated through the Best-worst type 3 methodology. Better potential community uptake may mean a better chance of any future conservation measures imposed being successfully implemented without objection from third party stakeholders. A greater preference towards *N. ustulata* for future conservation action may also indicate the viable use of *N. ustulata* as a 'flagship' species for the wider calcareous grassland flora (Caro, 2010). Utilising any positive preference towards the conservation of *N. ustulata* to benefit the wider floral ecology of calcareous grasslands.

The long-term changes brought on by anthropogenic climate change (Hansen and Stone, 2016) to the species ecology as assessed by chapter 3, identified that *N. ustulata* shifted its peak flowering dates in response to local climate and those changes correlated to increasing mean springtime temperatures, brought on by anthropogenic climate change. In isolation the changes to the flowering time of *N. ustulata* may lead to pollination asynchrony. However, the potential putative pollinators of *N. ustulata* also showed phenological shifts in response to the increasing mean springtime temperatures. With all species identified showing peak flowering/flight dates shifting earlier with increasing mean springtime temperatures, pollination asynchrony is unlikely (Wilcock and Neiland, 2002). Maintaining stable pollination phenology throughout the effects of anthropogenic climate change increasing the chances of stable reproductive success as mean springtime temperatures continue to increase (Hansen and Stone, 2016). Although no pollinator species is currently confirmed from the British Isles so as previously stated, the outcomes of this chapter are open to revision if evidence can contradict the viability of the putative pollinators identified as pollen vectors. The known pollen vector across the putative species range, *Tachina magnicornis*, shares similar morphometrics to the species identified in chapter 3. The ecology of the potential putative pollinators are also very similar to *T. magnicornis* so phenological shifts may also be seen in the continental pollen vector for *N. ustulata*. The potential stability of the interaction between plant/pollinator phenology of *N. ustulata* may provide some resilience towards any phenological changes caused by anthropogenic climate change as the effects continue to develop. However, the data collected as part of Chapter 3 has differing relevance to the *N. ustulata* populations within Kent. Of the two sub species only var. *ustulata* has been recorded within the county (Johnson, 2019). Chapter 3 treats all potential pollen vectors as viable species to act as pollen vectors for both sub species, with the evolutionary driver though to be different pollinator availability (Dave Roberts, per. comm.), *Leptura livida* has been recorded in Kent and is a known pollinator of *N. ustulata* var. *ustulata* in Germany (Woth, 1984, Mrvicka, 1991). No specific knowledge of the pollination ecology of *N. ustulata* within the British Isles is known so all potential pollinator species have been treated the same. Herbarium data was also unable to identify the two subspecies of *N. ustulata* as different so all herbarium records were treated as the same.

Despite the data collected through the research in chapters 2 and 3, the research needed to make long-term viability of *N, ustulata* both nationally and within Kent, still outweighs the current relevant knowledge. Although no literature on the subject is currently available, the decline experienced by *N. ustulata* through the 20th century, appears to have continued since 2004, with some populations in Wiltshire not having been record within the past decade (Bersweden, 2018, Pers. comm. June 2020), alongside the plants not having been recorded on Kentish sites in 2006 (Johnson, 2019). The lack of knowledge of the species' ecology, and the mechanisms and causes of the current declines will hamper any future conservation action (IUCN/SSC, 2013). While population trends are also poorly studied, the likely trend towards local extinction experienced by the species appears to continue unabated. Human intervention is inevitable if we do not wish to lose *N. ustulata* from our flora.

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Appendix

Chalk Grassland Flora Preference

My name is Jacob Coles and firstly many thanks for engaging in this questionnaire looking at the conservation of chalk grassland flora. This questionnaire forms part of my Masters by Research, undertaken at the University of Kent, into the conservation of an orchid species that has undergone a significant decline in Kent. In this part of my study, I aim to investigate how this species sits within the wider chalk grassland floral community that are competing for conservation resources. I will be using a Best-Worst preference experiment based on images and textual information on their ecology.

Following some demographics questions, the questionnaire is made up of 6 sections, follow by a chance to provide feedback. It is anticipated that the questionnaire should take no longer than 15 minutes.

The response should represent you own personal preferences. There are no right or wrong answers.

For any further information please contact:

Jacob Coles jmc89@kent.ac.uk

Dr Dave Roberts (supervisor) d.l.roberts@kent.ac.uk

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* Required

Prior Informed Consent

I volunteer to participate in this MSc by Research questionnaire. I understand that this research aims to collect information on the public preference of chalk grassland flora. The data collected will be used for an MSc thesis.

1. I confirm that I have read this Prior Informed Consent form and fully understand the information that it contains
2. I understand that my participation in this questionnaire is voluntary. I will not be paid for my involvement. I am free to withdraw from the research at any time, without reason.
3. I have read and understand that all responses provided will be treated in strict confidence. Any personal information I provide will be kept securely, and will be destroyed at the completion of the research project, in accordance with the 1998 Data Protection Act
4. I understand that this questionnaire conforms to the University of Kent's ethics code
5. I have read and understood the explanation of the research project. I have had the opportunity to ask any questions and they have been answered to my satisfaction

By giving my prior informed consent to take this questionnaire, I agree to take part in this questionnaire and the above 5 statements. Any statements I have concern with I will discuss with the primary researcher prior to commencing

1. Prior Informed Consent *

Mark only one oval.

○ I give my consent for my answers to be used in this study

○ I do not give my consent for my answers to be used in this study

Your Details

All answers will remain confidential, and no information will be shared outside the study

2. Age *

Mark only one oval.

○ 18-24

○ 25-34

○ 35-44

○ 45-54

○ 55-64

○ 65+

3. Gender *

Mark only one oval.

○ Male

○ Female

○ Other

○ Prefer not to say

4. First half of your postcode

5. Discipline *

Mark only one oval.

- ○ Conservation
- ○ Anthropology
- ○ Environmental Social Science & Human Geography
- ○ Other

6. Role *

Mark only one oval.

- ○ Undergraduate
- ○ Taught Postgraduate
- ○ Postgraduate Researcher
- ○ Academic Staff
- ○ Other

Descriptive Best-Worst Section 1

For the following questions, please select your Most Preferred and Least Preferred species based on the information provided.

Each species is described using the following information: - The classification given by the British Red List of Vascular Plants describing the species' level of threat in the UK
- The nation distribution pattern of the species
- The change in reported instances of the species in the past 3 decades
- The percentage level of occupancy of the species in Kent

Set 1

Species A	**Species B**	**Species C**	**Species D**
Vulnerable	Not Listed	Least Concern	Vulnerable
Endemic to the South East	Nationally Widespread	Widespread Across England	Nationally Widespread
Stable	22% Decline	42% Increase	57% Increase
Found in 1% of Kent	Found in 11% of Kent	Found in 21% of Kent	Found in 0.10% of Kent

7. Set 1 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

Set 1 Least Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

Set 2

Species A	**Species B**	**Species C**	**Species D**
Vulnerable	Near Threatened	Least Concern	Vulnerable
Endemic to Kent	Mostly Found in Southern England	Nationally Widespread	Mostly Found in Southern England
100% Decline	20% Decline	13% Decline	25% Decline
Found in 1.4% of Kent	Found in 5% of Kent	Found in 30% of Kent	Found in 9% of Kent

8. Set 2 Most Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

9. Set 2 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

Set 3

Species A	**Species B**	**Species C**	**Species D**
Near Threatened	Endangered	Not Listed	Vulnerable
Mostly Found in Southern England	South-East Endemic	Nationally Widespread	Southern Endemic
10% Decline	38% Decline	Stable	273% Increase
Found in 7% of Kent	Found in 10% of Kent	Found in 47% of Kent	Found in 2% of Kent

10. Set 3 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

11. Set 3 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

Set 4

Species A	**Species B**	**Species C**	**Species D**
Vulnerable	Near Threatened	Vulnerable	Least Concern
Nationally Widespread	South-East Endemic	Widespread across England	Nationally Widespread
88% Increase	40% Increase	142% Increase	14% Increase
Found in 2% of Kent	Found in 3% of Kent	Found in 0.5% of Kent	Found in 35% of Kent

12. Set 4 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

13. Set 4 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

Set 5

Species A	**Species B**	**Species C**	**Species D**
Endangered	Least Concern	Vulnerable	Least Concern
Southern Endemic	Nationally Widespread	Widespread across England	South East Endemic
10% Decline	43% Increase	Stable	25% Increase
Found in 2% of Kent	Found in 26% of Kent	Found in 10% of Kent	Found in 4% of Kent

14. Set 5 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

15. Set 5 Least Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

Descriptive BestWorst Section 2

For the following questions, please select your Most Preferred and Least Preferred species based on the information provided.

Each species is described using the following information: - The classification given by the British Red List of Vascular Plants describing the species' level of threat in the UK
- The nation distribution pattern of the species
- The change in reported instances of the species in the past 3 decades
- The percentage level of occupancy of the species in Kent

Set 1

Species A	**Species B**	**Species C**	**Species D**	**Species E**
Near Threatened	Least Concern	Least Concern	Not Listed	Endangered
Widespread across England	Mostly Found in Southern England	Nationally Widespread	Southern Endemic	South-East Endemic
12% Decline	47% Decline	58% Decline	Stable	38% Decline
Found in 1.4% of Kent	Found in 13% of Kent	Found in 16% of Kent	Found in 0.7% of Kent	Found in 10% of Kent

17. Set 1 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D
○ Species E

18. Set 1 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D
○ Species E

Set 2

Species A	**Species B**	**Species C**	**Species D**	**Species E**
Vulnerable	Least Concern	Critically Endangered	Least Concern	Vulnerable
Southern Endemic	Nationally Widespread	Mostly found in Southern England	Widespread across England	Widespread across England
12% Decline	12% Decline	Stable	50% Decline	142% Decline
Found in 1.6% of Kent	Found in 12% of Kent	Found in 0.2% of Kent	Found in 16% of Kent	Found in 0.5% of Kent

19. Set 2 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D
○ Species E

20. Set 2 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D
○ Species E

Set 3

Species A	**Species B**	**Species C**	**Species D**	**Species E**
Vulnerable	Least Concern	Near Threatened	Least Concern	Not Listed
Mostly Found in Southern England	Southern Endemic	Southern Endemic	Nationally Widespread	Mostly Found in Southern England
25% Decline	484% Increase	32% Decline	Stable	46% Decline
Found in 25% of Kent	Found in 2% of Kent	Found in 0.6% of Kent	Found in 15% of Kent	Found in 5% of Kent

21. Set 3 Most Preferable *

Mark only one oval.

- ○ Species A
- ○ Species B
- ○ Species C
- ○ Species D
- ○ Species E

22. Set 3 Least Preferable *

Mark only one oval.

- ○ Species A
- ○ Species B
- ○ Species C
- ○ Species D
- ○ Species E

Set 4

Species A	**Species B**	**Species C**	**Species D**	**Species E**
Endangered	Least Concern	Least Concern	Endangered	Not Listed
Southern Endemic	Nationally Widespread	Southern Endemic	Southern Endemic	Nationally Widespread
72% Decline	47% Decline	45% Decline	10% Decline	47% Decline
Found in 7% of Kent	Found in 0.7% of Kent	Found in 5% of Kent	Found in 2% of Kent	Found in 8% of Kent

23. Set 4 Most Preferable *

Mark only one oval.

- ○ Species A
- ○ Species B
- ○ Species C
- ○ Species D
- ○ Species E

24. Set 4 Least Preferable *

Mark only one oval.

- ○ Species A
- ○ Species B
- ○ Species C
- ○ Species D
- ○ Species E

Set 5

Species A	**Species B**	**Species C**	**Species D**	**Species E**
Endangered	Least Concern	Not Listed	Near Threatened	Least Concern
Southern Endemic	Mostly Found in Southern England	Nationally Widespread	Widespread across England	Nationally Widespread
46% Decline	49% Decline	22% Decline	65% Decline	56% Decline
Found in 12% of Kent	Found in 1% of Kent	Found in 11% of Kent	Found in 2% of Kent	Found in 8% of Kent

25. Set 5 Most Preferable *

Mark only one oval.

- ○ Species A
- ○ Species B
- ○ Species C
- ○ Species D
- ○ Species E

26. Set 5 Least Preferable *

Mark only one oval.

- ○ Species A
- ○ Species B
- ○ Species C
- ○ Species D
- ○ Species E

Photographic Best-Worst Section 1

For the following questions, please select your Most Preferred and Least Preferred species based on the images provided.

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Set 1

Species A	**Species B**	**Species C**	**Species D**
			

27. Set 1 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

28. Set 1 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

Set 2

Species A	**Species B**	**Species C**	**Species D**
			

29. Set 2 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

30. Set 2 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

Set 3

Species A	**Species B**	**Species C**	**Species D**
			

31. Set 3 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

32. Set 3 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

Set 4

Species A	**Species B**	**Species C**	**Species D**
			

33. Set 4 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

34. Set 4 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

Set 5

Species A	Species B	Species C	Species D
 **Copyright** Jacob Coles			

35. Set 5 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

36. Set 5 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

Photographic Best-Worst Section 2

For the following questions, please select your Most Preferred and Least Preferred species based on the images provided.

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Set 1

Species A	**Species B**	**Species C**	**Species D**	**Species E**
				

37. Set 1 Most Preferable *

Mark only one oval.

- ○ Species A
- ○ Species B
- ○ Species C
- ○ Species D
- ○ Species E

38. Set 1 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D
○ Species E

Set 2

Species A	**Species B**	**Species C**	**Species D**	**Species E**
				

39. Set 2 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D
○ Species E

40. Set 2 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D
○ Species E

Set 3

Species A	**Species B**	**Species C**	**Species D**	**Species E**
				

41. Set 3 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D
○ Species E

42. Set 3 Least Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

○ Species E

Set 4

Species A	**Species B**	**Species C**	**Species D**	**Species E**
 **Copyright** Surrey Botanical Society			 **Copyright** Jacob Coles	

43. Set 4 Most Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

○ Species E

44. Set 4 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D
○ Species E

Set 5

Species A	**Species B**	**Species C**	**Species D**	**Species E**
 **Copyright** L'herbier en photos				

45. Set 5 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D
○ Species E

46. Set 5 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D
○ Species E

Photographic Best-Worst Section 3

For the following questions, please select your Most Preferred and Least Preferred species based on the images provided.

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Set 1

Species A	**Species B**	**Species C**	**Species D**
			

47. Set 1 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

48. Set 1 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

Set 2

Species A	**Species B**	**Species C**	**Species D**
			

49. Set 2 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

50. Set 2 Least Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

Set 3

Species A	**Species B**	**Species C**	**Species D**
			

51. Set 3 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D

52. Set 3 Least Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

Set 4

Species A	**Species B**	**Species C**	**Species D**
			

53. Set 4 Most Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

54. Set 4 Least Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

Set 5

Species A	**Species B**	**Species C**	**Species D**
			

55. Set 5 Most Preferable *

Mark only only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

56. Set 5 Least Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

Photographic Best-Worst Section 4

For the following questions, please select your Most Preferred and Least Preferred species based on the images provided.

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Set 1

Species A	**Species B**	**Species C**	**Species D**	**Species E**
				

57. Set 1 Most Preferable *

Mark only one oval.

○ Species A
○ Species B
○ Species C
○ Species D
○ Species E

58. Set 1 Least Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

○ Species E

Set 2

Species A	**Species B**	**Species C**	**Species D**	**Species E**
				

59. Set 2 Most Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

○ Species E

60. Set 2 Least Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

○ Species E

Set 3

Species A	**Species B**	**Species C**	**Species D**	**Species E**
				

61. Set 3 Most Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

○ Species E

62. Set 3 Least Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

○ Species E

Set 4

Species A	Species B	Species C	Species D	Species E
				

63. Set 4 Most Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

○ Species E

64. Set 4 Least Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

○ Species E

Set 5

Species A	**Species B**	**Species C**	**Species D**	**Species E**
				

65. Set 5 Most Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

○ Species E

66. Set 5 Least Preferable *

Mark only one oval.

○ Species A

○ Species B

○ Species C

○ Species D

○ Species E