THE MYSTERIOUS SEDGE OF THE RIVER NAVER

The UK has arguably one of the most intensively studied floras in the world. This makes it all the more exciting when a new coloniser is discovered on our beautiful isles. Nestled away in the north-west of the Scottish highlands amongst the rolling mountains and beguiling lochs, a small sedge species has made Scotland its home.

Carex salina Walenb. or commonly the Saltmarsh Sedge, is a stable hybrid species which derives from its closely related parents, *C. paleacea* and *C. subspathacea*. It's a small to mid-sized species (10 – 30cm in height) and distinguishable from others outside the section via its biconvex utricle shape and two stigmas. All three species have a fascinating trans-Atlantic distribution and experience a typical range, spanning the north-eastern seaboard of North America, across to Scandinavia and stretching further east to Russia. Until 2006 none of the three species had been recorded from Britain.

However in 2004, while undertaking a saltmarsh survey at Morvich for National Trust Scotland, Keith Hutcheon found a new and unidentifiable *Carex* species. After review by a team of experts, this new, plucky, little plant was confirmed as *C. salina* and thus the search for more populations was afoot. By 2012, five additional Scottish populations of this sedge had been identified: Strontian and Loch Sunart (VC97), Loch Nevis (VC97), Loch Long (VC105) and Invernaver (VC108).

During surveying in 2018, immediately after having sampled the majority of other UK *C. salina* populations, Paul Ashton, Professor of Botany at Edge Hill University, Lancashire, surveyed the population at Invernaver. Observations of overall morphology led him to question whether this sedge species was indeed *C. salina*. Upon further inspection, Dr Mary Dean, another sedge enthusiast who earned her PhD studying this group of sedges, and Paul Ashton proposed that this population wasn't that of *C. salina* at all, but rather a closely related species called *C. vacillans* or Swinging Sedge. If this were to be the case, then it would be yet another new coloniser of the UK.

However, there have been some conflicting opinions regarding this Invernaver sedge. Samples sent to Scandinavian *Carex* expert, Prof. Reidar Elven, led him to identify it as Common Sedge *C. nigra*. In contrast, UK *Carex* referee Mike Porter contested the latter identification as "not like any *C. nigra* [he had] ever seen". Thus, a research opportunity was born! To establish the true identity of this mystery *Carex* species situated on the north coast of Scotland, through means of not just morphometrical, but also genetically based identification techniques. Before I could do this, however, I first needed some plant material.

The drive from Edge Hill University to Invernaver is a long one. However, it is far from tedious as it's a journey of exceptional viewpoints and wholesome eateries certainly making the drive an enjoyable part of the whole botanical expedition. After a couple days of travel and a total of eleven hours driving, my marvellous field assistant and photographer, Mark Ashton and I, finally made it to the River Naver, our home for the next few days.

Travelling 18 miles from Loch Naver, the river meets the sea at Bettyhill, a small village situated nearby. A Special Area of Conservation, the river Naver is home to two Annex II species, Freshwater Pearl Mussel and Atlantic Salmon and, unsurprisingly, is considered one of Scotland's best rivers for salmon fishing. Encapsulating the sea mouth are highly resistant metamorphic rocks of the Moine (c. 420-415 Ma old), as well as Moine Schists making up the glacially scoured bedrock ridge in the centre of the bay, a goldmine of interest for any geologist.

Bettyhill provided an excellent base as surveying next to the sea mouth was more appropriate due to the increase of salinity, something this group of maritime sedges appreciates. Our mystery *Carex* has established itself on both sides of this river and since the extensive survey in 2018 is showing no signs of a decline. This is important given that we wanted to collect leaf material for DNA analysis without harming the population. In addition to collecting leaf samples, physical characteristics of a good sample were also needed for morphological analysis.

As part of her PhD, Mary Dean collected a wealth of measurements from six species belonging to the *Carex* section *Phacocystis,* the group to which *C. salina* and *C. vacillans* belong. It was this dataset that enabled us to make the comparison between the morphological measurements of each species. Following Mary's approach, we spent three days randomly selecting individuals and measuring each for 21 different morphometric characters, from stem length to utricle beak size. Once we had collected and collated our data, we slowly travelled back to the university via the north-west coast and a couple of island hops to Lewis and Mull; these detours served as a chance to investigate the Water Sedge *C. aquatilis* populations growing here, but that species is a different story all together!

Upon our return, data analysis ensued. The morphometric measurements were easy enough in themselves, as, thankfully, data analysing technology can do almost anything (as long as you input the data correctly in the first place). The genetic side, alas, takes a few more steps from the initial DNA extraction, through to gene amplification, sequencing and finally raw data input. Thankfully, this was done with relative ease and once I've collated all of the genetic information from samples collected from Norway, a genetic comparison can be made. For now however, the morphometric information has yielded some insights though still leaves some questions unanswered.

To tackle the genetic aspect of this identification quest, two approaches have been taken. Firstly, DNA samples of the Bettyhill *Carex* and other close relations (such as *C. salina* and *C. vacillans*) have been analysed using microsatellites. These are typically utilised when investigating aspects such as pollen movement, long distance dispersal and the relatedness of individual plants in the same population.

The other method will involve analysing specific segments of chloroplast DNA (cpDNA). This differs from the nucleic microsatellite DNA in that this special cpDNA only exists inside the chloroplast organelles within the plant's cells. Mutations within cpDNA are much less common than those in microsatellites, as it is a more slowly evolving gene. As cpDNA is only maternally inherited, it is a useful tool when wanting to investigate aspects relating to genetic heritage.

A final thread of evidence comes from the work of Edge Hill University dissertation student Andy Weaver, who is investigating leaf anatomy as an added source of information. When we combine the DNA approaches, alongside the morphological data this will hopefully finally provide an indication of what species is growing in the mud at Invernaver.

So alas, at this stage we still don't have the definitive answer. However, we are definitely closer than we were this time last year (and will hopefully have a more satisfying end to this question very soon!). Thus we owe a great debt of gratitude to the Wild Flower Society for funding this research, enabling us to travel to the Highlands and complete our data collection. Areas of research such as this greatly benefit from the assistance and funding of organisations such as yours and make a real difference to advancing our understanding of the natural world (and by helping researchers accomplish and enjoy something we truly love!). Thank you for all of your support!

JENNIFER CLAYTON-BROWN

Jennifer hopes to have finished the analysis this summer and has promised to keep us updated as to the identity of this mystery sedge. With luck we should be able to give you the results in the autumn issue of the Wild Flower Magazine.

Below is an abridged report received from David Roberts, another recipient of a WFS grant, on his observations on the pollinators of Burnt Orchid.

OBSERVATIONS ON INSECT VISITORS TO BURNT ORCHID

Although small in stature, Burnt Orchid *Neotinea ustulata* is, at least in my opinion, one of the UK's most beautiful orchids. Unfortunately, it is also one of the UK's most threatened orchids having suffered significant declines across the country, with fewer than ten sites having more than 200 flowering individuals. In Kent this species has declined to a single site where only two individuals are known to have flowered in recent years. However, Parsonage Down in Hampshire is home to much of the UK population with an estimated 30,000 flowering individuals. While, *N. ustulata* is of conservation interest, being listed as Nationally Scarce, it is also of evolutionary interest due to its flowering behaviour, having an early and late flowering form (*N. ustulata* var. *ustulata* and *N. ustulata* var. *aestivalis* respectively), with the former being far more common than the latter. What drives this divergence is unclear. However, it is thought to be differences in pollinator communities, with recent studies implicating two fly species (*Tachina fera* and *Nowickia ferox* respectively) in the divergence; although a variety of insects are known to be occasional pollinators. The purpose of this study, funded by the Wild Flower Society, was to provide further understanding of the pollination ecology of this rare and curious orchid in a UK context.
This involved pollinator observations at the Mount Caburn population, near Lewes, of *N. ustulata* var. *ustulata* and the High and Over population, near Seaford, of *N. ustulata* var. *aestivalis*.

Observations
The early flowering form, at Mount Caburn, was visited in late May to early June where approximately 130 flowering individuals were recorded. During this period it was reported that a number of plants had been illegally dug up from the population, although no evidence was found. During 58.5 hours of inflorescence observations, a wide range of insect taxa were recorded, although the majority were flies. The 14 visitors included ten flies, a beetle, possibly the Small Copper butterfly and an *Andrena* bee species, resulting in a visitation rate of 0.24 visitors per hour.

Interestingly, one visitor, a male *Sarcophaga* sp., was observed to be carrying what looked like pollinia on the end of its abdomen. While pollinia were observed, for a taxon to be considered a pollinator, pollinia must be recorded to have been removed and deposited. In this case, neither event was observed. However, we can be confident the pollinia were of *N. ustulata* var. *ustulata*, as the only other orchid flowering at the same time was the larger Chalk Fragrant-orchid *Gymnadenia conopsea*.

At the High and Over population, near Seaford, approximately 40 plants of the late flowering form were recorded at the beginning of July. During 52.0 hours of inflorescence observations, a much more restricted visitor community was recorded. All visitors were diptera. Of those that could be identified (eight out of ten) all were of the Calyptratae and all but one from the closely related families of the Tachinidae and Sarcophagidae. Based on these observations, the visitation rate was 0.19 visitors per hour. None carried pollinia, although two visitors were *Nowickia ferox*, a known pollinator of the late flowering form.

In both cases, no reward in the form of nectar was observed and the low visitation rates, far lower than those observed in related *Orchis* species in the UK, suggest that pollination is likely to be through deception. Further, it has recently been suggested that the 'burnt-tip' serves a biological function in attracting flies. However, it is interesting to speculate whether the 'burnt-tip' serves as food-deception for the adult fly or brood-deception as a place to lay eggs by the adult flies.

If we assume *N. ustulata* is predominantly fly-pollinated by Tachinid and Sarcophagid flies, this raises an interesting conservation question. Tachinid flies are parasites of other invertebrates, while Sarcophagid flies' larvae feed, as their name 'flesh flies' suggests, on carrion, as well as faeces and

associated bacteria films. This is a far cry from the more charismatic insect fauna, such as bees and butterflies, and given their more putrid ecology is likely to make them a conservation challenge!

DR DAVID L ROBERTS