Genetic diversity and connectivity in populations of *Rhinanthus minor* L.

Dr Elizabeth Sullivan, Edge Hill University, Department of Biology, Edge Hill University. S. Helens Road, Ormskirk, L39 4QP
Email: Sullivae@edgehill.ac.uk

This article describes research carried out as part of a PhD project on hay meadow vegetation in which the genetic diversity and connectivity of *Rhinanthus minor* were investigated in nine Worcestershire meadows. The results were compared to those from a similar investigation in the Forest of Bowland in Lancashire which has contrasting land-use and topography.

Background

There has been a drastic decline in species rich hay meadows since the middle of the 20th century. Many of the remaining meadows are managed for conservation through agri-environment agreements and the majority are protected through statutory designations such as sites of special scientific interest (SSSIs) but the distribution of these important habitats is fragmented (Hodgson *et al.*, 2005; Sullivan *et al.*, 2017). This fragmentation, and the fact that many sites are small, individual fields, has led to concerns about a loss of genetic diversity within populations of plant species and other taxa, and about reduced gene flow between populations. The focus of conservation management has shifted from a site-based approach to one which also addresses conservation at the landscape scale. Studies which aim to quantify levels of connectivity between populations are, therefore, of particular importance in this context.

Research questions

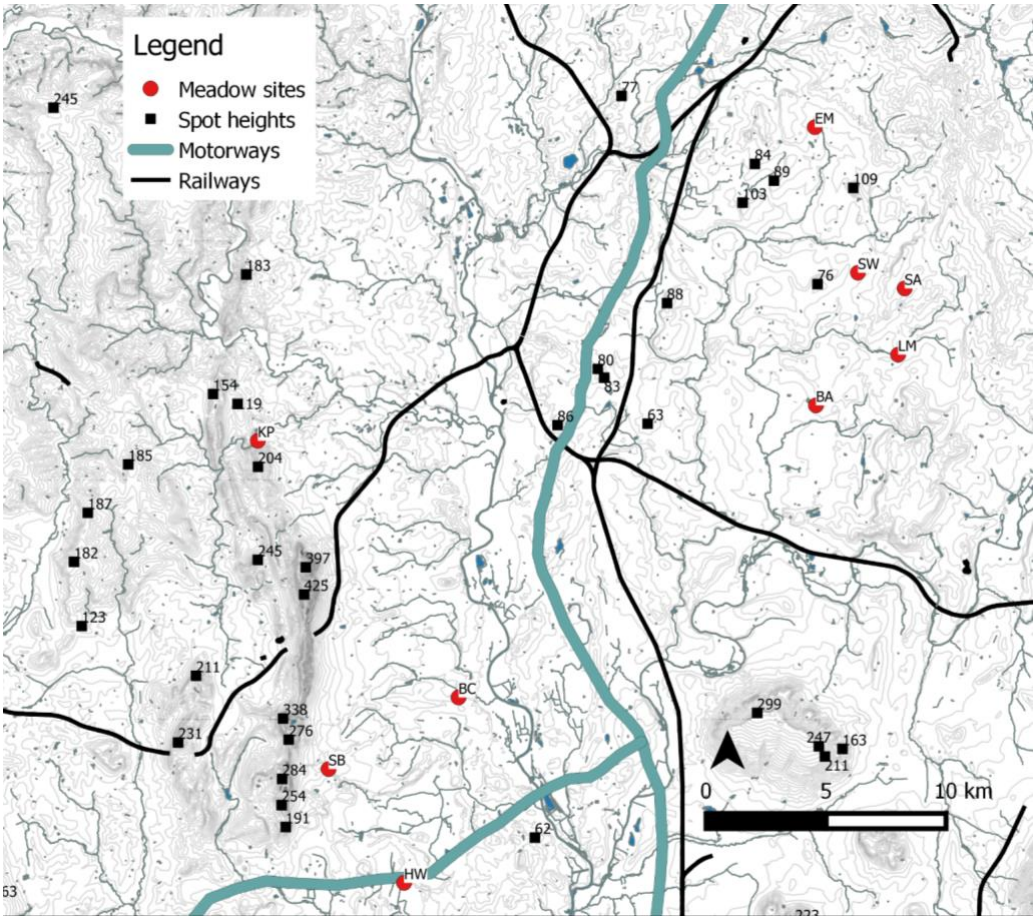
The research investigated the following questions:
What is the level of genetic diversity within populations of *R. minor* in species rich hay meadows?
Is there evidence for gene flow between hay meadow sites?
How does genetic diversity and connectivity in a lowland region (Worcestershire) compare with that of an upland region (Forest of Bowland)?

Study species

R. minor (Yellow Rattle) is an annual species and so would be expected to reflect the genetic impact of recent fragmentation more readily than long-lived perennial plants. It has a widespread distribution in the UK and much of Europe and North America (Westbury, 2004). It is found in a range of grassland habitats but is most commonly associated with meadows (Coulson *et al.*, 2001). More intensive grassland management, such as that which requires an early cut for silage, limits the ability of seed production so *R. minor* has seen a decline with the changes in agricultural practices since the mid 20th Century. It is a diploid species (2n = 22) and is either insect- or self-pollinated with the main pollinators being *Bombus* spp. (Natalis and Wesselingh, 2012). Seeds do not persist in the seed bank and seed dispersal is poor with most seeds located <1.5m from the parent plant (Westbury, 2004).

Methods

Nine meadow sites were selected in Worcestershire (see map 01 and photographs 02, 03, and 04)). All of these sites were known to support populations of *R. minor*, all were owned/managed by the Worcestershire Wildlife Trust and the degree of isolation from other known meadow sites varied from 0.8 to 3.1 km (see Table 1). Leaves from approximately 30 plants were sampled in each meadow and dried in silica gel. The plants were a minimum of 5m apart. A similar approach was used in the upland meadows (in Bowland) although sample sizes varied more widely due to differences in population sizes.



01 Map of Worcestershire study sites

Table 1 Details of study sites in Worcestershire and Bowland

Worcestershire sites	Altitude (m. a. s. l.)	Size (ha)	*N*	Nearest protected meadow
BA	35	1.07	27	1.6
BC	20	6.84	32	1.3
EM	85	7.0	32	2.2
HW	15	11.41	32	0.8
KP	75	3.91	32	2.4
LM	50	5.12	32	2.6
SA	90	4.67	32	3.1
SB	45	2.21	30	1.9
SW	55	1.75	32	1.50
Bowland sites	Altitude (m. a.s.l)	Size (ha)	*N*	Nearest protected meadow
BG	180	5.47	21	1.97
BS	150	7.65	88	0.52
CB	60	0.54	12	3.49
FH	105	1.63	20	3.49
FHM	210	3.33	21	0.46
LCM	190	5.26	18	0.46
MM	155	9.09	40	1.29
NI	125	2.09	12	6.1
TB	155-180	11.87	22	11.59

m. a. s. l = metres above sea level; ha = hectares; *N* = number of samples; Nearest protected meadow = closest protected meadow site in km.

02. Baynall Meadow

03. Boynes Coppice Meadow

04. Eades Meadow

DNA was extracted from the leaves using a crude extraction method and molecular markers (microsatellites) were used to amplify particular regions of DNA. Fragment analysis was carried out and this resulted in genotypic data which were then subjected to statistical analysis. Data analysis included levels of expected heterozygosity – which is a measure of genetic diversity and F_{ST} which gives an estimate of the extent to which populations are genetically differentiated. This degree of differentiation can then be used to indicate gene flow.

Results

Moderate levels of genetic diversity were recorded in both the Worcestershire populations and the Bowland populations. Expected heterozygosity values range from 0 to 1 and the overall levels for *R. minor* in the Worcestershire meadows were 0.35 and were 0.39 in Bowland. These results are similar to those in a study of *R. minor* at sites across the UK (see 02) but higher than the values for expected heterozygosity in studies in Estonia and the Rocky Mountains (Hargreaves, *et al*., 2015; Houston and Wolff, 2012; Talve *et al*., 2013) It should be noted that *R. minor* is known to self-pollinate which will result in lower levels of genetic diversity when compared with an outcrossing species. Estimates of inbreeding for both the Worcestershire and Bowland meadows were relatively high across all the meadows, and some sites, e.g., Baynhall Meadow, had particularly high numbers of the same genotype. There was not a significant correlation between site size (in ha) and expected heterozygosity.

The analysis of gene flow found that there was more evidence of population differentiation in the Worcestershire meadows than in the Bowland ones. F_{ST} values of 0.15 for Worcestershire and 0.07 for Bowland were recorded. Interpretation of values of F_{ST} is debated in the literature but a value of 0.07 would generally be considered to indicate high levels of gene flow (low population differentiation) and 0.15 would be moderate gene flow (Hartl and Clark, 1997). Figures above 0.15 suggest low gene flow although this can vary according to the species and means of analysis. The results for Bowland and Worcestershire were confirmed in further analyses using spatial and genetic data.

Comments

The indications of moderate genetic variation and gene flow in populations of *R. minor* in the Worcestershire meadows suggest that conservation is being relatively successful. The high levels of inbreeding, which result from a high frequency of self-pollination, may indicate a lack of availability of pollinators which may be a cause for concern for conservation. However, studies have shown that selfing can occur in *R. minor* even when pollinators are known to be present and causes for self-pollination in plants are still unclear (Angeloni *et al*. 2011).

The differences in levels of gene flow between the Worcestershire meadows and those in the Bowland region are interesting. The number of species rich meadows in Worcestershire is much greater than in Bowland. Indeed the nine meadows included in this study represent all of the protected meadow sites (SSSIs) in the Bowland region and some of these sites are separated by an area of upland rising to over 550m in altitude. There are, however, significant differences in land use in the two regions with Worcestershire characterised by more intensive agriculture and features such as the M5 motorway and River Severn presenting possible barriers to pollinators. The Bowland region is an upland region with low intensity land use including permanent grassland and heather moorland.

In summary, the findings from this research suggest that the populations of *R. minor* in the Worcestershire meadows are large enough and sufficiently well connected in terms of pollinator networks to maintain moderate levels of genetic diversity and connectivity. However, connectivity could be improved by the provision of more favourable conditions for pollinators in areas of higher intensity land use, for example by enhancing nectar sources in roadside verges, community spaces or other suitable habitats. Continuing with low intensity meadow management and grassland restoration such as that carried out by Worcestershire Wildlife Trust is also essential to maintaining genetic diversity and connectivity. Similar research on other meadow species including perennials and wind pollinated and wind dispersed species would give a more complete picture of diversity and connectivity in this important habitat.

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