

A population genetics approach to evaluating weed movement and the role for area wide weed management

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Summary This study used a population genetics approach to assess weed movement within and across each of three regions in Australia – the Darling Downs, the Riverina, and Sunraysia. Populations of three weed species (fleabane, feathertop Rhodes grass and annual ryegrass) were collected across varying land uses and gene flow was investigated using a genotyping by sequencing approach.

Annual ryegrass populations were very similar, with very little genetic differentiation across sites sampled in the Riverina region. This suggests high levels of mixing within the region. Feathertop Rhodes grass populations sampled across the Darling Downs were also genetically similar, indicating high levels of gene flow and movement in this weed species. While some evidence of long-distance dispersal between regions was found, fleabane populations revealed surprising evidence of genetic differences within the Riverina region, and between the Sunraysia and Riverina regions. The research suggests that herbicide resistance in annual ryegrass and feathertop Rhodes grass is moving widely across the regional landscape.

Keywords Population genetics, gene flow, weed, area wide management.

INTRODUCTION

Understanding how far and how quickly a weed species can spread is important in planning an area wide weed management programme. Highly mobile weeds more rapidly become shared problems, especially as increasing numbers of weed species are becoming resistant to key herbicides such as glyphosate. The increase and spread of herbicide resistance are seen as key issues by growers in the regions investigated in this study (Height *et al.* 2022).

This study was designed to investigate the movement of key weeds in the three regions. Weed species were selected based on high potential mobility, the occurrence of herbicide resistance, and concerns of growers in these regions (Height *et al.* 2022). The three species selected were fleabane

(*Conyza bonariensis*), annual ryegrass (*Lolium rigidum*), and feathertop Rhodes grass (*Chloris virgata*).

Fleabane has high fecundity and high dispersal capacity due to its wind (and potentially water) dispersed seeds (Wu 2007). Glyphosate resistance was first detected in Queensland in 2006 (Walker *et al.* 2011), but by 2018 all populations of fleabane screened for glyphosate resistance across Queensland were resistant (Jalaludin *et al.* 2019).

Feathertop Rhodes grass was always difficult to control with glyphosate and was considered tolerant of the herbicide, but widespread use of glyphosate has resulted in the evolution of several different target site resistance mutations in this species (Ngo *et al.* 2018) making it even harder to control.

In contrast to the other two species, annual ryegrass is a self-incompatible, outcrossing weed species. Pollen mediated gene flow of herbicide resistance genes has been measured up to the maximum tested distance of 3,000m in an experiment in Western Australia (Busi *et al.* 2008). The species is diploid, favouring the evolution of target site herbicide resistance, and outcrossing has led to the widespread occurrence of populations resistant to multiple modes of action (Matzrafi *et al.* 2021).

The goal of this study was to assess the movement of these three weed species by comparing the genetics of weeds sampled at the same sites across two seasons (2020; 2021). This paper reports the results of the genotyping of the populations collected in 2020 and assesses population structure within and between regions to infer patterns of gene flow and mobility.

MATERIALS AND METHODS

In 2020, fleabane was collected in the Riverina and Sunraysia, feathertop Rhodes grass was collected in the Darling Downs, and annual ryegrass was sampled in the Riverina. At each site 32 individuals were sampled, and leaf material was placed directly into silica gel for DNA preservation.

DNA was extracted from all samples using a CTAB buffer and spin column extraction (Ridley *et al.* 2016). Genome-wide single nucleotide polymorphisms (snps) data were generated using a genotyping-by-sequencing method. The protocol and adaptor regime can be found online at <http://www.jameshereward.org/GBS.html>. We pooled 288 individuals per sequencing lane and sequenced the libraries with PE150 Illumina sequencing at Novogene (Beijing, China).

The sequence data were demultiplexed, assembled, and snps were called using STACKS (Catchen *et al.* 2013). A variant call format (vcf) file of the genotype data was output with any marker having a heterozygosity above 0.65 discarded. The genotypes were then filtered using vcftools (Danecek *et al.* 2011) to a minor allele count of three (one heterozygote and one homozygote), to conservatively remove singleton snps that are likely to be errors. We also set a minimum depth of five and kept only biallelic snps. We removed missing data in three steps. First, any marker missing more than 50% data was discarded to remove the markers most affected by missing data. Second, any individual that had missing data at more than 50% of the markers was discarded to remove the individuals that had bad quality genotyping. Finally, any marker missing more than 5% data was discarded to produce a final dataset with relatively little missing data (~3%).

For each species and each region, a principal component analysis (PCA) was conducted using the adegenet package in R (Jombart 2008). We then assessed gene flow and genetic clustering by performing a STRUCTURE analysis for each species in each region, this program uses a Bayesian algorithm to assign individuals to each of K hypothetical populations based on allele frequencies (Pritchard *et al.* 2000). For each species and each region, we performed 10 runs of the program using different starting seeds. For fleabane in the Riverina, we assumed K=3 populations, and for all the others K=2, the algorithm was run using the ‘admixture’ model and with 100,000 iterations of ‘burn-in’ followed by 1,000,000 iterations.

RESULTS

Fleabane. When the fleabane samples from the Riverina and Sunraysia were compared to each other, the samples from each region largely clustered independently (Fig. 1). Two individuals from Sunraysia placed within the Riverina cluster, and these likely represent movement of weeds between regions. The genotypic diversity recovered was quite high, indicating that fleabane populations do outcross under field conditions (although likely at very low frequencies).

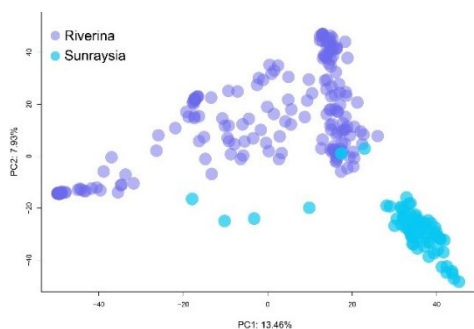


Figure 1. Genetic clustering (principal component analysis axes one and two) for all fleabane samples (Riverina and Sunraysia).

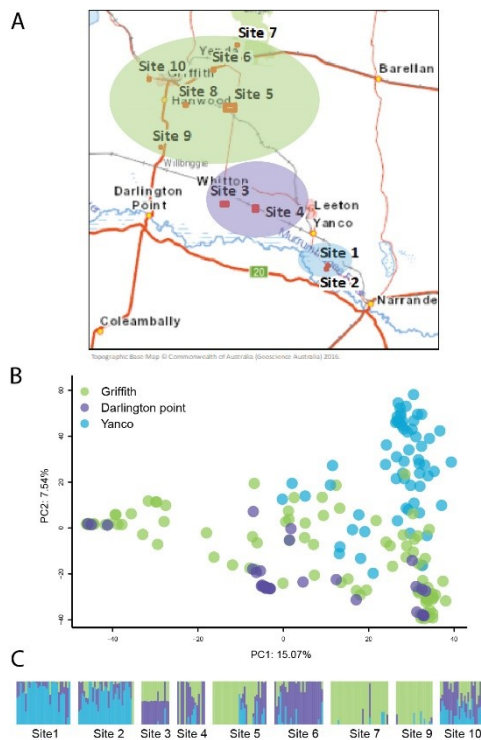


Figure 2. Genetic analysis of fleabane populations from the Riverina region, A; sampling locations, B; plot of principal components analysis of the genetic data showing axes one and two, C; plot showing the results of the STRUCTURE analysis.

In the Riverina region, individuals from around Yanco (sites 1 and 2, Fig. 2A) clustered together in the top right of the PCA plot (Fig. 2B). These sites were also assigned to a separate cluster from the other sites in the STRUCTURE analysis (Fig. 2C). Overall, the STRUCTURE plot indicated high levels of admixture as indicated by many individuals with a posterior probability of being assigned to more than

one cluster. In the PCA (Fig. 2B), sites around Darlington were clustered with sites from around Griffith. In the STRUCTURE analysis, sites four six and ten were mostly assigned to one cluster, and sites five, seven and nine to another, with individuals at site three mostly having a 50/50 posterior probability of being assigned to these two clusters.

Feathertop Rhodes grass. Genetic and genotypic diversity was lower for feathertop Rhodes grass than fleabane. Most individuals from all sites were clustered closely together in the top right of the PCA plot (Fig. 3B) with only a few individuals being separated by principal components one and two. In the STRUCTURE analysis there was very little evidence for any geographic genetic structure associated with the sampling sites with all individuals having a high posterior probability of belonging to one genetic population (Fig. 3C).

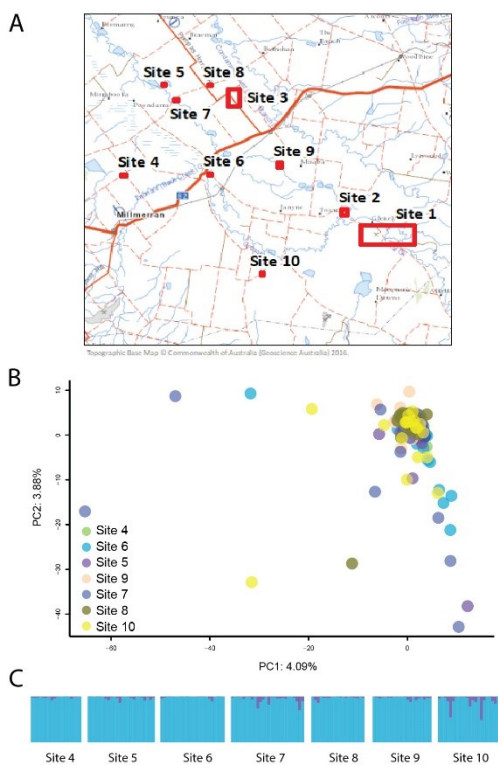


Figure 3. Genetic analysis of feathertop Rhodes grass, A; sampling locations B; plot of principal components analysis axes one and two, C; STRUCTURE analysis.

Annual Ryegrass. Samples were collected from ten sites during 2020 with the genetic analysis of three of them presented here. Site one (near Yanco), site two

(near Griffith) and site three (near Darlington Point) were all clustered together in the PCA (Fig. 4B). The STRUCTURE analysis indicated that all three sites represented a single genetic population with every individual having around 10% posterior probability of being assigned to one population and 90% posterior probability of being assigned to the second one (Fig. 4C). There was very little evidence of any admixture in the structure analysis.

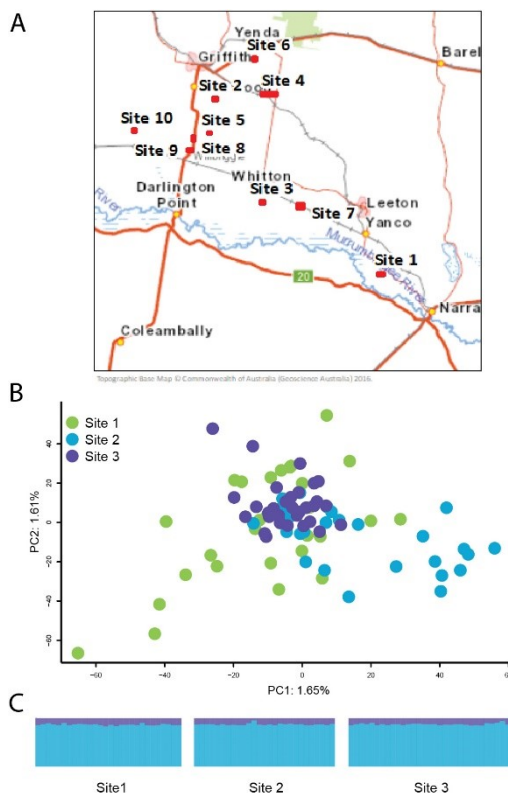


Figure 4. Genetic analysis of annual ryegrass populations from the Riverina region, A; sampling locations B; plot of principal components analysis axes one and two, C; STRUCTURE analysis.

DISCUSSION

Despite its reputation for long distance dispersal by wind, we detected local genetic population structure in fleabane. While some evidence of long-distance dispersal was identified, substantial genetic differences were found between the Sunraysia and Riverina regions. Most notably, genetic differences were found within the Riverina region and, to a lesser extent, Sunraysia. Our results suggest that local dispersal may be a more important driver of fleabane population genetics than long-distance dispersal. The extremely high seed production of fleabane may mean that a few long-distance dispersal events tend

not to contribute significantly to local population dynamics, the short life of the seed bank also leads to rapid population turnover in this species. The spread of glyphosate resistance across the whole of Queensland within 12 years of its first detection highlights the importance of weed movement in spreading herbicide resistance in this species (Walker *et al.* 2011, Jalaludin *et al.* 2019).

We found very little evidence for genetic structure in feathertop Rhodes grass populations within the Darling Downs region, and none for annual ryegrass within the Riverina. This indicates very high levels of movement of these two species within the regions investigated here. Our results for annual ryegrass are consistent with previous studies indicating very long-distance dispersal capability of resistance genes via pollen in this obligately cross-pollinating species (Busi *et al.* 2008). Previous population genetic analysis of feathertop Rhodes grass suggests that glyphosate resistance has evolved at least 12 times in this species, with one of the resistance alleles having been recorded at sites 700km apart from each other (Hereward unpublished data). Although this species is regarded as both cross and self-pollinating, our data indicated a close clustering of genotypes, suggesting that self-pollination has been predominant in feathertop Rhodes grass populations, as self-pollination tends to reduce genotypic diversity. Further research on the extent of cross or self-pollination in this species is warranted.

The high mobility of these three weed species within regions will lead to the rapid spread of herbicide resistance genes across the landscape (Preston *et al.* 2022), highlighting the importance of early detection and elimination of herbicide resistant populations. Co-ordinated efforts to control herbicide survivors and eliminate resistant populations across land uses would have area wide community benefits by reducing the spread of herbicide resistance, especially at the regional scale.

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