

Herbicides are used across the world to control weeds in agricultural settings. As a result, there is strong selection for weed populations to evolve resistance to herbicides. Indeed, herbicide resistance now extremely common – over 500 unique cases known (Heap 2021) - and is therefore a growing threat to food security. Despite its ubiquity, it remains difficult to predict or prevent resistance from evolving (Kreiner et al. 2018). Better understanding the population genetics of herbicide resistance therefore has the potential to help reduce cases of resistance from arising in the future and presents an interesting opportunity to study the genetics of rapid adaptation (Kreiner et al. 2018).

Kreiner et al. (2019) investigate the evolution of herbicide resistance in *Amaranthus tuberculatus*. *A. tuberculatus* is known to have evolved resistance to glyphosate, the active ingredient of Round-Up Ready. As Kreiner et al. (2019) explain, Round-Up Ready was introduced in 1996 and resistance quickly followed by 2005 in Missouri. Similarly, agricultural-associated *A. tuberculatus* appeared in Ontario in the early 2000s and evolved resistance within a decade. Kreiner et al. (2019) set out to discover just how resistance evolved. In particular, they ask what the genetic basis of resistance is, how many times it has independently arisen, and how it has spread geographically.

Kreiner et al. (2019) collected seeds from 163 *A. tuberculatus* individuals from 19 agricultural fields across Missouri, Illinois, and Ontario (Essex Country and Walpole Island). They also sampled 10 individuals from non-agricultural settings in Ontario as a natural comparison. In order to study the population genetics of this sample, the authors first built a high-quality reference genome using 1 individual and then resequenced all sampled individuals. To

understand how these genomes correspond to resistance they also raised individuals in the greenhouse with glyphosate and measured resistance (visually) on a scale of 0 to 5.

The authors first sought to describe the demographic history of this species. Using the computer program STRUCTURE, they deduced that there are two main lineages, var. *rudis* and var. *tuberculatus*. These previously hypothesized lineages historically correspond to populations on either side of the Mississippi River. The Missouri populations appear to be nearly homogenous for *rudis* ancestry while the natural Ontario populations appear homogeneous for *tuberculatus* ancestry. Importantly, they find the Essex populations composed mostly of *rudis* ancestry, indicating gene flow from the western part of the range, while the Walpole populations are mostly *tuberculatus*, suggesting recent colonization of agricultural settings from surrounding riparian habitat. However, the Walpole populations also have considerable *rudis* ancestry, such that resistance genes may have introgressed from elsewhere.

To explore the genetic basis of resistance in Walpole, Kreiner et al. (2019) looked for regions of the genome that most differentiated resistant Walpole populations from surrounding susceptible natural populations. Doing so revealed a number of important genes, including CYP450s and glycosyltransferases, both of which are known to be involved in detoxifying herbicides, and both of which have increased in copy number in Walpole. However, the copy number of these genes did not significantly correlate with greenhouse-measured resistance, suggesting these genes might confer resistance to other herbicides.

Based on previous work, the authors suspected that EPSPS genes may also be involved in resistance. They therefore compared EPSPS copy number with resistance and found that it explained roughly 20-50% of the variance. A mutation in EPSPS explained a further 10%. Together, this suggests there are mutations elsewhere in the genome that are contributing to

resistance as well. To further investigate selection on the EPSPS region, the authors used a program called SweepFinder2 to look for evidence of selection on EPSPS. Interestingly, they found the strongest evidence of selection in Walpole populations, suggesting a hard selective sweep (a single new mutation rises in frequency; Hermisson & Pennings 2005) there, but soft selective sweeps (multiple mutations simultaneously rising in frequency; Hermisson & Pennings 2005) elsewhere. Finally, the authors compared EPSPS copy number to a phylogenetic tree and compared copy number profiles between individuals to infer how many times copy number mutations have arisen in this species. These analyses also suggest one unique mutation in Walpole and multiple unique mutations elsewhere.

In summary, Kreiner et al. (2019) suggest that *A. tuberculatus* has evolved resistance to herbicides multiple times via multiple genetic pathways, and that gene flow between populations helps resistance spread. Reducing gene flow is therefore one way to reduce resistance in this system but is unlikely to prevent it entirely. One outstanding question arising from this work is whether the resistance mutation in Walpole arose there on a non-agricultural genetic background or was introgressed from an unsampled agricultural population elsewhere.

References:

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