ON THE NATURE OF THINGS: ESSAYS

New Ideas and Directions in Botany

The remarkable repeated evolution of herbicide resistance[1]

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Perhaps, when teaching students about plant defense, you show pictures of the long spines of the African acacia tree or use caffeine as an example of a plant toxin. Plants have evolved a mind-boggling array of solutions to the problem of being damaged, and these extreme examples of defense are useful and engaging when initiating students into the remarkable world of botany. In my opinion, there is no example of plant defense quite as intriguing as the evolution of herbicide resistance. Herbicides are chemicals that disrupt major plant physiological processes. They work by targeting a range of mechanisms: they disrupt the production of amino acids and fatty acids or arrest microtubule formation or the electron transport chain of photosynthesis (Délye et al., 2013). Herbicides impose extreme selection as they are designed to remove between 90–99% of the plant population (Jasieniuk et al., 1996; Delye, 2013). As a result, plants have evolved resistance to all of the commonly used herbicide classes, and there are currently over 240 herbicide resistant species, with many distantly related species resistant to the same herbicide (Heap, 2015). Beyond providing students an engaging topic that scales from cell biology, physiology, to genetics, the rapid evolution of herbicide resistance also allows for a "real time" approach to the study of evolutionary processes. Here I highlight how the many repeated cases of herbicide resistance provide a unique opportunity to study the mechanics of a phenomenon that has long intrigued evolutionary biologists—that of phenotypic convergence, or the independent evolution of the same trait among distinct evolutionary lineages (Losos, 2011). I briefly discuss the current state of our understanding of the genetics underlying herbicide resistance, then I contextualize how future research on herbicide resistance evolution could address the mechanics of evolutionary convergence more broadly.

Biologists since Darwin have been fascinated by evolutionary repetition (Darwin, 1859). Such repetition is viewed as evidence that natural selection produces the optimal phenotype following repeated challenges with the same environment (Simpson, 1953; Endler, 1986). Others see convergent phenotypes among species as evidence for shared biases in the production of variation, also known as genomic constraints (Wake, 1991; Gould, 2002). Phenotypic convergence may be due to changes in the same or different locus/loci—when the same locus is responsible, the phenomenon is considered "genetic parallelism" (Martin and Orgogozo, 2013). Examples of pesticide and herbicide resistance have been referred to as cases of "extreme parallelism" since the same locus can be implicated to underlie resistance in multiple distantly related species (Martin and Orgogozo, 2013). Thus, these cases are used to bolster the contention that genomic constraint is responsible for phenotypic convergence in nature (Martin and Orgogozo, 2013). While the many documented cases of herbicide resistance do in fact provide firm evidence that parallel genetic changes cause convergent phenotypes, parallelism at the genetic level is not always the case—there are a number of examples wherein the mechanism underlying resistance to the same herbicide differs among and even within species (Yu et al., 2007; Preston and Wakelin, 2008; Preston et al., 2009).

The current convention in weed science is to group the mechanisms that underlie herbicide resistance into two broad categories: target site resistance (TSR) and nontarget site resistance (NTSR) (Table 1). Briefly, TSR describes the phenomenon wherein a structural change to the binding site of the herbicide molecule confers resistance or the target site is overexpressed, generally through gene amplification (Délye et al., 2013). Nontarget site resistance mechanisms are diverse and vary within and among species, but generally function to reduce the number of herbicide molecules that reach the herbicide target site (see Table 1; Délye, 2013). Thus far, the evidence for extreme parallelism comes from TSR, with the most striking example documented in weeds that are resistant to herbicides that inhibit acetolactate synthase (ALS) (Tranel and Wright, 2002; Heap, 2015). An exceptional number of species (157) are resistant to this herbicide class, and the mutations underlying resistance to ALS inhibitors have been identified through sequencing the ALS gene of 145 resistant biotypes of a diverse range of flowering plants (biotype refers to a variant; there can be multiple biotypes per species) (Heap, 2015). Summarized over 58 species, there are >140 substitutions in the ALS gene that are either linked or conclusively shown through functional tests to be responsible

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TABLE 1. Summary of the known physiological and genetic mechanisms underlying resistance to three herbicide classes. While the genetic basis of NTSR has yet to be conclusively identified in any weed species, there are indications that glutathione-*S*-transferases, members of the cytochrome P450 gene family, esterases, hydrolyases, and transporter proteins are involved (reviewed by Délye, 2013).

	Target site resistance (TSR)			Nontarget site resistance (NTSR)	
Levels of convergence	**Gene**	**Herbicide type**	**No. of species**	**Gene**	**Herbicide type**
Genetic basis	Acetolactate synthase (ALS)	ALS inhibitors	58	Unknown	ALS inhibitors
	Acetyl coenzyme A carboxylase (ACC	ACCase inhibitors	7	Unknown	ACCase inhibitors
	5-Enolpyruvylshikimate-3-phosphate synthase (EPSP)	Glyphosate	3	Unknown	Glyphosate
Physiological mechanism(s)	• Reduced binding to the target protein			• Reduced penetration of herbicide • Altered translocation of herbicide • Enhanced metabolism • Protection against oxidative damage	

for resistance (see ALS mutation table and references in Heap, 2015). Thirty-four of these species exhibit a change at amino acid proline 197 of the mature protein sequence that confers greater than 10-fold resistance compared with the wild type. Thus, mutations in the ALS gene provide evidence of genetic parallelism (as depicted in Fig. 1A, B) as well as solid evidence of a genetic hotspot within the ALS locus responsible for resistance (Fig. 1A). This pattern, albeit with fewer examples, is also seen in cases of resistance to two other herbicide groups, the acetyl-CoA caroboxylase (ACCase) inhibitors and herbicides that inhibit 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase) (Table 1; Heap, 2015). Our current understanding of TSR thus strongly supports the idea that parallel genetic changes—and thus genomic constraints—are responsible for convergent evolution of the resistance phenotype.

Alternatively, examples of NTSR show that resistance can arise through different genetic and physiological mechanisms both within and among species (Fig. 1C). Biotypes of *Lolium rigidium* (rigid ryegrass) resistant to the herbicide glyphosate, for example, exhibit a TSR mutation in the EPSP synthase locus (position Pro106) (Wakelin and Preston, 2006); however, other biotypes of this species are resistant due to reduced translocation (i.e., movement) of the herbicide, an NTSR mechanism (Preston and Wakelin, 2008). Strikingly, both mechanisms have been discovered within the same population (Yu et al., 2007; Preston et al., 2009). Altered translocation has been identified as an NTSR mechanism in glyphosate resistant biotypes of the closely related *Lolium multiflorum* as has reduced penetration of glyphosate into the leaf surface, another NTSR mechanism (Michitte et al., 2007; Nandula et al., 2007). Biotypes of this species also exhibit the conserved Pro106 TSR mutation, indicating the presence of TSR as well as NTSR mechanisms. We currently know very little about the genetic basis of NTSR, but reports indicate that its control can be either monogenic (Yu et al., 2009) or polygenic (Petit et al., 2010), depending on the species and herbicide in question. These examples, along with others reviewed by Powles and Yu (2010) show that convergent phenotypes are not necessarily due to the same genetic bases and that the "extreme parallelism" of TSR is not always the case.

Many questions remain unanswered about NTSR. For example, how often do species evolve the same or different NTSR mechanisms? Even though NTSR provides an alternate path to resistance beyond TSR, are particular NTSR mechanisms preferred for a given species/herbicide combination? Such a scenario would support the idea that while there is not a single constrained path to resistance, there are still a limited number of ways resistance may evolve. Further, what are the initial steps underlying the evolution of NTSR mechanisms? With some notable exceptions (Neve and Powles, 2005; Baucom and Mauricio, 2008), most examinations focus on species that exhibit very high resistance, or survival at 5–10× the field-dose of herbicide compared with susceptible lineages, meaning that the initial stages of resistance evolution are rarely considered. Does it take multiple NTSR mechanisms working in concert to confer high resistance, and, if NTSR mechanisms work synergistically, do some NTSR mechanisms work together better than others? Are the majority of NTSR mechanisms under the control of multiple loci, or few?

Answering these questions could address one of the central challenges in the study of phenotypic convergence more broadly: Why in

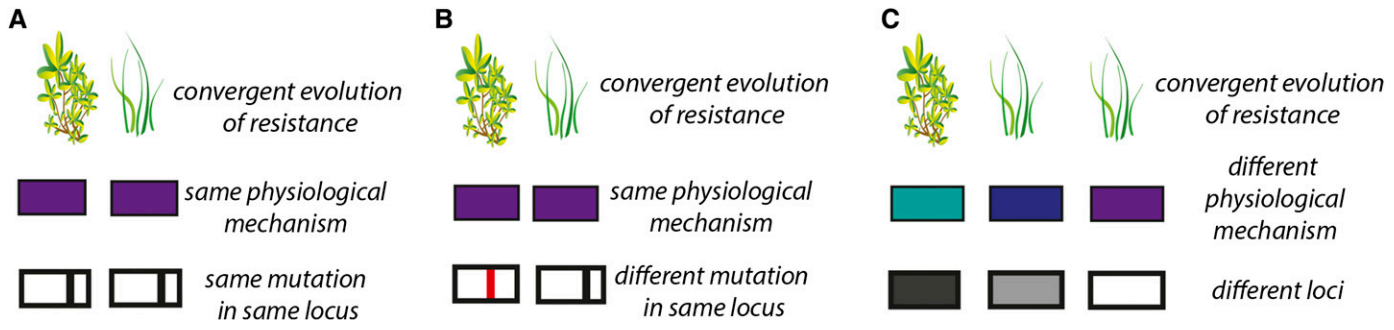

FIGURE 1 The convergent evolution of resistance among species can be due to the (A) the same mutation in the same locus among species (TSR), (B) different mutations in the same locus among species (TSR), or (C) through widely different physiological and genetic mechanisms, such as herbicide detoxification or translocation, or a mix of NTSR and TSR among and/or within species.

some cases do we see parallel genetic changes that confer the same phenotype, but in other cases we do not, as is the case for *Lolium* species? The likelihood of repeated phenotypes across the landscape is dependent on the mutation rate of the locus (or loci) underlying the trait, as well as population genetics parameters such as, but not limited to, the migration rate, population size, and fitness effects of alleles (Ralph and Coop, 2015; Stern, 2013). Do we see NTSR mechanisms more often in cases where the mutation rate of the target-site locus is low? This scenario could explain why NTSR mechanisms, rather than TSR, are responsible for the majority of glyphosate-resistant weeds. Perhaps, as theoretical work would suggest, species that evolve different NTSR mechanisms or a combination of NTSR and TSR exhibit low dispersal among subpopulations—these populations may adapt via new mutations before resistance alleles from another population could arrive by migration (Ralph and Coop, 2015). Finally, some NTSR mechanisms, such as detoxification and the reduction of oxidative stress are hypothesized to stem from general plant stress responses. A scenario in which ancestral variation of a co-opted stress response contributed to the same NTSR mechanism in multiple populations would support the hypothesis that genomic constraints underlie repeated evolution of resistance. Alternatively, perhaps different NTSR mechanisms are beneficial in their local environment due to some other abiotic selective agent, or past history of selection via another selective regime. This would produce a pattern of convergent resistance phenotypes with different underlying genetic control, and support the hypothesis that natural selection produces the optimal solution for the challenge at hand.

The phenomenon of herbicide resistance represents one of the best current examples of "evolution in action", and as such makes for a great system to study the genetics of convergent phenotypes. We know when weed populations were first exposed to selection by specific herbicides, as well as the strength and frequency of herbicide use. Further, there are usually many replicated populations of the same weed exposed to the same herbicide across the landscape, as well as a broad range of flowering plant species exposed to the same herbicides. Thus, herbicide resistance, writ large, is well positioned to examine the genetics of convergence both within and among species. It is important to note, however, that our current understanding of TSR—and likewise the conclusion that parallel genetic changes underlie resistance among species—is based almost entirely on candidate gene sequencing, as there are strikingly few genetic investigations of herbicide resistance that use less-biased techniques such as QTL mapping or population genomics. This scenario should change rapidly since weed biologists have taken renewed interest in NTSR mechanisms (Délye, 2013), and the genomic tools to perform QTL mapping, gene expression surveys, and surveys of loci under selection are now possible with nonmodel plants given recent advances in massively parallel sequencing and computational technologies. In conclusion, the study of herbicide resistance evolution has been made simpler by the use of a known selective agent; because of this and the progress weed biologists have made documenting repeated cases of resistance, we can now place a practical problem at the forefront of a long-standing and central topic in evolutionary biology.

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