DR. REGINA S BAUCOM (Orcid ID : 0000-0001-7960-498X)
DR. JULIA KREINER (Orcid ID : 0000-0002-8593-1394)
DR. SARAH YAKIMOWSKI (Orcid ID : 0000-0002-2465-5314)

Article type : Special Issue

Resistance evolution, from genetic mechanism to ecological context

Regina S. Baucom[1], Veronica Iriart[2], Julia M. Kreiner[3], and Sarah Yakimowski[4]

[1]Ecology and Evolutionary Biology Department, University of Michigan, Ann Arbor, Michigan, USA
[2]Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania, USA
[3]Biodiversity Research Centre & Department of Botany, The University of British Columbia, Vancouver, BC V6T 1Z4
[4]Department of Biology, Queen's University, Kingston, ON K7L 3N6

Correspondence
Regina S. Baucom, Ecology and Evolutionary Biology Department, University of Michigan, Ann Arbor, Michigan, 48109.
Email: rsbaucom@umich.edu

*Authors contributed equally

Pesticide use by humans has induced strong selective pressures, reshaping evolutionary trajectories, ecological networks, and even influencing ecosystem dynamics. The evolution of pesticide resistance across weeds, insects, and fungi often leads to negative impacts on both human health and the economy while concomitantly providing excellent systems for studying the process of evolution. In fact, the study of pesticide resistance has been a feature of evolutionary biology since the Evolutionary Synthesis, with Dobzhansky noting in his book *The Genetics and Origins of Species* (1937) that cyanide resistance in the California red scale constituted the "best proof of the effectiveness of natural selection yet obtained". Following the pioneering work of James Crow and others in the 1950's—which greatly expanded our knowledge of the genetics underlying adaptation—the study of pesticide resistance has shed light on a variety of topics, such as the repeatability of phenotypic evolution across the landscape, 'hotspots' of evolution across the genome, and information on the number and type of genetic solutions that populations may employ to strong selection pressures.

Landscape level approaches have come to the forefront over the last 20 years of resistance evolution research, often taking advantage of the fact that replicated populations of the same species are exposed to the same pesticide. Further, the resistance evolution field is turning more attention to the ecological context within which resistance evolution occurs, likely stemming, at least in part, from an historical focus on fitness costs (Cousens & Fournier-Level 2018; Baucom 2019). This special feature, '*Resistance evolution, from genetic mechanism to ecological context'* in Molecular Ecology captures the current state of resistance evolution with contributions broadly addressing the question '*What has the rapid evolution of pesticide resistance taught us about genome dynamics and adaptation as well as the ecological context within which resistance evolution occurs?'* Below, we contextualize the manuscripts in this special feature that provide insight into the state of the art investigations of resistance evolution across various species of insects, weeds and fungi.

The genomic basis of adaptation: target site and non-target site resistance mechanisms

Pesticides are designed to target particular genes in functional pathways of pest organisms. For example, quinone-outside inhibitor fungicides (QoIs) target the products of the fungal gene cytochrome b, a critical component of aerobic respiration. In plants, the herbicide glyphosate targets the protein product of EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), a central gene in the shikimate acid pathway. One type of pesticide resistance—termed target site resistance—occurs when a mutation in the gene targeted by the pesticide alters the conformation of the protein, reducing or completely eliminating the ability of the pesticide to bind to the protein's active site (Délye *et al.* 2013). A number of studies have examined target site resistance by sequencing the target locus and assessing the various mutations associated with resistance. Given *a priori* information about how pesticides work, thus providing obvious genetic candidates to explore, along with the relative simplicity of sequencing a single gene, we currently have a somewhat comprehensive understanding of the type and number of genetic variants associated with target site resistance, especially in herbicide resistant weeds (Tranel, Wright, & Heap 2021; Baucom 2016).

We still have much to learn about target-site resistance evolution, however, especially in the context of broader, genome-scale dynamics. In this special feature, Clarkson et al. (2021) move beyond investigating the dynamics of target-site resistance in isolation by explicitly examining the role of intragenic variation on resistance to pyrethroid, a class of insecticide that is used to control mosquito populations associated with malaria. Their investigation of whole-genome sequence data reveals 'a tale of two alleles': two widespread large-effect target-site resistance alleles within the voltage-gated sodium channel (VGSC) gene appear to be on different evolutionary trajectories. One allele, likely an early ancestral mutation, is associated (*i.e.,* in strong positive linkage disequilibrium) with a subsequent explosion of 13 secondary non-synonymous mutations, whereas the second allele is associated with fewer mutations. Further, most of these mutations are background-dependent, occurring nearly exclusively on distinct haplotypes—haplotypes that are associated with different signatures of selection despite harbouring the same focal resistance allele, implying important compensatory or enhancing allelic interactions for resistance evolution.

A major contribution to our understanding of the predictability of evolution stems from work examining the repeatability of target site changes that confer pesticide resistance across insect and weed species, respectively (Martin & Orgogozo 2013). However, whether or not parallel genetic changes lead to resistance among fungal plant pathogens has yet to be succinctly summarized. In this issue, Hawkins and Fraaije (2021) investigate the extent of parallel evolution of individual mutations in target genes among species of fungal pathogens. Focusing on mutations associated with four classes of fungicide, they show that the target-genes vary substantially in the diversity of mutations detected. For two fungicide classes (QoIs and MBCs) the same mutations are observed repeatedly across species. In contrast, a greater diversity of resistance mutations was uncovered within genes targeted by azole and SDHI fungicides, providing less evidence for extreme parallelism across species compared to QoIs and MBCs.

Another form of target-site resistance is from gene amplification, where increased copy number of the target locus leads to more functional protein and subsequent resistance. In a handful of weeds, an increase in the copy number of the EPSPS locus leads to high glyphosate resistance (*reviewed in* Gaines *et al.* 2019); while the underlying mechanism of this copy number increase has been described (Koo *et al.* 2018), we understand relatively little about the long-term maintenance of copy number variation (CNV) and how gene amplification may influence interactions with other loci. Yakimowski, Teitel and Caruso (2021) quantified patterns of variation of target gene copy number and resistance phenotypes within and among populations—the 'natural history' of a resistance CNV—to provide insight into the evolution of glyphosate resistance in the agricultural weed *Amaranthus palmeri* in the eastern United States. They detected a steep increase in phenotypic glyphosate resistance at a threshold value of ~15 gene copies, but also found that populations with the highest mean resistance contained some low copy number individuals (albeit at low-frequency). From 15 to 160 gene copies the level of resistance changed very little; however, the proportion of low-resistance phenotypes gradually decreased in populations with increased copy number, suggesting that dosage of the

target gene with increasing copy number might compensate for negative interactions with other loci. Potential positive interactions with other genes were also observed in populations from Georgia. Overall, target gene copy number variation explained a high proportion (~57%) of variation in phenotypic resistance among populations.

In another set of contributions to this special feature, Ravet et al. (2021) and Gaines et al. (2021) take landscape-level approaches to examine EPSPS copy number variation and its association with resistance in kochia (*Bassia scoparia*) and *Amaranthus palmeri,* respectively. In glyphosate resistant kochia, researchers paired an investigation of population structure *via* SSR markers with qPCR markers designed to examine the EPSPS repeat genotype across western lineages. They found low population structure in this species but evidence for independent evolutionary origins of the EPSPS duplication leading to resistance. In the landscape level assessment of *A. palmeri*, researchers found copy number variation of this target gene—like that found across lineages from the eastern United States by Yakimowski, Teitel and Caruso (2021)—present in populations from Brazil and Uruguay, indicating that copy number variation related to resistance is found broadly across the landscape in this species. Interestingly, however, resistance in Argentinian lineages of *A. palmeri* was due in large part to non-target site resistance mechanisms—*i.e.* resistance mechanisms that do not involve the target site, such as altered translocation or detoxification of the pesticide, among others—rather than elevated copy number of the EPSPS locus. These results show both genomic flexibility in solving the problem of herbicide exposure and the independent, novel evolution of resistance across geography.

Thus, in addition to target site resistance mechanisms, organisms can also evolve resistance through non-target site mechanisms. Non-target site resistance mechanisms, which are often thought to be due to polygenic variation, can both confer resistance as well as potentially supplement target-site effects. While both target site and non-target site mechanisms have previously been uncovered within the same herbicide resistant weed species (as in Gaines et al. 2020), the relative contribution of either type of mechanism has yet to be

clearly delineated in any weed species. Using another glyphosate resistant *Amaranthus* species, *A. tuberculatus* (common waterhemp), Kreiner et al. (2021) uncovered the cryptic contribution of genome-wide alleles to glyphosate resistance. On the genomic background of agricultural populations harbouring high frequencies of target-site resistance mechanisms (Kreiner *et al.* 2019), the authors illustrated a near-equal importance of non-target and target-site mechanisms. Further, they uncovered hundreds of alleles associated with non-target site resistance that show not only evidence of recent strong selection from herbicides but a classic trade-off between effect size and allele frequency that implicates pleiotropy as a key constraint to the evolution of herbicide resistance.

As our understanding of the genetic architecture of pesticide resistance and governing selective processes deepens, a key question will be how consistently such alleles are involved across geographic scales. This question is addressed by Hartmann et al. (2020), who investigated the architecture of azole fungicide in a key wheat pathogen, *Zymoseptoria tritici* across three continents. They uncovered a suite of azole resistance-related loci across the genome including a novel large-effect gene, DHHC palmitoyl transferase. Along with key alleles conferring resistance to three other chemical classes of fungicides, the authors find evidence that the genomic architecture of fungicide resistance is largely distinct across continents, with the exception of large-effect genes that act as hotspots for convergence. Overall, this collection of work characterizing the genetic architecture of pesticide resistance uncovers remarkable complexity in monogenic and polygenic contributions and the processes that govern their assemblage across genomes, from background- and population-specific constraints to the potential for pleiotropic tradeoffs.

Ecological context and the influence of interactions on the evolution of resistance

Pesticide use can have impacts far beyond their intended target organism by influencing the ecology and evolution of organisms with which the target species interact. Contributions in

this issue explore the ecological context of resistance evolution by assessing how pesticide application may affect interactions between target and non-target organisms, which may influence downstream eco-evolutionary feedback dynamics.

Iriart et al. (2020) set the stage for our understanding of the ecological context of resistance evolution by reviewing the role of herbicides in driving the ecology and evolution of plants and plant-associates (*e.g.* pollinators, soil microbes, herbivores, and parasitoids) living in communities at the agro-ecological interface. They synthesize what is known about how herbicides can alter plant phenotypes from plastic or genetic changes and how plant-associates may be directly or indirectly (*via* interactions) affected by herbicides. Building off this knowledge, they demonstrate that herbicides can induce sufficiently rapid change in plants and plant-associates to alter both evolution and ecological dynamics over the same timescales, thus producing eco-evolutionary feedbacks. From these insights, they provide suggestions for future research into herbicide catalyzed eco-evolutionary dynamics, with the goal of deciphering the effects of herbicides on plant and plant-associates' traits, on species interactions, and on the composition of the broader ecological community.

Herbicide application may alter eco-evolutionary dynamics by selecting for traits that are correlated to resistance, such as earlier flowering time or altered mating patterns (among other changes) thereby potentially modifying mutualistic interactions between plants and their associates. For example, glyphosate resistant populations of the common morning glory (*Ipomoea purpurea*) exhibit higher selfing rates compared to susceptible populations (Kuester et al 2017), perhaps due to reproductive assurance associated with being both highly selfing and herbicide resistant. However, an association between the mating system and resistance would not be expected to be maintained over time if the resistant, selfing types exhibited inbreeding depression. In this issue, Van Etten et al. (2021) combined growth chamber and field studies with transcriptome surveys to ask whether genetic lines of *Ipomoea purpurea* selected for increased glyphosate resistance exhibited signs of inbreeding depression (*i.e.* poorer performance of inbred versus outcross progeny) compared to both non-selected control lines

and lines selected for increased susceptibility. Interestingly, they found that while plants from non-selected control lines and susceptible lines exhibited evidence of inbreeding depression, plants from resistant lines provided no evidence for inbreeding depression in most characters. Rather, in the presence of herbicide, resistant lines tended to show *outbreeding depression*: seeds from resistant lines that were produced *via* selfing germinated more and grew to be larger plants than those from resistant lines that were produced from outcrossing. Additionally, the authors showed that the expression of genes within the transcriptome mirrored the phenotypic patterns—resistant, inbred plants showed higher expression of genes involved in translation and DNA replication compared to resistant, outcrossed progeny in the presence of glyphosate. Thus, in this case study, continued resistance evolution would support higher self-fertilization and decreased outcrossing. In this way, the maintenance of plant-pollinator interactions could be negatively altered over time in herbicide-exposed populations of *I. purpurea*.

As with herbicides, the evolution of resistance to insecticides has the potential to alter ecological interactions in crop ecosystems, as shown by Paddock et al. (2021). Their study investigated whether the microbial communities differed between herbivorous western corn rootworms (a widespread agricultural pest) that were susceptible or had evolved resistance to the insecticide *Bacillus thuringiensis* (Bt) produced by genetically-modified maize. Their results supported different enteric microbiomes between resistant and susceptible western corn rootworm in that resistant individuals had less rich and diverse bacterial communities. Additionally, western corn rootworm digesting the insecticide caused a severe shift towards more simplified bacterial communities in susceptible western corn rootworm, but not resistant western corn rootworm, suggesting an effect of host-microbial interactions in the evolution of resistance to Bt. Together, these results contribute to our budding understanding of the role that ecology can play in resistance evolution to modern stressors, further perpetuating eco-evolutionary feedbacks and dynamics in natural communities.

Conclusion

The manuscripts in this special feature cover both a wide conceptual range and a broad group of organisms. The common thread between studies is that they each investigate applied systems to broaden our understanding of either the ecological or genetic factors that influence adaptation. While many researchers are likely drawn to the study of pesticide resistance evolution for its perceived simplicity—*e.g,* there is a known agent of selection as well as (typically) a known biochemical and often simple genetic target of the pesticide—contributions in this special feature show that both the genetic and ecological context of resistance evolution remains to be deepened in important ways. For example, the population genomic approaches used in these papers provide increased resolution into the number and interactions of alleles involved in resistance, timescale-dependent signatures of selection, heterogeneity among populations in the control of resistance, and gene expression differences associated with both resistance and the mating system. Additionally, the work in this special feature highlights that resistance evolution does not occur in a vacuum; while many examinations of resistance focus on the evolutionary trajectory of a single species exposed to pesticide, the overall adaptation and persistence of a population will concomitantly be influenced by, and likewise influence, other community members such as pollinators, herbivores and the microbial community. Ultimately, deepening the context of resistance evolution by both broadening our genetic toolkits and by assessing community dynamics will allow us to better understand how genetics and ecology are linked and how such linkages can then influence larger-scale ecosystem dynamics.

References

Baucom RS (2016) The remarkable repeated evolution of herbicide resistance. *American Journal of Botany*, **103**, 181–183.

Baucom RS (2019) Evolutionary and ecological insights from herbicide-resistant weeds: what have we learned about plant adaptation, and what is left to uncover? *The New Phytologist*, **223**, 68-82.

Clarkson CS, Miles A, Harding NJ *et al.* (2021) The genetic architecture of target-site resistance

to pyrethroid insecticides in the African malaria vectors *Anopheles gambiae* and *Anopheles coluzzii*. *Molecular Ecology* XXX-XXX (THIS SPECIAL FEATURE).

Cousens RD, Fournier-Level A (2018) Herbicide resistance costs: what are we actually measuring and why?: Herbicide resistance costs. *Pest Management Science*, **75**, 349.

Délye C, Jasieniuk M, Le Corre V (2013) Deciphering the evolution of herbicide resistance in weeds. *Trends in Genetics*, **29**, 649–658.

Dobzhansky T (1937) *Genetics and the Origin of Species*. Columbia University Press.

Gaines TA, Patterson EL, Neve P (2019) Molecular mechanisms of adaptive evolution revealed by global selection for glyphosate resistance. *The New Phytologist*, **223**, 1770–1775.

Gaines T, Slavov G, Hughes D *et al.* (2020) Investigating the origins and evolution of a glyphosate-resistant weed invasion in South America. *Molecular Ecology* XXX-XXX (THIS SPECIAL FEATURE).

Hartmann FE, Vonlanthen T, Singh NK *et al.* (2020) The complex genomic basis of rapid convergent adaptation to pesticides across continents in a fungal plant pathogen. *Molecular Ecology*.

Hawkins NJ, Fraaije BA (2021) Contrasting levels of genetic predictability in the evolution of resistance to major classes of fungicides. *Molecular Ecology* XXX-XXX (THIS SPECIAL FEATURE).

Iriart, V, Baucom, RS, Ashman, T-L. Herbicides as anthropogenic drivers of eco-evo feedbacks in plant communities at the agro-ecological interface. *Molecular Ecology* XXX-XXX (THIS SPECIAL FEATURE).

Koo D-H, Molin WT, Saski CA *et al.* (2018) Extrachromosomal circular DNA-based amplification and transmission of herbicide resistance in crop weed *Amaranthus palmeri*. *Proceedings of the National Academy of Sciences of the United States of America*, **115**, 3332–3337.

Kreiner JM, Giacomini DA, Bemm F *et al.* (2019) Multiple modes of convergent adaptation in the spread of glyphosate-resistant *Amaranthus tuberculatus*. *Proceedings of the National Academy of Sciences of the United States of America*, **116**, 21076–21084.

Kreiner JM, Tranel PJ, Weigel D, Stinchcombe JR, Wright SI (2021) The genetic architecture and population genomic signatures of glyphosate resistance in *Amaranthus tuberculatus*.

Molecular Ecology XXX-XXX (THIS SPECIAL FEATURE).

Martin A, Orgogozo V (2013) The loci of repeated evolution: a catalog of genetic hotspots of phenotypic variation. *Evolution*, **67**, 1235–1250.

Paddock KJ, Pereira AE, Finke DL *et al.* (2021) Host resistance to *Bacillus thuringiensis* is linked to altered bacterial community within a specialist insect herbivore. *Molecular Ecology* XXX-XXX (THIS SPECIAL FEATURE).

Ravet K, Sparks C, Dixon A, et al. (2021) Genomic-based epidemiology reveals gene flow and independent origins of glyphosate resistance in Bassia scoparia populations across North America. *Molecular Ecology* XXX-XXX (THIS SPECIAL FEATURE).

Tranel PJ, Wright TR, Heap IM (2021) Mutations in herbicide-resistant weeds to ALS inhibitors. *International Survey of Herbicide Resistant Weeds*. Online

Van Etten ML, Soble A, Baucom RS (2021) Variable inbreeding depression may explain associations between the mating system and herbicide resistance in the common morning glory. *Molecular Ecology* XXX-XXX (THIS SPECIAL FEATURE).

Yakimowski SB, Teitel Z, Caruso CM (2021) Defense by duplication: The relation between phenotypic glyphosate resistance and EPSPS gene copy number variation in *Amaranthus palmeri*. *Molecular Ecology* XXX-XXX (THIS SPECIAL FEATURE).