

# 1      The International Weed Genomics Consortium: Community

## 2      Resources for Weed Genomics Research

### 3

4      Jacob S. Montgomery<sup>1</sup>, Sarah Morran<sup>1</sup>, Dana R. MacGregor<sup>2</sup>, J. Scott McElroy<sup>3</sup>, Paul Neve<sup>4</sup>,  
5      Célia Neto<sup>4</sup>, Martin M. Vila-Aiub<sup>5</sup>, Maria Victoria Sandoval<sup>5</sup>, Analia I. Menéndez<sup>6</sup>, Julia M.  
6      Kreiner<sup>7</sup>, Longjiang Fan<sup>8</sup>, Ana L. Caicedo<sup>9</sup>, Peter J. Maughan<sup>10</sup>, Bianca Assis Barbosa Martins<sup>11</sup>,  
7      Jagoda Mika<sup>11</sup>, Alberto Collavo<sup>11</sup>, Aldo Merotto Jr.<sup>12</sup>, Nithya K. Subramanian<sup>13</sup>, Muthukumar  
8      V. Bagavathiannan<sup>13</sup>, Luan Cutti<sup>14</sup>, Md. Mazharul Islam<sup>15</sup>, Bikram S Gill<sup>16</sup>, Robert Cicchillo<sup>17</sup>,  
9      Roger Gast<sup>17</sup>, Neeta Soni<sup>17</sup>, Terry R. Wright<sup>18</sup>, Gina Zastrow-Hayes<sup>18</sup>, Gregory May<sup>18</sup>, Jenna M.  
10     Malone<sup>19</sup>, Deepmala Sehgal<sup>20</sup>, Shiv Shankhar Kaundun<sup>20</sup>, Richard P. Dale<sup>20</sup>, Barend Juan  
11     Vorster<sup>21</sup>, Bodo Peters<sup>11</sup>, Jens Lerchl<sup>22</sup>, Patrick J. Tranel<sup>23</sup>, Roland Beffa<sup>24</sup>, Mithila Jugulam<sup>15</sup>,  
12     Kevin Fengler<sup>18</sup>, Victor Llaca<sup>18</sup>, Eric L. Patterson<sup>14</sup>, Todd Gaines<sup>1</sup>

13

14     <sup>1</sup>Department of Agricultural Biology, Colorado State University, Fort Collins, Colorado, USA.

15     <sup>2</sup>Protecting Crops and the Environment, Rothamsted Research, Harpenden, Hertfordshire, UK.

16     <sup>3</sup>Department of Crop, Soil, and Environmental Sciences, Auburn University, Auburn, Alabama,  
17     USA.

18     <sup>4</sup>Department of Plant and Environmental Sciences, University of Copenhagen, Taastrup,  
19     Denmark.

20     <sup>5</sup>IFEVA-Conicet-Department of Ecology, University of Buenos Aires, Buenos Aires, Argentina.

21     <sup>6</sup>Department of Ecology, Faculty of Agronomy, University of Buenos Aires, Buenos Aires,  
22     Argentina.

23 <sup>7</sup>Department of Botany, The University of British Columbia, Vancouver, British Columbia,  
24 Canada.

25 <sup>8</sup>Institute of Crop Sciences, Zhejiang University, Hangzhou, China.

26 <sup>9</sup>Department of Biology, University of Massachusetts Amherst, Massachusetts, USA.

27 <sup>10</sup>Department of Plant and Wildlife Sciences, Brigham Young University, Provo, Utah, USA.

28 <sup>11</sup>Bayer AG, Weed Control Research, Frankfurt, Germany.

29 <sup>12</sup>Department of Crop Sciences. Federal University of Rio Grande do Sul, Porto Alegre, Rio  
30 Grande do Sul, Brazil.

31 <sup>13</sup>Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas, USA.

32 <sup>14</sup>Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing,  
33 Michigan, USA.

34 <sup>15</sup>Department of Agronomy, Kansas State University, Manhattan, Kansas, USA.

35 <sup>16</sup>Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA.

36 <sup>17</sup>Crop Protection Discovery and Development, Corteva Agriscience, Indianapolis, Indiana,  
37 USA.

38 <sup>18</sup>Genome Center of Excellence, Corteva Agriscience, Johnston, Iowa, USA.

39 <sup>19</sup>School of Agriculture, Food and Wine, University of Adelaide, Glen Osmond, South Australia,  
40 Australia.

41 <sup>20</sup>Jealott's Hill International Research Centre, Syngenta Ltd, Bracknell, Berkshire, UK.

42 <sup>21</sup>Department of Plant and Soil Sciences, University of Pretoria, Pretoria, South Africa  
43 <sup>22</sup>BASF SE, Ludwigshafen am Rhein, Germany.

44 <sup>23</sup>Department of Crop Sciences, University of Illinois, Urbana, Illinois, USA.

45 <sup>24</sup>Senior Scientist Consultant, Herbicide Resistance Action Committee / CropLife International,  
46 Liederbach, Germany.

47

48 Author for correspondence: Todd Gaines, Associate Professor, Colorado State University, 1177  
49 Campus Delivery, Fort Collins, CO 80523, USA. (Email: [Todd.Gaines@ColoState.edu](mailto:Todd.Gaines@ColoState.edu))

50

51 Keywords: Weed science, Reference genomes, Rapid adaptation, Herbicide resistance, Public  
52 resources

53

## 54 **Abstract**

55 The International Weed Genomics Consortium is a collaborative group of researchers focused on  
56 developing genomic resources for the study of weedy plants. Weeds are attractive systems for  
57 basic and applied research due to their impacts on agricultural systems and capacity to swiftly  
58 adapt in response to anthropogenic selection pressures. Our goal is to use genomic information to  
59 develop sustainable and effective weed control methods and to provide insights about biotic and  
60 abiotic stress tolerance to assist crop breeding. Here, we outline resources under development by  
61 the consortium and highlight areas of research that will be impacted by these enabling resources.

62

## 63 **Introduction**

64 Each year globally, agricultural producers and landscape managers spend billions of US  
65 dollars [1, 2] and countless hours attempting to control weedy plants and reduce their adverse  
66 effects. These management methods range from low-tech (e.g., pulling plants from the soil by  
67 hand) to extremely high-tech (e.g., computer vision-controlled spraying of herbicides).

68 Regardless of technology level, effective control methods serve as strong selection pressures on  
69 weedy plants, and often result in rapid evolution of weed populations resistant to such methods  
70 [3-7]. Thus, humans and weeds have been locked in an arms race, where humans develop new or  
71 improved control methods and weeds adapt and evolve to circumvent such methods.

72 Applying genomics to weed science will enable the development of more sustainable and  
73 effective control methods and offer a unique opportunity to study rapid adaptation and  
74 evolutionary rescue of diverse weedy species in the face of widespread and powerful selective  
75 pressures. Furthermore, lessons learned from these studies may also help to improve crop  
76 breeding efforts in the face of our ever-changing climate. While other research fields have used  
77 genetics and genomics to uncover the basis of many biological traits [8-11] and to understand  
78 how ecological factors affect evolution [12, 13], the field of weed science has lagged behind in  
79 the development of genomic tools essential for such studies [14]. As research in human and crop  
80 genetics pushes into the era of pangenomics, (i.e., multiple chromosome scale genome  
81 assemblies for a single species [15, 16]) publicly available genomic information is still lacking or  
82 severely limited for the majority of weed species. In fact, a recent review of current weed  
83 genomes identified just 26 weed species with sequenced genomes [17] – many assembled to a  
84 sub-chromosome level.

85 The International Weed Genomics Consortium (IWGC) is an open collaboration between  
86 academic, government, and industry researchers focused on producing genomic tools for weedy  
87 species from around the world. Through this collaboration, our initial aim is to provide  
88 chromosome-level reference genome assemblies for at least 50 important weedy species from  
89 across the globe. Each genome will include annotation of gene models and repetitive elements  
90 and will be free to the public with no intellectual property restrictions. Species were chosen

91 based on member input, economic impact, and global prevalence (Figure 1). Additionally, future  
92 funding of the IWGC will focus on supplementing these reference genomes with tools that  
93 increase their utility.

94 The IWGC held its first conference in Kansas City, Missouri, USA in September of 2021. At  
95 this meeting, guest speakers highlighted successful examples of using genomics to address  
96 questions in weed science [5, 18-20]. Training workshops taught commonly used bioinformatic  
97 pipelines, and oral and poster sessions showcased current research activities in weed genomics.  
98 At the conclusion of this meeting, attendees participated in a forward-looking discussion about  
99 the future of genomics in weed science and how the IWGC can help facilitate its successful  
100 implementation. In this paper, we summarize the goals of the IWGC and how we plan to provide  
101 support around the resources being developed to ensure they are widely accessible and utilized  
102 by the research community. We go on to highlight areas of research where these tools can be  
103 applied with hopes of attracting researchers from other fields to integrate weed science with the  
104 many other research areas where genomic tools are being successfully utilized, enabling new  
105 research towards adaptation, evolution, herbicide resistance, and genome biology.

106

## 107 **Development of Weed Genomics Resources by the IWGC**

### 108 *Reference genomes and data analysis tools*

109 The first objective of the IWGC is to provide high quality genomic resources for  
110 agriculturally important weeds. The IWGC therefore created two main resources for information  
111 about, access to, or analysis of weed genomic data (Figure 1). The IWGC website [21]  
112 communicates the status and results of genome sequencing projects, information on training and  
113 funding opportunities, upcoming events, and news in weed genomics. It also contains details of

114 all sequenced species including genome size, ploidy, chromosome number, herbicide resistance  
115 status, and reference genome assembly statistics. The IWGC either compiles existing data on  
116 genome size, ploidy, and chromosome number, or obtains the data using flow cytometry and  
117 cytogenetics (Figure 1; Additional File 1). Through this website, users can create an account to  
118 access our second main resource, an online genome database called WeedPedia. WeedPedia  
119 hosts IWGC-generated and other relevant publicly accessible genomic data as well as a suite of  
120 bioinformatic tools. Unlike what is available for other fields, weed science did not have a  
121 centralized hub for genomics information, data, and analysis prior to the IWGC. Our intention in  
122 creating WeedPedia is to encourage collaboration and equity of access to information across the  
123 research community.

124 WeedPedia is a cloud-based omics database management platform built from the software  
125 'CropPedia', and licensed from KeyGene (Wageningen, The Netherlands). The interface allows  
126 users to access, visualize, and download genome assemblies along with structural and functional  
127 annotation. The platform includes a genome browser, comparative map viewer, pangenome  
128 tools, RNA-sequencing data visualization tools, genetic mapping and marker analysis tools, and  
129 alignment capabilities that allow searches by keyword or sequence. Additionally, genes encoding  
130 known target sites of herbicides have been specially annotated, allowing users to quickly identify  
131 and compare these genes of interest. The platform is flexible, making it compatible with future  
132 integration of other data types such as epigenetic or proteomic information. As an online  
133 platform with a graphical user interface, WeedPedia provides user-friendly, intuitive tools that  
134 encourage users to integrate genomics into their research. We aspire for WeedPedia to mimic the  
135 success of other public genomic databases such as NCBI, CoGe, Phytozome, InsectBase, and  
136 Mycocosm to name a few. Additionally, all genome assemblies and annotations produced

137 through the IWGC will be uploaded to NCBI GenBank upon release (Table 1). WeedPedia  
138 currently hosts 36 reference genomes with additional genomes in the pipeline to reach a currently  
139 planned total of 50 reference genomes (Table 1). These genomes include both *de novo* reference  
140 genomes generated or in progress by the IWGC (31 species; Table 1), and publicly available  
141 genome assemblies of 19 weedy or related species (Table 2). As of June 2023, WeedPedia has  
142 over 250 registered users representing 27 countries spread across 6 continents.

143 The IWGC reference genomes are generated in partnership with the Corteva Agriscience  
144 Genome Center of Excellence (Johnston, Iowa) using a combination of single molecule long read  
145 sequencing, optical genome maps, and chromosome conformation mapping. This strategy has  
146 yielded highly contiguous, phased, chromosome-level assemblies for 20 weed species, with  
147 additional assemblies currently in progress (Table 1). The IWGC assemblies have been  
148 completed as single or haplotype-resolved double-haplotype pseudomolecules in inbreeding and  
149 outbreeding species, respectively, with multiple genomes being near gapless. For example, the *de*  
150 *novo* assemblies of the allohexaploids *Conyza sumatrensis* and *Chenopodium album*, have all  
151 chromosomes captured in single scaffolds and most chromosomes being gapless from telomere  
152 to telomere. Complementary full-length isoform (IsoSeq) sequencing of RNA collected from  
153 diverse tissue types and developmental stages assists in the development of gene models during  
154 annotation. Finally, the use of PacBio Revio has enabled the re-sequencing of 80 relevant  
155 accessions, which is enabling initial pangenomic analysis for some of the IWGC-selected  
156 species.

157 As with accessibility of data, a core objective of the IWGC is to facilitate open access to  
158 sequenced germplasm for all featured species. Historically, the weed science community has  
159 rarely shared or adopted standard germplasm (e.g., specific weed accessions). The IWGC has

160 selected a specific accession of each species for reference genome assembly (typically  
161 susceptible to herbicides). In collaboration with a parallel effort by the Herbicide Resistant Plants  
162 committee of the Weed Science Society of America, seeds of the sequenced weed accessions  
163 will be deposited in the United States Department of Agriculture Germplasm Resources  
164 Information Network [22] for broad access by the scientific community. The IWGC ensures that  
165 sequenced accessions are collected and documented to comply with the Nagoya Protocol on  
166 access to genetic resources and the fair and equitable sharing of benefits arising from their  
167 utilization under the Convention on Biological Diversity and related Access and Benefit Sharing  
168 Legislation [23]. As additional accessions of weed species are sequenced (e.g., pangenomes are  
169 obtained) the IWGC will facilitate germplasm sharing protocols to support collaboration.  
170 Further, to simplify the investigation of herbicide resistance, the IWGC will link WeedPedia  
171 with the International Herbicide-Resistant Weed Database [24], an already widely known and  
172 utilized database for weed scientists.

173

174 *Training and collaboration in weed genomics*

175 Beyond producing genomic tools and resources, a priority of the IWGC is to enable the  
176 utilization of these resources across a wide range of stakeholders. A holistic approach to training  
177 is required for weed science generally [25], and we would argue even more so for weed  
178 genomics. To accomplish our training goals, the IWGC is developing and delivering programs  
179 aimed at the full range of IWGC stakeholders and covering a breadth of relevant topics. We have  
180 taken care to ensure our approaches are diverse as to provide training to researchers with all  
181 levels of existing experience and differing reasons for engaging with these tools. Throughout, the

182 focus is on ensuring that our training and outreach result in impacts that benefit a wide range of  
183 stakeholders.

184 Although recently developed tools are incredibly enabling and have great potential to replace  
185 antiquated methodology [26] and to solve pressing weed science problems [14], specialized  
186 computational skills are required to fully explore and unlock meaning from these highly complex  
187 datasets. Collaboration with, or training of, computational biologists equipped with these skills  
188 and resources developed by the IWGC will enable weed scientists to expand research programs  
189 and better understand the genetic underpinnings of weed evolution and herbicide resistance. To  
190 fill existing skill gaps, the IWGC is developing summer bootcamps and online modules directed  
191 specifically at weed scientists that will provide training on computational skills (Figure 1).

192 Because successful utilization of the IWGC resources requires more than general computational  
193 skills, we have also created three additional targeted workshops that teach practical skills related  
194 to genomics databases, molecular biology, and population genomics (available at [27]).

195 Engagement opportunities during undergraduate degrees has been shown to improve  
196 academic outcomes [28, 29]. Therefore, the IWGC sponsors opportunities for undergraduates to  
197 undertake 10-week Research Experiences for Undergraduates (REU). These REU include an  
198 introduction to bioinformatics, a plant genomics research project that results in a presentation,  
199 and access to career building opportunities in diverse workplace environments. To increase  
200 equitable access to conferences and professional communities, we supported early career  
201 researchers to attend the first two IWGC conferences in the USA as well as workshops and  
202 bootcamps in Europe and South America. These hybrid or in-person travel grants are  
203 intentionally designed to remove barriers and increase participation of individuals from  
204 backgrounds and experiences currently underrepresented within weed/plant science or genomics

205 [30]. Recipients of these travel awards gave presentations and gained the measurable benefits  
206 that come from either virtual or in-person participation in conferences [31]. Moving forward,  
207 weed scientists must amass skills associated with genomic analyses and collaborate with other  
208 area experts to fully leverage resources developed by the IWGC.

209

## 210 **Evolution of Weediness: Potential Research Utilizing New Weed Genomics**

### 211 **Tools**

212 Weeds can evolve from non-weed progenitors through wild colonization, crop de-  
213 domestication, or crop-wild hybridization [32]. Because the time span in which weeds have  
214 evolved is necessarily limited by the origins of agriculture, these non-weed relatives often still  
215 exist and can be leveraged through population genomic and comparative genomic approaches to  
216 identify the adaptive changes that have driven the evolution of weediness. The ability to rapidly  
217 adapt, persist, and spread in agroecosystems are defining features of weedy plants, leading many  
218 to advocate agricultural weeds as ideal candidates for studying rapid plant adaptation [33-36].  
219 The insights gained from applying plant ecological approaches to the study of rapid weed  
220 adaptation will move us towards the ultimate goals of mitigating such adaptation and increasing  
221 the efficacy of crop breeding and biotechnology [14].

222

### 223 *Biology and ecological genomics of weeds*

224 The impressive community effort to create and maintain resources for *Arabidopsis thaliana*  
225 ecological genomics provides a motivating example for the emerging study of weed genomics  
226 [37-40]. *Arabidopsis thaliana* was the first flowering plant species to have its genome fully  
227 sequenced [41] and rapidly became a model organism for plant molecular biology. As weedy

228 genomes become available, collection, maintenance, and resequencing of globally distributed  
229 accessions of these species will help to replicate the success found in ecological studies of *A.*  
230 *thaliana* [42-48]. Evaluation of these accessions for traits of interest to produce large phenomics  
231 data sets (as in [49-53]) enables genome-wide association studies and population genomics  
232 analyses aimed at dissecting the genetic basis of variation in such traits [54]. Increasingly, these  
233 resources (e.g the 1001 genomes project [42]) have enabled *A. thaliana* to be utilized as a model  
234 species to explore the eco-evolutionary basis of plant adaptation in a more realistic ecological  
235 context. Weedy species should supplement lessons in eco-evolutionary genomics learned from  
236 these experiments in *A. thaliana*.

237 Untargeted genomic approaches for understanding the evolutionary trajectories of  
238 populations and the genetic basis of traits as described above rely on the collection of genotypic  
239 information from across the genome of many individuals. While whole-genome resequencing  
240 accomplishes this requirement and requires no custom methodology, this approach provides  
241 more information than is necessary and is prohibitively expensive in species with large genomes.  
242 Development and optimization of genotype-by-sequencing methods for capturing reduced  
243 representations of newly sequenced genomes like those described by [55-57] will reduce the cost  
244 and computational requirements of genetic mapping and population genetic experiments.  
245 Additionally, the species sequenced by the IWGC do not currently have protocols for stable  
246 transformation, a key development in the popularity of *A. thaliana* as a model organism and a  
247 requirement for many functional genomic approaches. Functional validation of genes/variants  
248 believed to be responsible for traits of interest in weeds has thus far relied on transiently  
249 manipulating endogenous gene expression [58, 59] or ectopic expression of a transgene in a  
250 model system [60-62]. While these methods have been successful, few weed species have well-

251 studied viral vectors to adapt for use in virus induced gene silencing and spray induced gene  
252 silencing is relatively ineffective without the use of nanocarriers [63], which require specialized  
253 equipment and expertise. Furthermore, traits with complex genetic architecture divergent  
254 between the researched and model species may not be amenable to functional genomic  
255 approaches using transgenesis techniques in model systems. Developing protocols for reduced  
256 representation sequencing, stable transformation, and gene editing/silencing in weeds will allow  
257 for more thorough characterization of candidate genetic variants underlying traits of interest.

258 Beyond rapid adaptation, some weedy species offer an opportunity to better understand co-  
259 evolution, like that between plants and pollinators and how their interaction leads to the spread of  
260 weedy alleles (Additional File 2). A suite of plant-insect traits has co-evolved to maximize the  
261 attraction of the insect pollinator community and the efficiency of pollen deposition between  
262 flowers ensuring fruit and seed production in many weeds [64, 65]. Genetic mapping  
263 experiments have identified genes and genetic variants responsible for many floral traits  
264 affecting pollinator interaction including petal color [66-69], flower symmetry and size [70-72],  
265 and production of volatile organic compounds [73-75] and nectar [76-78]. While these studies  
266 reveal candidate genes for selection under co-evolution, herbicide resistance alleles may also  
267 have pleiotropic effects on the ecology of weeds [79], altering plant-pollinator interactions [80].

268 Discovery of genes and genetic variants involved in weed-pollinator interaction and their  
269 molecular and environmental control may create opportunities for better management of weeds  
270 with insect-mediated pollination. For example, if management can disrupt pollinator  
271 attraction/interaction with these weeds, the efficiency of reproduction may be reduced.

272 A more complete understanding of weed ecological genomics will undoubtedly elucidate  
273 many unresolved questions regarding the genetic basis of various aspects of weediness. For

274 instance, when comparing populations of a species from agricultural and non-agricultural  
275 environments, is there evidence for contemporary evolution of weedy traits selected by  
276 agricultural management or were ‘natural’ populations pre-adapted to agroecosystems? Where  
277 there is differentiation between weedy and natural populations, which traits are under selection  
278 and what is the genetic basis of variation in those traits? When comparing between weedy  
279 populations, is there evidence for parallel versus non-parallel evolution of weediness at the  
280 phenotypic and genotypic levels? Such studies may uncover fundamental truths about weediness.  
281 For example, is there a common phenotypic and/or genotypic basis for aspects of weediness  
282 amongst diverse weed species? As genomic tools developed by the IWGC enable researchers to  
283 address these questions, knowledge gained will help predict the potential development of newly  
284 important weed species in new environments and cropping systems.

285

286 *Population and Comparative Genomics*

287 A fundamental attribute of locally adaptive genetic variation is that adaptive alleles are  
288 overrepresented in their home environment relative to elsewhere [81]. This is a key motivation of  
289 genotype-by-environment association (GEA) and selective sweep scan approaches, which allow  
290 researchers to resolve the molecular basis of multi-dimensional adaptation [82, 83]. GEA  
291 approaches, in particular, have been widely used on landscape-wide resequencing collections to  
292 quantify the genetic basis of climate adaptation (e.g., [40, 84, 85]), but have yet to be fully  
293 exploited to diagnose the genetic basis of the various aspects of weediness [86]. Armed with data  
294 on environmental dimensions of agricultural settings, such as focal crop, soil quality, herbicide  
295 use, and climate, GEA approaches can help disentangle how discrete farming practices have  
296 influenced the evolution of weediness and resolve broader patterns of local adaptation across a

297 weed's range. Although non-weedy relatives are not technically required for GEA analyses,  
298 inclusion of environmental and genomic data from weed progenitors can further distinguish  
299 genetic variants underpinning weed origins from those involved in local adaptation.

300 Even without environmental or phenotypic data, the application of selective sweep scan  
301 approaches to comparisons of weed and non-weed relatives facilitates powerful inference of  
302 weed adaptation on micro- or macro-evolutionary scales. Two recent within-species examples  
303 include weedy rice, where population differentiation between weedy and domesticated  
304 populations was used to identify the genetic basis of weedy de-domestication [87], and common  
305 waterhemp, where consistent allelic differences among natural and agricultural collections  
306 resolved a complex set of agriculturally adaptive alleles [88, 89]. A recent comparative  
307 population genomic study of weedy barnyardgrass and crop millet species has demonstrated how  
308 inter-specific investigations can resolve the signatures of crop and weed evolution [90] (also see  
309 [91] for a non-weed climate adaptation example). Multiple sequence alignments across numerous  
310 species provide complementary insight into adaptive convergence over deeper timescales, even  
311 with just one genomic sample per species (e.g., [92, 93]). Thus, the new IWGC weed genomes  
312 combined with genomes available for closely related crops (outlined by [14, 94]) and an effort to  
313 identify other non-weed wild relatives will be invaluable in characterizing the genetic  
314 architecture of weed adaptation and evolution across diverse species.

315

316 *Herbicide resistance*

317 Herbicide resistance is among the numerous weedy traits that can evolve in plant populations  
318 exposed to agricultural selection pressures. Over-reliance on herbicides to control weeds, along  
319 with low diversity and lack of redundancy in weed management strategies, has resulted in

320 globally widespread herbicide resistance [95]. To date, 268 herbicide-resistant weed species have  
321 been reported worldwide, and at least one resistance case exists for 21 of the 31 existing  
322 herbicide sites of action [24] – significantly limiting chemical weed control options available to  
323 agriculturalists. This limitation of control options is exacerbated by the recent lack of discovery  
324 of herbicides with new sites of action [96].

325 Herbicide resistance may result from several different physiological mechanisms. Such  
326 mechanisms have been classified into two main groups, target-site resistance (TSR) [4, 97] and  
327 non-target-site resistance (NTSR) [4, 98]. The first group encompasses changes that reduce  
328 binding affinity between a herbicide and its target [99]. These changes may provide resistance to  
329 multiple herbicides that have a common biochemical target [100] and can be effectively  
330 managed through mixture and/or rotation of herbicides targeting different sites of action [101].  
331 The second group (NTSR), includes alterations in herbicide absorption, translocation,  
332 sequestration, and/or metabolism that may lead to unpredictable pleotropic cross-resistance  
333 profiles where structurally and functionally diverse herbicides are rendered ineffective by one or  
334 more genetic variant(s) [60]. This mechanism of resistance threatens not only the efficacy of  
335 existing herbicidal chemistries, but also ones yet to be discovered. While TSR is well understood  
336 because of the ease of identification and molecular characterization of target site variants, NTSR  
337 mechanisms are significantly more challenging to research because they are often polygenic, and  
338 the resistance causing element(s) are not well understood [102].

339 Improving the current understanding of metabolic NTSR mechanisms is not an easy task,  
340 since genes of diverse biochemical functions are involved, many of which exist as extensive  
341 gene families [100, 103]. Expression changes of NTSR genes have been implicated in several  
342 resistance cases where the protein products of the genes are functionally equivalent across

343 sensitive and resistant plants, but their relative abundance leads to resistance. Thus, regulatory  
344 elements of NTSR genes have been scrutinized to understand their role in NTSR mechanisms  
345 [104]. Similarly, epigenetic modifications have been hypothesized to play a role in NTSR, with  
346 much remaining to be explored [105-107]. Untargeted approaches such as genome-wide  
347 association, selective sweep scans, linkage mapping, RNA-sequencing, and metabolomic  
348 profiling have proven helpful to complement more specific biochemical- and chemo-  
349 characterization studies towards the elucidation of NTSR mechanisms as well as their regulation  
350 and evolution [60, 108-115]. Due to their complexity and importance, the IWGC has begun  
351 addressing this subject by manually curating the annotation of NTSR genes and developing a  
352 standard nomenclature for the gene families often involved in NTSR. This standardization will  
353 allow researchers to quickly identify true orthologous genes between weedy species, which is a  
354 hurdle for current research of these complex and often vast gene families.

355 High-quality weed genome assemblies and gene model annotations have helped and will be  
356 crucial for investigating the landscape of NTSR genes in weeds. They can also be used to predict  
357 the protein structure for herbicide target site and metabolism genes to predict the efficacy and  
358 selectivity of new candidate herbicides *in silico* to increase herbicide discovery throughput.

359 Knowledge of the genetic basis of NTSR will aid the rational design of herbicides by 1)  
360 screening new compounds in the presence of newly discovered NTSR proteins during early  
361 research phases; 2) identifying conserved chemical structures that interact with these proteins;  
362 and 3) optimizing herbicide molecular design to lower potential for resistance evolution and  
363 increase potency/spectrum of control.

364 Moving forward, genomic resources will be increasingly needed and used not only for the  
365 design of conventional small molecule herbicides, but also for next generation technologies for

366 sustainable weed management. Proteolysis targeting chimeras (PROTACs) have the potential to  
367 bind desired targets with great selectivity and degrade proteins by utilizing natural protein  
368 ubiquitination and degradation pathways within plants [116]. The combination of nanoparticles  
369 with oligonucleotides has recently shown potential to be used in spray applications towards gene  
370 silencing in weeds, which paves the way for a new, innovative, and sustainable method for weed  
371 management [117, 118]. Additionally, success in the field of pharmaceutical drug discovery in  
372 the development of molecules modulating protein-protein interactions offers another potential  
373 avenue towards the development of herbicides with novel targets [119, 120]. High-quality  
374 genomic references allow for the design of new weed management technologies like the ones  
375 listed here that are specific to – and effective across – weed species but have a null effect on non-  
376 target organisms. The tools being developed by the IWGC will have a crucial role in enabling the  
377 development of next generation weed management strategies that will reduce our reliance on the  
378 few chemical control options currently available to agriculturalists.

379

#### 380 *Genome Biology*

381 The genomes of weed species are as diverse as weed species themselves. Many weed species  
382 belong to unique plant families with no phylogenetically close model or crop species relatives for  
383 comparison. On all measurable metrics, weed genomes run the gamut. Some have smaller  
384 genomes like *Cyperus* spp. (~0.26 Gb) while others are larger, such as *Avena fatua* (~11.1 Gb)  
385 (Table 1). Some have high heterozygosity in terms of single nucleotide polymorphisms,  
386 repetitive DNA, and structural variants, such as the *Amaranthus* spp., while others are primarily  
387 self-pollinated and quite homozygous, such as *Poa annua* [121, 122]. Some are diploid such as  
388 *Conyza canadensis* and *Echinochloa haploclada* while others are polyploid such as *C.*

389 *sumetrensis*, *E. crus-galli*, and *E. colona* [90]. The availability of genomic resources in these  
390 diverse, unexplored branches of the tree of life allows us to identify consistencies and anomalies  
391 in the field of genome biology.

392 The weed genomes published so far have focused mainly on weeds of agronomic crops, and  
393 studies have revolved around their ability to resist key herbicides. For example, genomic  
394 resources were vital in the elucidation of herbicide resistance cases involving target site gene  
395 copy number variants (CNVs). Gene CNVs of 5-enolpyruvylshikimate-3-phosphate synthase  
396 (*EPSPS*) have been found to confer resistance to the herbicide glyphosate in diverse weed  
397 species. To date, nine species have independently evolved *EPSPS* CNVs, and species achieve  
398 increased *EPSPS* copy number via different mechanisms [123]. For instance, the *EPSPS* CNV in  
399 *Bassia scoparia* is caused by tandem duplication, which is accredited to transposable element  
400 insertions flanking *EPSPS* and subsequent unequal crossing over events [124, 125]. In *Eleusine*  
401 *indica*, a *EPSPS* CNV was caused by translocation of the *EPSPS* locus into the subtelomere  
402 followed by telomeric sequence exchange [126]. One of the most fascinating genome biology  
403 discoveries in weed science has been that of extra-chromosomal circular DNAs (eccDNAs) that  
404 harbor the *EPSPS* gene in the weed species *Amaranthus palmeri* [127, 128]. In this case, the  
405 eccDNAs autonomously replicate separately from the nuclear genome and do not reintegrate into  
406 chromosomes, which has implications for inheritance, fitness, and genome structure [129]. These  
407 discoveries would not have been possible without reference assemblies of weed genomes, next-  
408 generation sequencing, and collaboration with experts in plant genomics and bioinformatics.

409 Another question that is often explored with weedy genomes is the nature and composition of  
410 gene families that are associated with NTSR. Gene families under consideration often include  
411 cytochrome P450s (CYPs), glutathione-S-transferases (GSTs), ABC transporters, etc. Some

412 questions commonly considered with new weed genomes include: how many genes are in each  
413 of these gene families, where are they located, and which weed accessions and species have an  
414 over-abundance of them that might explain their ability to evolve resistance so rapidly [19, 90,  
415 130, 131]? Weed genome resources are necessary to answer questions about gene family  
416 expansion or contraction during the evolution of weediness, including the role of polyploidy in  
417 NTSR gene family expansion as explored by [132].

418

419 *Translational Research and Communication with Weed Management Stakeholders*

420 Whereas genomics of model plants is typically aimed at addressing fundamental questions in  
421 plant biology, and genomics of crop species has the obvious goal of crop improvement, goals of  
422 genomics of weedy plants also include the development of more effective and sustainable  
423 strategies for their management. Weed genomics assists with these objectives by providing novel  
424 molecular ecological and evolutionary insights from the context of intensive anthropogenic  
425 management (which is lacking in model plants), and offers knowledge and resources for trait  
426 discovery for crop improvement, especially given that many wild crop relatives are also  
427 important agronomic weeds (e.g. [133]). For instance, crop-wild relatives are valuable for  
428 improving crop breeding for marginal environments [134]. Thus, weed genomics presents unique  
429 opportunities and challenges relative to plant genomics more broadly. It should also be noted that  
430 although weed science at its core is a very applied discipline, it draws broadly from many  
431 scientific disciplines such as, plant physiology, chemistry, ecology, and evolutionary biology, to  
432 name a few. The successful integration of weed-management strategies, therefore, requires  
433 extensive collaboration among individuals collectively possessing the necessary expertise [135].  
434 Consequently, a major objective of the IWGC is to ensure that basic findings arising from weed

435 genomics are translated to advances in weed management and crop breeding by collaborating  
436 broadly with breeders, applied weed scientists, outreach specialists, and practitioners.

437 To accomplish this objective, the IWGC must facilitate communication of weed genomics  
438 findings to relevant stakeholders (Figure 1). With the growing complexity of herbicide resistance  
439 management, practitioners are beginning to recognize the importance of understanding resistance  
440 mechanisms to inform appropriate management tactics [14]. Although weed science practitioners  
441 do not need to understand the technical details of weed genomics, their appreciation of the power  
442 of weed genomics - together with their unique insights from field observations - will yield novel  
443 opportunities for applications of weed genomics to weed management. In particular, combining  
444 field management history with information on weed resistance mechanisms is expected to  
445 provide novel insights into evolutionary trajectories [e.g., 6, 136], which can be utilized for  
446 disrupting evolutionary adaptation. It can be difficult to obtain field history information from  
447 practitioners, but developing an understanding among them of the importance of such  
448 information can be invaluable. To address these aspects, the IWGC can provide funding, or at  
449 least coordinate teams, to build extension/education programs focused on weed genomics.  
450 Factsheets and easy-to-understand infographics can be developed and communicated to various  
451 stakeholders through traditional and electronic media.

452

## 453 **Conclusions**

454 Weeds are unique and fascinating plants, having significant impacts on agriculture and  
455 ecosystems; and yet, aspects of their biology, ecology, and genetics remain poorly understood.  
456 Weeds represent a unique area within plant biology, given their repeated rapid adaptation to  
457 sudden and severe shifts in the selective landscape of anthropogenic management practices. The

458 production of a public genomics database with reference genomes for over 50 weed species  
459 represents a substantial step forward towards research goals that improve our understanding of  
460 the biology and evolution of weeds. Future work is needed to improve annotations, particularly  
461 for complex gene families involved in herbicide detoxification, structural variants, and mobile  
462 genetic elements, given the evidence to date of the generation of adaptive genetic variation in  
463 weeds through structural variation. As reference genome assemblies become available; standard,  
464 affordable methods for gathering genotype information will allow for the identification of  
465 genetic variants underlying traits of interest. Further, development of methods for functional  
466 gene validation and hypothesis testing is needed in weeds to validate the effect of genetic  
467 variants detected through such experiments, including systems for transformation, gene editing,  
468 and transient gene silencing and expression. Future research should focus on utilizing weed  
469 genomes to investigate questions about the evolutionary biology, ecology, and genetics of weedy  
470 traits and weed population dynamics. The IWGC plans to continue the public-private partnership  
471 model to continue to host the WeedPedia database, integrate new datasets such as genome  
472 resequencing and transcriptomes, conduct trainings, and serve as a research coordination  
473 network to ensure that advances in weed science from around the world are shared across the  
474 research community (Figure 1). Bridging basic plant genomics with translational applications in  
475 weeds is needed to deliver on the potential of weed genomics to improve weed management and  
476 crop breeding.

477

## 478 **Availability of data and materials**

479 The datasets supporting the conclusions of this article is included within the article and its  
480 additional files.

481

482 **Competing interests**

483 The authors declare that they have no competing interests.

484

485 **Acknowledgements**

486 The International Weed Genomics Consortium is supported by BASF SE, Bayer AG, Syngenta  
487 Ltd, Corteva Agriscience, CropLife International, the Foundation for Food and Agriculture  
488 Research (Award DSnew-024), and a conference grant from USDA-NIFA (Award number 2021-  
489 67013-33570).

490

491 **References**

- 492 1. Gianessi LP, Nathan PR. The Value of Herbicides in U.S. Crop Production. *Weed Technol.* 2007;21(2):559-66.
- 494 2. Pimentel D, Lach L, Zuniga R, Morrison D. Environmental and Economic Costs of  
495 Nonindigenous Species in the United States. *BioScience.* 2000;50(1):53-65.
- 496 3. Barrett SH. Crop mimicry in weeds. *Econ Bot.* 1983;37(3):255-82.
- 497 4. Powles SB, Yu Q. Evolution in action: plants resistant to herbicides. *Annu Rev Plant  
498 Biol.* 2010;61:317-47.
- 499 5. Thurber CS, Reagon M, Gross BL, Olsen KM, Jia Y, Caicedo AL. Molecular evolution  
500 of shattering loci in U.S. weedy rice. *Mol Ecol.* 2010;19(16):3271-84.
- 501 6. Comont D, Lowe C, Hull R, Crook L, Hicks HL, Onkokesung N, et al. Evolution of  
502 generalist resistance to herbicide mixtures reveals a trade-off in resistance management. *Nat  
503 Commun.* 2020;11(1):3086.

504 7. Ashworth MB, Walsh MJ, Flower KC, Vila-Aiub MM, Powles SB. Directional selection  
505 for flowering time leads to adaptive evolution in *Raphanus raphanistrum* (wild radish). *Evol  
506 Appl.* 2016;9(4):619-29.

507 8. Chan EK, Rowe HC, Kliebenstein DJ. Understanding the evolution of defense  
508 metabolites in *Arabidopsis thaliana* using genome-wide association mapping. *Genetics.*  
509 2010;185(3):991-1007.

510 9. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, et al. A  
511 common variant in the *FTO* gene is associated with body mass index and predisposes to  
512 childhood and adult obesity. *Science.* 2007;316(5826):889-94.

513 10. Harkess A, Zhou J, Xu C, Bowers JE, Van der Hulst R, Ayyampalayam S, et al. The  
514 asparagus genome sheds light on the origin and evolution of a young Y chromosome. *Nat  
515 Commun.* 2017;8(1):1279.

516 11. Periyannan S, Moore J, Ayliffe M, Bansal U, Wang X, Huang L, et al. The gene *Sr33*, an  
517 ortholog of barley *Mla* genes, encodes resistance to wheat stem rust race Ug99. *Science.*  
518 2013;341(6147):786-8.

519 12. Ågren J, Oakley CG, McKay JK, Lovell JT, Schemske DW. Genetic mapping of  
520 adaptation reveals fitness tradeoffs in *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A.*  
521 2013;110(52):21077-82.

522 13. Schartl M, Walter RB, Shen Y, Garcia T, Catchen J, Amores A, et al. The genome of the  
523 platyfish, *Xiphophorus maculatus*, provides insights into evolutionary adaptation and several  
524 complex traits. *Nat Genet.* 2013;45(5):567-72.

525 14. Ravet K, Patterson EL, Krähmer H, Hamouzová K, Fan L, Jasieniuk M, et al. The power  
526 and potential of genomics in weed biology and management. Pest Manag Sci. 2018;74(10):2216-  
527 25.

528 15. Hufford MB, Seetharam AS, Woodhouse MR, Chougule KM, Ou S, Liu J, et al. *De novo*  
529 assembly, annotation, and comparative analysis of 26 diverse maize genomes. Science.  
530 2021;373(6555):655-62.

531 16. Liao W-W, Asri M, Ebler J, Doerr D, Haukness M, Hickey G, et al. A draft human  
532 pangenome reference. Nature. 2023;617(7960):312-24.

533 17. Huang Y, Wu D, Huang Z, Li X, Merotto A, Bai L, et al. Weed genomics: yielding  
534 insights into the genetics of weedy traits for crop improvement. aBIOTECH. 2023;4:20-30.

535 18. Bennetzen JL, Schmutz J, Wang H, Percifield R, Hawkins J, Pontaroli AC, et al.  
536 Reference genome sequence of the model plant *Setaria*. Nat Biotechnol. 2012;30(6):555-61.

537 19. Kreiner JM, Giacomini DA, Bemm F, Waithaka B, Regalado J, Lanz C, et al. Multiple  
538 modes of convergent adaptation in the spread of glyphosate-resistant *Amaranthus tuberculatus*.  
539 Proc Natl Acad Sci U S A. 2019;116(42):21076-84.

540 20. Yu Y, Hu H, Doust AN, Kellogg EA. Divergent gene expression networks underlie  
541 morphological diversity of abscission zones in grasses. New Phytol. 2020;225(4):1799-815.

542 21. International Weed Genomics Consortium Website. [weedgenomics.org](http://weedgenomics.org). Accessed 20  
543 June 2023.

544 22. USDA-ARS Germplasm Resources Information Network (GRIN). [www.ars-grin.gov](http://www.ars-grin.gov).  
545 Accessed 20 June 2023.

546 23. Buck M, Hamilton C. The Nagoya Protocol on Access to Genetic Resources and the Fair  
547 and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological

548 Diversity. Review of European Community & International Environmental Law. 2011;20(1):47-  
549 61.

550 24. The International Herbicide-Resistant Weed Database. [www.weedscience.org](http://www.weedscience.org). Accessed  
551 20 June 2023.

552 25. Chauhan BS, Matloob A, Mahajan G, Aslam F, Florentine SK, Jha P. Emerging  
553 challenges and opportunities for education and research in weed science. Front Plant Sci.  
554 2017;(8):1537.

555 26. Shah S, Lonhienne T, Murray CE, Chen Y, Dougan KE, Low YS, et al. Genome-Guided  
556 Analysis of Seven Weed Species Reveals Conserved Sequence and Structural Features of Key  
557 Gene Targets for Herbicide Development. Front Plant Sci. 2022;13:909073.

558 27. International Weed Genomics Consortium Training Resources.  
559 [www.weedgenomics.org/training-resources](http://www.weedgenomics.org/training-resources). Accessed 20 June 2023.

560 28. Blackford S. Harnessing the power of communities: career networking strategies for  
561 bioscience PhD students and postdoctoral researchers. FEMS Microbiol Lett. 2018;365(8).

562 29. Pender M, Marcotte DE, Sto Domingo MR, Maton KI. The STEM Pipeline: The Role of  
563 Summer Research Experience in Minority Students' Ph.D. Aspirations. Educ Policy Anal Arch.  
564 2010;18(30):1-36.

565 30. Burke A, Okrent A, Hale K. The State of U.S. Science and Engineering 2022. National  
566 Science Foundation. <https://ncses.nsf.gov/pubs/nsb20221>. 2022.

567 31. Wu J-Y, Liao C-H, Cheng T, Nian M-W. Using Data Analytics to Investigate Attendees'  
568 Behaviors and Psychological States in a Virtual Academic Conference. Educ Technol Soc.  
569 2021;24(1):75-91.

570 32. De Wet JMJ, Harlan JR. Weeds and domesticates: Evolution in the man-made habitat.

571 Econ Bot. 1975;29(2):99-108.

572 33. Mahaut L, Cheptou PO, Fried G, Munoz F, Storkey J, Vasseur F, et al. Weeds: Against

573 the Rules? Trends Plant Sci. 2020;25(11):1107-16.

574 34. Neve P, Vila-Aiub M, Roux F. Evolutionary-thinking in agricultural weed management.

575 New Phytol. 2009;184(4):783-93.

576 35. Sharma G, Barney JN, Westwood JH, Haak DC. Into the weeds: new insights in plant

577 stress. Trends Plant Sci. 2021;26(10):1050-60.

578 36. Vigueira CC, Olsen KM, Caicedo AL. The red queen in the corn: agricultural weeds as

579 models of rapid adaptive evolution. Heredity (Edinb). 2013;110(4):303-11.

580 37. Donohue K, Dorn L, Griffith C, Kim E, Aguilera A, Polisetty CR, et al. Niche

581 construction through germination cueing: life-history responses to timing of germination in

582 *Arabidopsis thaliana*. Evolution. 2005;59(4):771-85.

583 38. Exposito-Alonso M. Seasonal timing adaptation across the geographic range of

584 *Arabidopsis thaliana*. Proc Natl Acad Sci U S A. 2020;117(18):9665-67.

585 39. Fournier-Level A, Korte A, Cooper MD, Nordborg M, Schmitt J, Wilczek AM. A map of

586 local adaptation in *Arabidopsis thaliana*. Science. 2011;334(6052):86-9.

587 40. Hancock AM, Brachi B, Faure N, Horton MW, Jarymowycz LB, Sperone FG, et al.

588 Adaptation to climate across the *Arabidopsis thaliana* genome. Science. 2011;334(6052):83-6.

589 41. TAG Initiative. Analysis of the genome sequence of the flowering plant *Arabidopsis*

590 *thaliana*. Nature. 2000;408(6814):796-815.

591 42. Alonso-Blanco C, Andrade J, Becker C, Bemm F, Bergelson J, Borgwardt KM, et al.  
592 1,135 Genomes reveal the global pattern of polymorphism in *Arabidopsis thaliana*. *Cell*.  
593 2016;166(2):481-91.

594 43. Durvasula A, Fulgione A, Gutaker RM, Alacakaptan SI, Flood PJ, Neto C, et al. African  
595 genomes illuminate the early history and transition to selfing in *Arabidopsis thaliana*. *Proc Natl  
596 Acad Sci U S A*. 2017;114(20):5213-18.

597 44. Frachon L, Mayjonade B, Bartoli C, Hautekèete N-C, Roux F. Adaptation to plant  
598 communities across the genome of *Arabidopsis thaliana*. *Mol Biol Evol*. 2019;36(7):1442-56.

599 45. Fulgione A, Koornneef M, Roux F, Hermisson J, Hancock AM. Madeiran *Arabidopsis  
600 thaliana* reveals ancient long-range colonization and clarifies demography in Eurasia. *Mol Biol  
601 Evol*. 2018;35(3):564-74.

602 46. Fulgione A, Neto C, Elfarargi AF, Tergemina E, Ansari S, Göktay M, et al. Parallel  
603 reduction in flowering time from *de novo* mutations enable evolutionary rescue in colonizing  
604 lineages. *Nat Commun*. 2022;13(1):1461.

605 47. Kasulin L, Rowan BA, León RJC, Schuenemann VJ, Weigel D, Botto JF. A single  
606 haplotype hyposensitive to light and requiring strong vernalization dominates *Arabidopsis  
607 thaliana* populations in Patagonia, Argentina. *Mol Ecol*. 2017;26(13):3389-404.

608 48. Picó FX, Méndez-Vigo B, Martínez-Zapater JM, Alonso-Blanco C. Natural genetic  
609 variation of *Arabidopsis thaliana* is geographically structured in the Iberian Peninsula. *Genetics*.  
610 2008;180(2):1009-21.

611 49. Atwell S, Huang YS, Vilhjálmsson BJ, Willems G, Horton M, Li Y, et al. Genome-wide  
612 association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. *Nature*.  
613 2010;465(7298):627-31.

614 50. Flood PJ, Kruijer W, Schnabel SK, van der Schoor R, Jalink H, Snel JFH, et al.

615 Phenomics for photosynthesis, growth and reflectance in *Arabidopsis thaliana* reveals circadian

616 and long-term fluctuations in heritability. *Plant Methods*. 2016;12(1):14.

617 51. Marchadier E, Hanemian M, Tisné S, Bach L, Bazakos C, Gilbault E, et al. The complex

618 genetic architecture of shoot growth natural variation in *Arabidopsis thaliana*. *PLoS Genet*.

619 2019;15(4):e1007954.

620 52. Tisné S, Serrand Y, Bach L, Gilbault E, Ben Ameur R, Balasse H, et al. Phenoscope: an

621 automated large-scale phenotyping platform offering high spatial homogeneity. *Plant J*.

622 2013;74(3):534-44.

623 53. Tschiersch H, Junker A, Meyer RC, Altmann T. Establishment of integrated protocols for

624 automated high throughput kinetic chlorophyll fluorescence analyses. *Plant Methods*.

625 2017;13:54.

626 54. Chen X, MacGregor DR, Stefanato FL, Zhang N, Barros-Galvão T, Penfield S. A VEL3

627 histone deacetylase complex establishes a maternal epigenetic state controlling progeny seed

628 dormancy. *Nat Commun*. 2023;14(1):2220.

629 55. Choi M, Scholl UI, Ji W, Liu T, Tikhonova IR, Zumbo P, et al. Genetic diagnosis by

630 whole exome capture and massively parallel DNA sequencing. *Proc Natl Acad Sci U S A*.

631 2009;106(45):19096-101.

632 56. Davey JW, Blaxter ML. RADSeq: next-generation population genetics. *Brief Funct*

633 *Genomics*. 2010;9(5-6):416-23.

634 57. Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, et al. A robust,

635 simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One*.

636 2011;6(5):e19379.

637 58. MacGregor DR. What makes a weed a weed? How virus-mediated reverse genetics can  
638 help to explore the genetics of weediness. *Outlooks Pest Manag.* 2020;31(5):224-29.

639 59. Mellado-Sánchez M, McDiarmid F, Cardoso V, Kanyuka K, MacGregor DR. Virus-  
640 mediated transient expression techniques enable gene function studies in blackgrass. *Plant*  
641 *Physiol.* 2020;183(2):455-59.

642 60. Dimaano NG, Yamaguchi T, Fukunishi K, Tominaga T, Iwakami S. Functional  
643 characterization of Cytochrome P450 *CYP81A* subfamily to disclose the pattern of cross-  
644 resistance in *Echinochloa phylloplagon*. *Plant Mol Biol.* 2020;102(4-5):403-16.

645 61. de Figueiredo MRA, Küpper A, Malone JM, Petrovic T, de Figueiredo ABTB,  
646 Campagnola G, et al. An in-frame deletion mutation in the degron tail of auxin coreceptor IAA2  
647 confers resistance to the herbicide 2,4-D in *Sisymbrium orientale*. *Proc Natl Acad Sci U S A.*  
648 2022;119(9):e2105819119.

649 62. Patzoldt WL, Hager AG, McCormick JS, Tranel PJ. A codon deletion confers resistance  
650 to herbicides inhibiting protoporphyrinogen oxidase. *Proc Natl Acad Sci U S A.*  
651 2006;103(33):12329-34.

652 63. Schwartz SH, Hendrix B, Hoffer P, Sanders RA, Zheng W. Carbon dots for efficient  
653 small interfering RNA delivery and gene silencing in plants. *Plant Physiol.* 2020;184(2):647-57.

654 64. Fattorini R, Glover BJ. Molecular mechanisms of pollination biology. *Annu Rev Plant*  
655 *Biol.* 2020;71:487-515.

656 65. Rollin O, Benelli G, Benvenuti S, Decourtey A, Wratten SD, Canale A, et al. Weed-  
657 insect pollinator networks as bio-indicators of ecological sustainability in agriculture. A review.  
658 *Agron Sustain Dev.* 2016;36(1):8.

659 66. Irwin RE, Strauss SY. Flower color microevolution in wild radish: evolutionary response  
660 to pollinator-mediated selection. *Am Nat.* 2005;165(2):225-37.

661 67. Ma B, Wu J, Shi T-L, Yang Y-Y, Wang W-B, Zheng Y, et al. Lilac (*Syringa oblata*)  
662 genome provides insights into its evolution and molecular mechanism of petal color change.  
663 *Commun Biol.* 2022;5(1):686.

664 68. Xing A, Wang X, Nazir MF, Zhang X, Wang X, Yang R, et al. Transcriptomic and  
665 metabolomic profiling of flavonoid biosynthesis provides novel insights into petals coloration in  
666 Asian cotton (*Gossypium arboreum* L.). *BMC Plant Biol.* 2022;22(1):416.

667 69. Zheng Y, Chen Y, Liu Z, Wu H, Jiao F, Xin H, et al. Important roles of key genes and  
668 transcription factors in flower color differences of *Nicotiana alata*. *Genes (Basel).*  
669 2021;12(12):1976.

670 70. Krizek BA, Anderson JT. Control of flower size. *J Exp Bot.* 2013;64(6):1427-37.

671 71. Powell AE, Lenhard M. Control of organ size in plants. *Curr Biol.* 2012;22(9):R360-7.

672 72. Spencer V, Kim M. Re"CYC"ling molecular regulators in the evolution and development  
673 of flower symmetry. *Semin Cell Dev Biol.* 2018;79:16-26.

674 73. Amrad A, Moser M, Mandel T, de Vries M, Schuurink RC, Freitas L, et al. Gain and loss  
675 of floral scent production through changes in structural genes during pollinator-mediated  
676 speciation. *Curr Biol.* 2016;26(24):3303-12.

677 74. Delle-Vedove R, Schatz B, Dufay M. Understanding intraspecific variation of floral scent  
678 in light of evolutionary ecology. *Ann Bot.* 2017;120(1):1-20.

679 75. Pichersky E, Gershenzon J. The formation and function of plant volatiles: perfumes for  
680 pollinator attraction and defense. *Curr Opin Plant Biol.* 2002;5(3):237-43.

681 76. Ballerini ES, Kramer EM, Hodges SA. Comparative transcriptomics of early petal  
682 development across four diverse species of *Aquilegia* reveal few genes consistently associated  
683 with nectar spur development. *BMC Genom.* 2019;20(1):668.

684 77. Corbet SA, Willmer PG, Beament JW, Unwin DM, Prys-Jones OE. Post-secretory  
685 determinants of sugar concentration in nectar. *Plant Cell Environ.* 1979;2(4):293-308.

686 78. Galliot C, Hoballah ME, Kuhlemeier C, Stuurman J. Genetics of flower size and nectar  
687 volume in petunia pollination syndromes. *Planta.* 2006;225(1):203-12.

688 79. Vila-Aiub MM, Neve P, Powles SB. Fitness costs associated with evolved herbicide  
689 resistance alleles in plants. *New Phytol.* 2009;184(4):751-67.

690 80. Baucom RS. Evolutionary and ecological insights from herbicide-resistant weeds: what  
691 have we learned about plant adaptation, and what is left to uncover? *New Phytol.*  
692 2019;223(1):68-82.

693 81. Kawecki TJ, Ebert D. Conceptual issues in local adaptation. *Ecol Lett.* 2004;7(12):1225-  
694 41.

695 82. Lasky JR, Josephs EB, Morris GP. Genotype–environment associations to reveal the  
696 molecular basis of environmental adaptation. *Plant Cell.* 2023;35(1):125-38.

697 83. Lotterhos KE. The effect of neutral recombination variation on genome scans for  
698 selection. *G3-Genes Genom Genet.* 2019;9(6):1851-67.

699 84. Lovell JT, MacQueen AH, Mamidi S, Bonnette J, Jenkins J, Napier JD, et al. Genomic  
700 mechanisms of climate adaptation in polyploid bioenergy switchgrass. *Nature.*  
701 2021;590(7846):438-44.

702 85. Todesco M, Owens GL, Bercovich N, Légaré J-S, Soudi S, Burge DO, et al. Massive  
703 haplotypes underlie ecotypic differentiation in sunflowers. *Nature.* 2020;584(7822):602-07.

704 86. Revolinski SR, Maughan PJ, Coleman CE, Burke IC. Preadapted to adapt: Underpinnings  
705 of adaptive plasticity revealed by the downy brome genome. *Commun Biol.* 2023;6:326.

706 87. Qiu J, Zhou Y, Mao L, Ye C, Wang W, Zhang J, et al. Genomic variation associated with  
707 local adaptation of weedy rice during de-domestication. *Nat Commun.* 2017;8(1):15323.

708 88. Kreiner JM, Caballero A, Wright SI, Stinchcombe JR. Selective ancestral sorting and *de*  
709 *novo* evolution in the agricultural invasion of *Amaranthus tuberculatus*. *Evolution.*  
710 2022;76(1):70-85.

711 89. Kreiner JM, Latorre SM, Burbano HA, Stinchcombe JR, Otto SP, Weigel D, et al. Rapid  
712 weed adaptation and range expansion in response to agriculture over the past two centuries.  
713 *Science.* 2022;378(6624):1079-85.

714 90. Wu D, Shen E, Jiang B, Feng Y, Tang W, Lao S, et al. Genomic insights into the  
715 evolution of *Echinochloa* species as weed and orphan crop. *Nat Commun.* 2022;13(1):689.

716 91. Yeaman S, Hodgins KA, Lotterhos KE, Suren H, Nadeau S, Degner JC, et al. Convergent  
717 local adaptation to climate in distantly related conifers. *Science.* 2016;353(6306):1431-33.

718 92. Haudry A, Platts AE, Vello E, Hoen DR, Leclercq M, Williamson RJ, et al. An atlas of  
719 over 90,000 conserved noncoding sequences provides insight into crucifer regulatory regions.  
720 *Nat Genet.* 2013;45(8):891-98.

721 93. Sackton TB, Grayson P, Cloutier A, Hu Z, Liu JS, Wheeler NE, et al. Convergent  
722 regulatory evolution and loss of flight in paleognathous birds. *Science.* 2019;364(6435):74-78.

723 94. Ye CY, Fan L. Orphan crops and their wild relatives in the genomic era. *Mol Plant.*  
724 2021;14(1):27-39.

725 95. Bourguet D, Delmotte F, Franck P, Guillemaud T, Reboud X, Vacher C, et al.  
726 Heterogeneity of selection and the evolution of resistance. *Trends Ecol Evol.* 2013;28(2):110-8.

727 96. Powles S. Herbicide discovery through innovation and diversity. *Adv Weed Sci.*  
728 2022;40(spe1): e020220074.

729 97. Murphy BP, Tranel PJ. Target-site mutations conferring herbicide resistance. *Plants*  
730 (Basel). 2019;8(10):382.

731 98. Gaines TA, Duke SO, Morran S, Rigon CAG, Tranel PJ, Küpper A, et al. Mechanisms of  
732 evolved herbicide resistance. *J Biol Chem.* 2020;295(30):10307-30.

733 99. Lonhienne T, Cheng Y, Garcia MD, Hu SH, Low YS, Schenk G, et al. Structural basis of  
734 resistance to herbicides that target acetohydroxyacid synthase. *Nat Commun.* 2022;13(1):3368.

735 100. Comont D, MacGregor DR, Crook L, Hull R, Nguyen L, Freckleton RP, et al. Dissecting  
736 weed adaptation: Fitness and trait correlations in herbicide-resistant *Alopecurus myosuroides*.  
737 *Pest Manag Sci.* 2022;78(7):3039-50.

738 101. Neve P. Simulation modelling to understand the evolution and management of glyphosate  
739 resistance in weeds. *Pest Manag Sci.* 2008;64(4):392-401.

740 102. Torra J, Alcántara-de la Cruz R. Molecular mechanisms of herbicide resistance in weeds.  
741 *Genes (Basel).* 2022;13(11):2025.

742 103. Délye C, Gardin JAC, Boucansaud K, Chauvel B, Petit C. Non-target-site-based  
743 resistance should be the centre of attention for herbicide resistance research: *Alopecurus*  
744 *myosuroides* as an illustration. *Weed Res.* 2011;51(5):433-37.

745 104. Chandra S, Leon RG. Genome-wide evolutionary analysis of putative non-specific  
746 herbicide resistance genes and compilation of core promoters between monocots and dicots.  
747 *Genes (Basel).* 2022;13(7):1171.

748 105. Margaritopoulou T, Tani E, Chachalis D, Travlos I. Involvement of epigenetic  
749 mechanisms in herbicide resistance: The case of *Conyza canadensis*. *Agriculture.* 2018;8(1):17.

750 106. Pan L, Guo Q, Wang J, Shi L, Yang X, Zhou Y, et al. *CYP81A68* confers metabolic  
751 resistance to ALS and ACCase-inhibiting herbicides and its epigenetic regulation in *Echinochloa*  
752 *crus-galli*. *J Hazard Mater.* 2022;428:128225.

753 107. Sen MK, Hamouzová K, Košnarová P, Roy A, Soukup J. Herbicide resistance in grass  
754 weeds: Epigenetic regulation matters too. *Front Plant Sci.* 2022;13:1040958.

755 108. Han H, Yu Q, Beffa R, González S, Maiwald F, Wang J, et al. Cytochrome P450  
756 *CYP81A10v7* in *Lolium rigidum* confers metabolic resistance to herbicides across at least five  
757 modes of action. *Plant J.* 2021;105(1):79-92.

758 109. Kubis GC, Marques RZ, Kitamura RS, Barroso AA, Juneau P, Gomes MP. Antioxidant  
759 enzyme and Cytochrome P450 activities are involved in horseweed (*Conyza sumatrensis*)  
760 resistance to glyphosate. *Stress.* 2023;3(1):47-57.

761 110. Qiao Y, Zhang N, Liu J, Yang H. Interpretation of ametryn biodegradation in rice based  
762 on joint analyses of transcriptome, metabolome and chemo-characterization. *J Hazard Mater.*  
763 2023;445:130526.

764 111. Rouse CE, Roma-Burgos N, Barbosa Martins BA. Physiological assessment of non-  
765 target site resistance in multiple-resistant junglerice (*Echinochloa colona*). *Weed Sci.*  
766 2019;67(6):622-32.

767 112. Abou-Khater L, Maalouf F, Jighly A, Alsamman AM, Rubiales D, Rispail N, et al.  
768 Genomic regions associated with herbicide tolerance in a worldwide faba bean (*Vicia faba* L.)  
769 collection. *Sci Rep.* 2022;12(1):158.

770 113. Gupta S, Harkess A, Soble A, Van Etten M, Leebens-Mack J, Baucom RS.  
771 Interchromosomal linkage disequilibrium and linked fitness cost loci associated with selection  
772 for herbicide resistance. *New Phytol.* 2023;238(3):1263-77.

773 114. Kreiner JM, Tranel PJ, Weigel D, Stinchcombe JR, Wright SI. The genetic architecture  
774 and population genomic signatures of glyphosate resistance in *Amaranthus tuberculatus*. *Mol*  
775 *Ecol.* 2021;30(21):5373-89.

776 115. Parcharidou E, Dücker R, Zöllner P, Ries S, Orru R, Beffa R. Recombinant glutathione  
777 transferases from flufenacet-resistant black-grass (*Alopecurus myosuroides* Huds.) form different  
778 flufenacet metabolites and differ in their interaction with pre- and post-emergence herbicides.  
779 *Pest Manag Sci.* 2023;in press.

780 116. Békés M, Langley DR, Crews CM. PROTAC targeted protein degraders: the past is  
781 prologue. *Nat Rev Drug Discov.* 2022;21(3):181-200.

782 117. Martin K, Gaines T, Aishwarya V. Pairing nanoparticles with FANA technology for  
783 spray-on gene silencing in weeds. *Weed Science Society of America Annual Meeting*; Arlington,  
784 VA. 2023.

785 118. Zabala-Pardo D, Gaines T, Lamego FP, Avila LA. RNAi as a tool for weed management:  
786 challenges and opportunities. *Adv Weed Sci.* 2022;40(spe1): e020220096.

787 119. Acuner Ozbabacan SE, Engin HB, Gursoy A, Keskin O. Transient protein-protein  
788 interactions. *Protein Eng Des Sel.* 2011;24(9):635-48.

789 120. Lu H, Zhou Q, He J, Jiang Z, Peng C, Tong R, et al. Recent advances in the development  
790 of protein-protein interactions modulators: mechanisms and clinical trials. *Signal Transduct*  
791 *Target Ther.* 2020;5(1):213.

792 121. Benson CW, Sheltra MR, Maughan PJ, Jellen EN, Robbins MD, Bushman BS, et al.  
793 Homoeologous evolution of the allotetraploid genome of *Poa annua* L. *BMC Genom.*  
794 2023;24(1):350.

795 122. Robbins MD, Bushman BS, Huff DR, Benson CW, Warnke SE, Maughan CA, et al.

796 Chromosome-scale genome assembly and annotation of allotetraploid annual bluegrass (*Poa*  
797 *annua* L.). *Genome Biol Evol*. 2022;15(1):evac180.

798 123. Patterson EL, Pettinga DJ, Ravet K, Neve P, Gaines TA. Glyphosate resistance and  
799 EPSPS gene duplication: Convergent evolution in multiple plant species. *J Hered*.  
800 2018;109(2):117-25.

801 124. Jugulam M, Niehues K, Godar AS, Koo DH, Danilova T, Friebe B, et al. Tandem  
802 amplification of a chromosomal segment harboring 5-enolpyruvylshikimate-3-phosphate  
803 synthase locus confers glyphosate resistance in *Kochia scoparia*. *Plant Physiol*.  
804 2014;166(3):1200-7.

805 125. Patterson EL, Saski CA, Sloan DB, Tranel PJ, Westra P, Gaines TA. The draft genome of  
806 *Kochia scoparia* and the mechanism of glyphosate resistance via transposon-mediated *EPSPS*  
807 tandem gene duplication. *Genome Biol Evol*. 2019;11(10):2927-40.

808 126. Zhang C, Johnson N, Hall N, Tian X, Yu Q, Patterson E. Subtelomeric 5-  
809 enolpyruvylshikimate-3-phosphate synthase (*EPSPS*) copy number variation confers glyphosate  
810 resistance in *Eleusine indica*. *Res Sq*. 2023;rs.3.rs-2587355.

811 127. Koo D-H, Molin WT, Saski CA, Jiang J, Putta K, Jugulam M, et al. Extrachromosomal  
812 circular DNA-based amplification and transmission of herbicide resistance in crop weed  
813 *Amaranthus palmeri*. *Proc Natl Acad Sci U S A*. 2018;115(13):3332-37.

814 128. Molin WT, Yaguchi A, Blenner M, Saski CA. The eccDNA replicon: A heritable,  
815 extranuclear vehicle that enables gene amplification and glyphosate resistance in *Amaranthus*  
816 *palmeri*. *Plant Cell*. 2020;32(7):2132-40.

817 129. Jugulam M. Can non-Mendelian inheritance of extrachromosomal circular DNA-  
818 mediated EPSPS gene amplification provide an opportunity to reverse resistance to glyphosate?  
819 Weed Res. 2021;61(2):100-05.

820 130. Wang L, Sun X, Peng Y, Chen K, Wu S, Guo Y, et al. Genomic insights into the origin,  
821 adaptive evolution, and herbicide resistance of *Leptochloa chinensis*, a devastating tetraploid  
822 weedy grass in rice fields. Mol Plant. 2022;15(6):1045-58.

823 131. Cai L, Comont D, MacGregor D, Lowe C, Beffa R, Neve P, et al. The blackgrass genome  
824 reveals patterns of non-parallel evolution of polygenic herbicide resistance. New Phytol.  
825 2023;237(5):1891-907.

826 132. Chen K, Yang H, Peng Y, Liu D, Zhang J, Zhao Z, et al. Genomic analyses provide  
827 insights into the polyploidization-driven herbicide adaptation in *Leptochloa* weeds. Plant  
828 Biotechnol J. 2023;in press.

829 133. Ohadi S, Hodnett G, Rooney W, Bagavathiannan M. Gene flow and its consequences in  
830 *Sorghum* spp. Crit Rev Plant Sci. 2017;36(5-6):367-85.

831 134. Renzi JP, Coyne CJ, Berger J, von Wettberg E, Nelson M, Ureta S, et al. How could the  
832 use of crop wild relatives in breeding increase the adaptation of crops to marginal environments?  
833 Front Plant Sci. 2022;13:886162.

834 135. Ward SM, Cousens RD, Bagavathiannan MV, Barney JN, Beckie HJ, Busi R, et al.  
835 Agricultural weed research: A critique and two proposals. Weed Sci. 2014;62(4):672-78.

836 136. Evans JA, Tranel PJ, Hager AG, Schutte B, Wu C, Chatham LA, et al. Managing the  
837 evolution of herbicide resistance. Pest Management Science. 2016;72(1):74-80.

838 137. Montgomery JS, Giacomini D, Waithaka B, Lanz C, Murphy BP, Campe R, et al. Draft  
839 genomes of *Amaranthus tuberculatus*, *Amaranthus hybridus* and *Amaranthus palmeri*. *Genome*  
840 *Biol Evol*. 2020;12:1988-93.

841 138. Jeschke MR, Tranell PJ, Rayburn AL. DNA content analysis of smooth pigweed  
842 (*Amaranthus hybridus*) and tall waterhemp (*A. tuberculatus*): implications for hybrid detection.  
843 *Weed Sci*. 2003;51(1):1-3.

844 139. Rayburn AL, McCloskey R, Tatum TC, Bollero GA, Jeschke MR, Tranell PJ. Genome  
845 size analysis of weedy *Amaranthus* species. *Crop Sci*. 2005;45(6):2557-62.

846 140. Mulligan GA. Chromosome numbers of Canadian weeds. I. *Canad J Bot*.  
847 1957;35(5):779-89.

848 141. Meyer L, Causse R, Pernin F, Scalone R, Bailly G, Chauvel B, et al. New gSSR and  
849 EST-SSR markers reveal high genetic diversity in the invasive plant *Ambrosia artemisiifolia* L.  
850 and can be transferred to other invasive *Ambrosia* species. *PLOS ONE*. 2017;12(5):e0176197.

851 142. Pustahija F, Brown SC, Bogunić F, Bašić N, Muratović E, Ollier S, et al. Small genomes  
852 dominate in plants growing on serpentine soils in West Balkans, an exhaustive study of 8  
853 habitats covering 308 taxa. *Plant Soil*. 2013;373(1):427-53.

854 143. Kubešová M, Moravcová L, Suda J, Jarošík V, Pyšek P. Naturalized plants have smaller  
855 genomes than their non-invading relatives: a flow cytometric analysis of the Czech alien flora.  
856 *Preslia*. 2010;82(1):81-96.

857 144. Thébaud C, Abbott RJ. Characterization of invasive *Conyza* species (Asteraceae) in  
858 Europe: Quantitative trait and isozyme analysis. *Am J Bot*. 1995;82(3):360-68.

859 145. Garcia S, Hidalgo O, Jakovljević I, Siljak-Yakovlev S, Vigo J, Garnatje T, et al. New  
860 data on genome size in 128 Asteraceae species and subspecies, with first assessments for 40  
861 genera, 3 tribes and 2 subfamilies. *Plant Biosyst.* 2013;147(4):1219-27.

862 146. Bennett MD, Leitch IJ, Hanson L. DNA amounts in two samples of angiosperm weeds.  
863 *Ann Bot.* 1998;82:121-34.

864 147. Schulz-Schaeffer J, Gerhardt S. Cytotaxonomic analysis of the *Euphorbia* spp. (leafy  
865 spurge) complex. II: Comparative study of the chromosome morphology. *Biol Zentralbl.*  
866 1989;108(1):69-76.

867 148. Schaeffer JR, Gerhardt S. The impact of introgressive hybridization on the weediness of  
868 leafy spurge. 1989 Leafy Spurge Symposium. 1989: 97-105.

869 149. Bai C, Alverson WS, Follansbee A, Waller DM. New reports of nuclear DNA content for  
870 407 vascular plant taxa from the United States. *Ann Bot.* 2012;110(8):1623-29.

871 150. Aarestrup JR, Karam D, Fernandes GW. Chromosome number and cytogenetics of  
872 *Euphorbia heterophylla* L. *Genet Mol Res.* 2008;7(1):217-22.

873 151. Wang L, Sun X, Peng Y, Chen K, Wu S, Guo Y, et al. Genomic insights into the origin,  
874 adaptive evolution, and herbicide resistance of *Leptochloa chinensis*, a devastating tetraploid  
875 weedy grass in rice fields. *Molecular Plant.* 2022;15(6):1045-58.

876 152. Paril J, Pandey G, Barnett EM, Rane RV, Court L, Walsh T, et al. Rounding up the  
877 annual ryegrass genome: High-quality reference genome of *Lolium rigidum*. *Front Genet.*  
878 2022;13:1012694.

879 153. Weiss-Schneeweiss H, Greilhuber J, Schneeweiss GM. Genome size evolution in  
880 holoparasitic *Orobanche* (Orobanchaceae) and related genera. *Am J Bot.* 2006;93(1):148-56.

881 154. Towers G, Mitchell J, Rodriguez E, Bennett F, Subba Rao P. Biology & chemistry of  
882 *Parthenium hysterophorus* L., a problem weed in India. Biol Rev. 1977;48:65-74.

883 155. Moghe GD, Hufnagel DE, Tang H, Xiao Y, Dworkin I, Town CD, et al. Consequences of  
884 whole-genome triplication as revealed by comparative genomic analyses of the wild radish  
885 (*Raphanus raphanistrum*) and three other Brassicaceae species. Plant Cell. 2014;26(5):1925-37.

886 156. Zhang X, Liu T, Wang J, Wang P, Qiu Y, Zhao W, et al. Pan-genome of *Raphanus*  
887 highlights genetic variation and introgression among domesticated, wild, and weedy radishes.  
888 Mol Plant. 2021;14(12):2032-55.

889 157. Chytrý M, Danihelka J, Kaplan Z, Wild J, Holubová D, Novotný P, et al. Pladias  
890 database of the Czech flora and vegetation. Preslia. 2021;93(1):1-87.

891 158. Kersten S, Chang J, Huber CD, Voichek Y, Lanz C, Hagmaier T, et al. Standing genetic  
892 variation fuels rapid evolution of herbicide resistance in blackgrass. Proc Natl Acad Sci U S A.  
893 2023;120(16):e2206808120.

894 159. Chen J, Hall N, Saski C, Westra P, Gaines T, Patterson EL. *FHY3/FAR1* transposable  
895 elements generate adaptive genetic variation in the *Bassia scoparia* genome. bioRxiv. 2023;  
896 DOI:10.1101/2023.05.26.542497.

897 160. Jarvis DE, Sproul JS, Navarro-Domínguez B, Krak K, Jaggi K, Huang Y-F, et al.  
898 Chromosome-scale genome assembly of the hexaploid Taiwanese goosefoot “Djulis”  
899 (*Chenopodium formosanum*). Genome Biol Evol. 2022;14(8):evac120.

900 161. Laforest M, Martin SL, Bisaillon K, Soufiane B, Meloche S, Page E. A chromosome-  
901 scale draft sequence of the Canada fleabane genome. Pest Manag Sci. 2020;76:2158-69.

902 162. Guo L, Qiu J, Ye C, Jin G, Mao L, Zhang H, et al. *Echinochloa crus-galli* genome  
903 analysis provides insight into its adaptation and invasiveness as a weed. *Nat Commun.*  
904 2017;8(1):1031.

905 163. Sun J, Ma D, Tang L, Zhao M, Zhang G, Wang W, et al. Population genomic analysis  
906 and *de novo* assembly reveal the origin of weedy rice as an evolutionary game. *Mol Plant.*  
907 2019;12(5):632-47.

908 164. Mao Q, Huff DR. The evolutionary origin of *Poa annua* L. *Crop Sci.* 2012;52(4):1910-  
909 22.

910 165. Benson CW, Sheltra MR, Maughan JP, Jellen EN, Robbins MD, Bushman BS, et al.  
911 Homoeologous evolution of the allotetraploid genome of *Poa annua* L. *Res Sq.* 2023;DOI:  
912 10.21203/rs.3.rs-2729084/v1.

913 166. Thielen PM, Pendleton AL, Player RA, Bowden KV, Lawton TJ, Wisecaver JH.  
914 Reference genome for the highly transformable *Setaria viridis* ME034V. G3 (Bethesda, Md).  
915 2020;10(10):3467-78.

916 167. Yoshida S, Kim S, Wafula EK, Tanskanen J, Kim Y-M, Honaas L, et al. Genome  
917 sequence of *Striga asiatica* provides insight into the evolution of plant parasitism. *Curr Biol.*  
918 2019;29(18):3041-52.e4.

919 168. Qiu S, Bradley JM, Zhang P, Chaudhuri R, Blaxter M, Butlin RK, et al. Genome-enabled  
920 discovery of candidate virulence loci in *Striga hermonthica*, a devastating parasite of African  
921 cereal crops. *New Phytol.* 2022;236(2):622-38.

922 169. Nunn A, Rodríguez-Arévalo I, Tandukar Z, Frels K, Contreras-Garrido A, Carbonell-  
923 Bejerano P, et al. Chromosome-level *Thlaspi arvense* genome provides new tools for

924 translational research and for a newly domesticated cash cover crop of the cooler climates. Plant  
925 Biotechnol J. 2022;20(5):944-63.

926

927 Table 1. Genome assemblies of 31 weed species completed or ongoing by the International  
928 Weed Genomics Consortium.

Scientific name	Common name	Haplotypes in Assembly	Public Availability Date	Ploidy	x	n	Genome Size Estimate (Gbp)
<i>Amaranthus hybridus</i>	smooth pigweed	1; Previous version [137]	September 2023	diploid	16	16	0.509 [138]
<i>Amaranthus palmeri</i>	Palmer amaranth	Previous version [137]	June 2024	diploid	17	17	0.445 [139]
<i>Amaranthus retroflexus</i>	redroot pigweed		In progress	diploid	16	16	0.592 [139]
<i>Amaranthus tuberculatus</i>	common waterhemp	2; Previous version [137]	November 2023	diploid	16	16	0.694 [139]
<i>Ambrosia artemisiifolia</i>	common ragweed		In progress	diploid [140, 141]	18	18	1.152 [142]
<i>Ambrosia trifida</i>	giant ragweed		December 2023	diploid [140]	12	12	1.872 [143]
<i>Apera spica-venti</i>	loose silkybent	2	August 2023	diploid	7	7	4.622
<i>Avena fatua</i>	wild oat	1	August 2023	hexaploid (Additional file 1)	7	21	11.248
<i>Chenopodium album</i>	common lambsquarters	1	July 2023	hexaploid	9	27	1.59

<i>Cirsium arvense</i>	Canada thistle		In progress	diploid	17	17	1.415
<i>Convolvulus arvensis</i>	field bindweed		In progress	diploid <sup>+</sup>	12 <sup>+</sup>	12 <sup>+</sup>	0.652 [142]
<i>Conyza bonariensis</i> ( <i>Erigeron bonariensis</i> )	hairy fleabane		In progress	hexaploid [144]	9	27	2.043 [145]
<i>Conyza sumatrensis</i> ( <i>Erigeron sumatrensis</i> )	Sumatran fleabane	1	May 2023	hexaploid	9	27	1.874
<i>Cyperus esculentus</i>	yellow nutsedge	2	July 2023	diploid	54	54	0.588 [146]
<i>Cyperus rotundus</i>	purple nutsedge	2	December 2023	diploid	54	54	0.49 [146]
<i>Digitaria insularis</i>	sourgrass	1	September 2023	tetraploid	9	18	1.529
<i>Digitaria ischaemum</i>	hairy crabgrass		In progress	tetraploid	9	18	Unknown, in progress
<i>Echinochloa colona</i>	junglerice (weedy genotype)	See crop genotype assembly by [90]	In progress	hexaploid	9	27	1.372 [146]
<i>Euphorbia esula</i>	leafy spurge		In progress	hexaploid <sup>+</sup> [based on 147, 148]	10 <sup>+</sup>	60 <sup>+</sup>	2.3 [149]
<i>Euphorbia heterophylla</i>	wild poinsettia		July 2024	diploid [150]	14	14	Unknown, in progress
<i>Leptochloa chinensis</i>	Chinese sprangletop	2; See also [151]	August 2023	diploid	10	10	0.454
<i>Lolium rigidum</i>	annual ryegrass	2; See also [152]	August 2023	diploid (Additional file 1)	7	7	2.41

<i>Orobanche cernua</i>	nodding broomrape		In progress	diploid	19	19	1.421 [153]
<i>Orobanche crenata</i>	crenate broomrape		In progress	diploid	19	19	2.787 [153]
<i>Orobanche minor</i>	small broomrape		In progress	diploid	19	19	1.792 [153]
<i>Parthenium hysterophorus</i>	ragweed parthenium		In progress	diploid [154]	17	17	Unknown, in progress
<i>Phalaris minor</i>	little seed canary grass	1	August 2023	tetraploid (Additional file 1)	7	14	5.851
<i>Raphanus raphanistrum</i>	wild radish	Previous versions [155, 156]	In progress	diploid	9	9	0.515 [155]
<i>Salsola tragus</i>	Russian thistle	2	July 2023	tetraploid (Additional file 1)	9	18	1.319
<i>*Sorghum halepense</i>	johnsongrass	2	September 2023	tetraploid	10	20	1.752
<i>Verbascum blattaria</i>	moth mullein	1	December 2023	diploid	15	15	0.344 [157]

929

930 Table 1. Genome assemblies of 31 weed species completed or ongoing by the International  
931 Weed Genomics Consortium. All completed genomes are platinum assembly quality, defined as  
932 having chromosome-length scaffolds (i.e., 1-3 scaffolds per chromosome) for the assembly,  
933 unless indicated by \*. Genome size estimated from flow cytometry or published references as  
934 indicated. + indicates that verification is currently in progress for cytogenetic information.

935

936 Table 2. Genomic information for 19 weed species produced without assistance by the  
937 International Weed Genomics Consortium.

Scientific name	Common name	x	n	1n Genome Size Estimate (Gbp)	Genome Assembly Size (Gbp)
<i>Alopecurus myosuroides</i>	blackgrass	7	7	3.56 [158]	3.4-3.56 [131, 158]
<i>Bassia scoparia</i>	kochia	9	9	0.969 [159]	0.970 [159]
<i>Bromus tectorum</i>	cheatgrass	7	7		2.48 [86]
<i>Chenopodium formosanum</i> (domesticated genotype of <i>C. album</i> )	Djulis	9	27	1.69 [160]	1.59 [160]
<i>Conyza canadensis</i>	horseweed	9	9	0.425 [161]	0.426 [161]
<i>Echinochloa colona</i> (crop genotype)	junglerice	9	27	1.18 [90]	1.13 [90]
<i>Echinochloa crus-galli</i>	barnyardgrass	9	27	1.4 [162]	1.34 [90]
<i>Echinochloa oryzicola</i> (syn. <i>E. phyllopogon</i> )	late watergrass	9	18	1.0 [162]	0.95 [90]
<i>Eleusine indica</i>	goosegrass	9	9		0.510 [126]
<i>Ipomoea purpurea</i>	common	15	15	0.81 [113]	0.60 [113]

	morning-glory				
<i>Lolium perenne</i>	perennial ryegrass	7	7		2.63 (Bushman and Robbins, pers. comm.)
<i>Oryza sativa f. spontanea</i>	weedy rice	12	12		0.37 [163]
<i>Poa annua</i>	annual bluegrass	7	14	1.78 [122]	1.89 [164]
<i>Poa infirma</i>	early meadow-grass	7	7	1.17 [165]	1.13 [165]
<i>Poa supina</i>	supine bluegrass	7	7	0.66 [165]	0.64 [165]
<i>Setaria viridis</i>	green foxtail	9	9	0.40 [166]	0.40 [166]
<i>Striga asiatica</i>	red witchweed	12	12	0.6 [167]	0.47 [167]
<i>Striga hermonthica</i>	purple witchweed	10	20	1.48 [168]	0.96 [168]
<i>Thlaspi arvensis</i>	field pennycress	7	7	0.5 [169]	0.53 [169]

938

939 Table 2. Genomic information for 19 weed species produced without assistance by the  
940 International Weed Genomics Consortium. Haploid (1n) genome size estimations are either  
941 calculated through flow cytometry or k-mer estimation.

942

943 Figure 1. Process by which the International Weed Genomics Consortium operates.

944

945 **Additional Files**

946 Additional File 1 (.docx). Methods and results for visualizing and counting the metaphase  
947 chromosomes of (1A): diploid *Lolium rigidum*; (1B): hexaploid *Avena fatua*; (1C): diploid  
948 *Phalaris minor*; and (1D): tetraploid *Salsola tragus*.

949

950 Additional File 2 (.docx). List of completed and in-progress genome assemblies of weed species  
951 pollinated by insects.

