Review

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# Review: evolutionary drivers of agricultural adaptation in *Lolium* spp.

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## Abstract

**The genus *Lolium* comprises many species, of which *L. perenne* ssp. *multiflorum*, *L. perenne* ssp. *perenne*, and *L. rigidum* are of worldwide agricultural importance as both pasture crops and as weeds. These three species are inter-fertile, obligate outcrossers with a self-incompatible reproduction system. This combination contributes to high genetic diversity that supplies new variants during expansion to new natural areas and agricultural environments. Human dispersal, de-domestication and crop-weed hybridization events between *Lolium* spp., or with others such as *Festuca* spp., are likely associated with their distinct weediness abilities. Furthermore, new introductions followed by introgression may hasten adaptation to new environments. Most *Lolium*-related weed science studies have focused on adaptation leading to herbicide resistance, but other forms of adaptation may also occur. In this review, we explore how the wide genetic variation among *Lolium* species and hybridization with other species may contribute to range expansion, and adaptation to both new agricultural practices and future predicted climate change scenarios.**

**Keywords:** adaptation; evolution; genetic variation; herbicide resistance; hybridization; self-incompatibility

## 1 INTRODUCTION

### 1.1 Taxonomy and distribution *Lolium* spp.

The *Lolium* genus (Poaceae) is comprised of several perennial and annual species.[1] *Lolium* spp. are native to Europe, temperate Asia, and North Africa but have spread over the last 200 years to southern parts of Africa, Australia, South America, New Zealand, and North America.[2] *Lolium* spp. were introduced into new regions mainly as pasture plants, turf and cover crops, in contaminated commercial crop seed, and livestock feed. Plant morphology is very similar among members of the genus *Lolium*. Species distinction can be detected in the flowering stage, where the number of flowers in each spike, as well as the inflorescence shape and structure, are distinguishable features.[3] *L. perenne* L. ssp. *perenne* (synonym *Lolium perenne*, common name; perennial ryegrass), *L. perenne* L. ssp. *multiflorum* (synonym *Lolium multiflorum*, common name; Italian ryegrass), and *L. rigidum* (common name; rigid ryegrass) are the most distinctive members of the *Lolium* genus (hereinafter referred to collectively as *Lolium* spp.). As crops, *Lolium* spp. are adapted to different climates and, consequently, their use patterns are divergent depending on the region grown and end use. *L. perenne* is adapted primarily to temperate climates, possesses greater cold tolerance compared to the other two species,[4] and is widely adopted around the globe for either turf or pasture due to its high feed value and perenniality. Conversely, *L. multiflorum* and *L. rigidum* exhibit mainly annual behavior, and the majority of their use is for pasture in warmer regions (e.g. southern United States and Australia). However, in the process of domestication, a natural and deliberate adaptation has occurred for *Lolium* spp. to establish as pastures. Thus, as part of the domestication process, different cultivars have been breed for greater heat or cold tolerance. However, and most importantly, they are considered major weeds of worldwide importance in cropping systems that infest numerous agricultural and non-agricultural areas.

### 1.2 Yield reduction caused by *Lolium* spp.

Under severe infestation, *L. rigidum* can cause yield reductions of more than 30% in wheat.[5] *L. rigidum* is present in more than 800 000 ha of Spanish cereal fields where barley yield reductions of up to 85% were documented in field studies.[6] The evolution of herbicide resistance in *Lolium* populations makes control more difficult, thus increasing the likelihood of yield loss. Herbicide resistance to 14 herbicide mechanisms of action (MOA) has been reported in *Lolium* spp.[7] No other weed species has evolved herbicide resistance at this pace. A *L. rigidum* population originating from Western Australia evolved resistance to herbicides from seven different MOA, the most severe case of multiple herbicide resistance recorded to date. *Lolium* spp. have evolved both target site and non-target site mechanisms of resistance. Furthermore,

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different herbicide resistance mechanisms to the same MOA have been reported within a single population.[8]

It is clear that *Lolium* spp. possess a distinctive ability to rapidly adapt to new environments. However, in some parts of the world, *Lolium* spp. have colonized new areas where their presence had been previously prevented by environmental constraints.[9] We argue that the context in which *Lolium* spp. are dispersed, their mating system, and the genetic diversity inherent to this species complex play important roles in the current and future global spread. In this review, we (i) discuss the implication of the mating system of *Lolium* spp. and how it contributed to the wide genetic variation among populations, (ii) explore questions related to intra-specific and inter-specific hybridization of *Lolium* spp., (iii) discuss the effect of admixture among *Lolium* populations and its contribution to rapid evolution of complex traits, (iv) adaptation to both new agricultural practices and predicted climate change scenarios, and (v) future research directions.

## 2 SELF-INCOMPATIBILITY AND GENETIC VARIATION

Pollination and fertilization in flowering plants involves a series of interactive events between the male (pollen grain) and the female (stigma) structures. Pollen may be rejected or accepted by the stigma depending on underlying genetic factors inherent to the organisms. Self-incompatibility can depend entirely on the recognition of the genetic composition of the haploid pollen by the stigma (gametophytic), or can involve secretions from the stigmatic or the transmitting tissue that prevent the germination or growth of incompatible pollen. Gametophytic self-incompatibility is the mechanism present in *Lolium* spp. that reduces the frequency of successful fertilization of pollen with unmatched loci. Gametophytic self-incompatibility in flowering plants is controlled by several independent multi-allelic loci.[10]

### 2.1 Self-incompatibility

The gametophytic self-incompatibility system in *Lolium* spp. is governed by two multi-allelic independent loci, *S* and *Z*.[11] In contrast to gametophytic self-incompatibility mating systems in other grasses, not all alleles have to be matched in order to produce a self-incompatibility reaction in *Lolium* spp. Thus, differences in reproduction success rate may be dependent on the direction of reciprocal crosses and the degree of compatibility between individuals. Additional loci may be involved in the gametophytic self-incompatibility system in *Lolium* spp.,[12] thus the complexity of the gametophytic self-incompatibility mating system in *Lolium* spp. remains unclear. While the gametophytic self-incompatibility system is efficient under normal environmental conditions, under adverse conditions (e.g. high temperature) the self-pollination rate may be as high as 30%, a phenomenon known as pseudo-self-compatibility (Fig. 1).[13,14] Although these species are grown under mild temperatures during the vegetative stage,[4] late season seed production may occur under high temperatures where pseudo-self-compatibility is more likely to happen, considering these are winter annual species that flower as temperatures warm in the early summer. This fact may have been overlooked by weed scientists and may have been the cause for inconsistencies in some population genetics-related studies where the assumption is made that *Lolium* spp. are obligate outcrossers regardless of the environmental conditions. One possible outcome of that assumption is that crossing of a herbicide-susceptible and a herbicide-resistant biotype for inheritance studies at high temperatures may result in seeds originating from the susceptible or resistant individual that have undergone selfing and thus may shift the ratios of susceptible to resistant individuals in the progeny. This shift may lead to herbicide resistance inheritance ratios in the population not following Mendelian segregation, resulting in inaccurate herbicide evolution prediction models. Using multiple families, pollinating plants at cool temperatures, and emasculation may aid in avoiding these issues.

### 2.2 Effective population size

Due to their obligate outcrossing breeding systems, *Lolium* spp. are likely to exhibit a large effective population size (indexed size of a population that accounts for genetic events), with high heterozygosity and recombination rates resulting in large standing genetic variation. The effective population size of *Lolium* spp. has been inferred indirectly by means of linkage disequilibrium, because this estimate is dependent on the effective population size and the map distance between loci.[15] The gametophytic self-incompatibility breeding system reduces the accumulation of deleterious alleles that may cause inbreeding depression,[16]

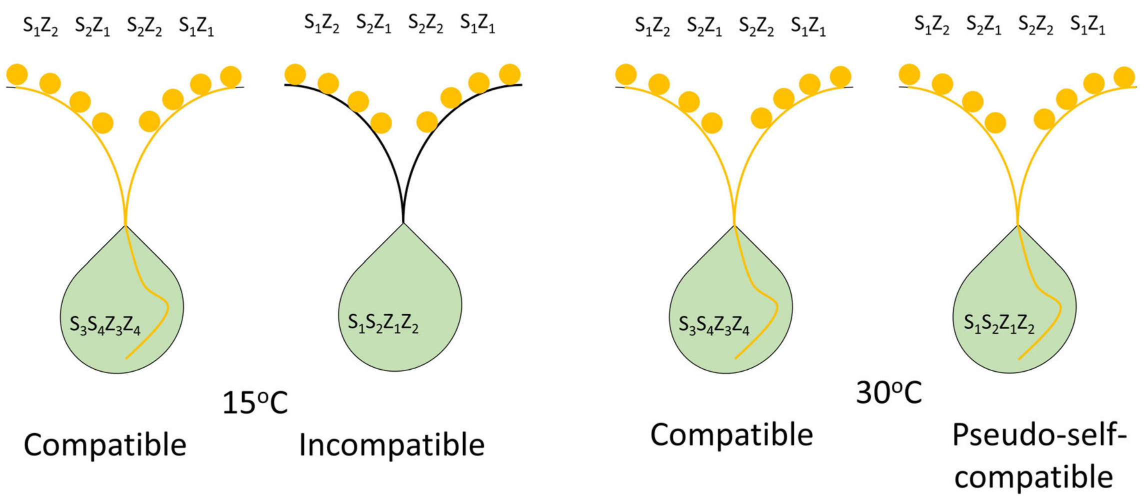

**Figure 1.** Effect of temperature on the reproduction system of *Lolium* spp. While under normal conditions *Lolium* spp. possess a gametophytic self-incompatibility system, under high temperatures self-pollination is enabled.

and is a key trait in the recovery of populations (i.e. evolutionary rescue) following population bottlenecks.

Alleles that confer adaptation may arise in a population via different evolutionary modes: (i) *de novo* mutations; (ii) selection on standing genetic variation; and (iii) immigration.[17] Standing genetic variation and initial allele frequency may explain the variation in life history traits within a population attributing to the fast adaptability of *Lolium* spp. Thus, although initial allele frequency and population size may contribute to the rate of adaptive evolution, allelic exchange through the gametophytic self-incompatibility mating system may have a more crucial role for adaptation processes. As obligate outcrossing species with wind-mediated pollen movement, *Lolium* spp. contain high genetic variation within populations but low genetic differentiation between populations.[18,19] Successive selection by the same stress agent will have less effectiveness in reducing the census population size. This will potentially lead to rapid restoration of populations to their original size, but with decreased genetic diversity and greater tolerance to the initial stress among individuals. A widespread example of this scenario is the selection of *Lolium* populations to herbicides. Although this adaptability may be specific to the stress agent, this is not always the case. For instance, recurrent selection with a herbicide may result in metabolic cross resistance to several other herbicides from different MOA.[20] Other examples are greater seed dormancy levels[21] evolved under intensive cultivation, where late-germinating individuals may avoid early season weed control, and selection for germination from greater soil depths to avoid shallow cultivation, and seedling avoidance of low-mobility pre-emergence herbicides. Differences in inter-species competitive abilities,[22] such as higher seed retention and seed production were also recorded in some populations of *L. rigidum*, in response to intensive cultivation using the harvest weed seed control. Thus, reducing the overall effectiveness of this new weed control tactic.

## 2.3 Genetic variation in *Lolium* spp.

As a pasture species, the genetic variability in populations of *L. perenne* was mainly studied for its effect on vegetative persistence[23] and morphology.[24] *L. multiflorum* and *L. rigidum* are also economically significant weeds of cropping systems, where genetic variation has been studied mainly in the context of genetic inheritance and variability of herbicide resistance mechanisms.[18,25] In addition to the evolution of herbicide resistance, high genetic variation may also lead to biotic and abiotic stress adaptation, which are highly valuable characteristics in a breeding program.[26,27] However, these traits may also increase the weediness of the species and thus result in crop yield losses. High adaptability resulting in reduced sensitivity to various chemical and non-chemical weed management approaches may be attributed to the large genetic variation observed among populations of *Lolium* spp. Evidence for this is in *L. multiflorum* and *L. rigidum* being reported among the ten weed species to evolve resistance to the greatest number of herbicides in a single field population.[7] These resistant populations exhibit both target site and non-target site mechanisms of resistance.

Genetic variation as a result of intentional crop improvement efforts likely plays an important role in the introgression of new traits into local populations. Several regions around the world exhibit appropriate conditions for the establishment of cool-season grass breeding programs. In Oregon, grass seed production dates back to the 1930s, when commercial production of *L. multiflorum* and *L. perenne* seed became popular, and by the 1960s, 11 varieties of both species were adopted by growers, where the majority were public varieties. In the 1980s, over 38 varieties were available and, currently, there are 76 *L. multiflorum* and 348 *L. perenne* certified varieties, produced and commercialized by many local grass seed farmers and breeding companies (https://www.oecd.org). In Australia, the early introductions of *Lolium* spp. were a combination of deliberate introductions and accidental introductions as contaminants. Starting in the early 20th century there was a more organized spread of *Lolium* spp. cultivars originally selected from sites where *Lolium* spp. had persisted and adapted to local conditions. Following this, there were deliberate introductions of new cultivars from South Africa, Tunisia and New Zealand.[28] Prior to 1990, the cultivars available for sowing were mainly *L. perenne* cultivars, but also included a small number of *L. multiflorum*, *L. perenne* × *L. multiflorum* hybrids and *L. rigidum* cultivars. There has been a marked increase in new cultivars since then. In 1993, there were six recommended cultivars of *L. perenne* including hybrids and three of *L. multiflorum*. Since 1993 there has been a large increase in new cultivars available in Australia, mainly from New Zealand, but also some from the United States and other countries and by 2013 there were 65 *L. perenne* and hybrid cultivars and 73 *L. multiflorum* and hybrid cultivars available. Around the world, there are over 2200 *L. perenne* and *L. multiflorum* varieties registered with the Organization for Economic Co-operation and Development (OECD, https://www.oecd.org). Overall, the main breeding objectives for *Lolium* spp. have included: improved persistence of *Lolium* spp. cultivars; increased dry matter production; shorter flowering time to suit dry environments; the presence of endophytes that do not induce mammalian toxicities; tolerance to abiotic stresses; rapid regrowth after grazing; high persistence, and pest and disease tolerance.[28] These traits are likely to confer improved competitive abilities to weedy populations, particularly to new, challenging environments.

## 2.4 Symbiosis with endophytes

*Epichloe* spp. are fungal organisms that form symbiotic associations with *Lolium* spp. They inhabit the intercellular space of the host organism, and are often associated with increases in production of secondary metabolites (e.g. molecules involved in defence against herbivores), protection against a range of fungal pathogens, and enhanced protection against abiotic stresses, such as drought. *Epichloe* spp. provide drought tolerance to *Lolium* spp. by enhancing antioxidant production that buffers the damaging effects of reactive oxygen species.[29] Endophytes were reported in symbiosis within 77% of *L. perenne* populations from Denmark[30] and 100% of the populations from Australia.[31] Likewise endophytes were identified in 100% of 299 populations of *L. rigidum* from Australia.[32]

There is limited research addressing the effects of fungal endophytes in *Lolium* spp. adaptation. Endophyte-host associations are generally specific, and deviations from this may result in negative effects for the host and/or endophyte. Furthermore, host genetic background, in particular in situations with genetic structure in isolated populations or populations that experienced genetic bottleneck, may affect the compatibility with endophyte organisms.[33] Endophytes common to *Lolium* spp. are transmitted asexually, and inherently the genetic variability of these organisms is low,[32] whereas the breeding system of *Lolium* spp. results in high genetic variability of host populations.

A model has been proposed to explain the correlation between host genetic diversity and symbiosis with endophytes.[33] When the endophyte-infected host population that possesses low levels of genetic variability, reduced fitness is expected. Despite the high host-endophyte compatibility in this scenario, reduced fitness is likely associated with the high energy costs of maintaining the fungal organisms and host fitness compromised due to inbreeding depression. At high levels of genetic diversity in the host population, symbiotic interactions are expected to reduce fitness, because of incompatibilities between host and endophyte. Finally, at intermediate levels of host genetic diversity, host fitness is expected to be at the highest level, likely associated with heterosis and reduction in the energy expenses with maintaining the endophyte.

It is still unclear why *L. rigidum* and *L. multiflorum* exhibit high levels of endophyte infections, while still maintaining high levels of genetic variability. A possible explanation is that endophytes common to these species are not as specific as observed in other hosts species. If that is the case, then even small, founder populations after bottlenecks may take advantage of the symbiotic associations with endophytes to increase their fitness under new environmental conditions, or under conditions of stress (e.g. drought, flooding, herbicide, herbivory, etc.). Small increments in plant fitness may not avert extinction under challenging new environments, but could delay extinction until evolution takes place (by introduction of new genotypes, new mutations, or epigenetics).

## 3 INTRA-SPECIFIC AND INTER-SPECIFIC HYBRIDIZATION AND EPIGENETIC EFFECTS IN *LOLIUM* SPP.

Human-mediated movement of *Lolium* spp. seed is an important component of the *Lolium* spp. allele dispersal network (Fig. 2). Although the center of origin of *L. multiflorum* and *L. perenne* is the Mediterranean basin,[2] the main seed production areas for commercial seed crops are in temperate regions of the globe, notably Oregon in the United States, Canada, Denmark, and New Zealand. These seed production areas typically host breeding programs to improve desirable traits for end users in *Lolium* spp., such as nitrogen content, digestibility, and pest resistance. From those countries, seeds have been shipped to the rest of the world, which is the main mechanism of long distance dispersal and source of new introduced genes to the gene pools of local *Lolium* populations. Dispersal of *Lolium* spp. seeds can also be via contaminated seed shipments. *Lolium* spp. seeds were found in wheat, barley and oilseed rape grain shipments imported from Western Australia to Japan, leading to plant dispersal around grain-importation ports.[34] Once new gene pools are introduced locally, short-distance dispersal by wind (via pollen) and commodity transport (via seed) are the predominant forms of the local allele dispersal network. With the free movement of alleles to new locations, de-domestication processes start to take place, as newly introduced genotypes may exchange genetic material with locally adapted genotypes.

Species in the genus *Lolium* may also hybridize with members of the genus *Festuca*, where *F. arundinacea* and *F. pratensis* are the two most important cultivated forage species. *Festuca* spp. exhibit a wide range of ploidy levels, ranging from diploids to octoploid species.[35] Similar, to *Lolium* spp., *Festuca* spp. are also native to the Mediterranean basin, and spontaneous hybridization may occur. *Lolium* spp. and *Festuca* spp. hybrids may form, and typically these crosses result in male sterility (indehiscent anthers), and a small percentage of female fertility.[36] *Festuca* spp. are generally regarded as better adapted to abiotic and biotic stresses compared to *Lolium* spp., such as tolerance to harsh winter and

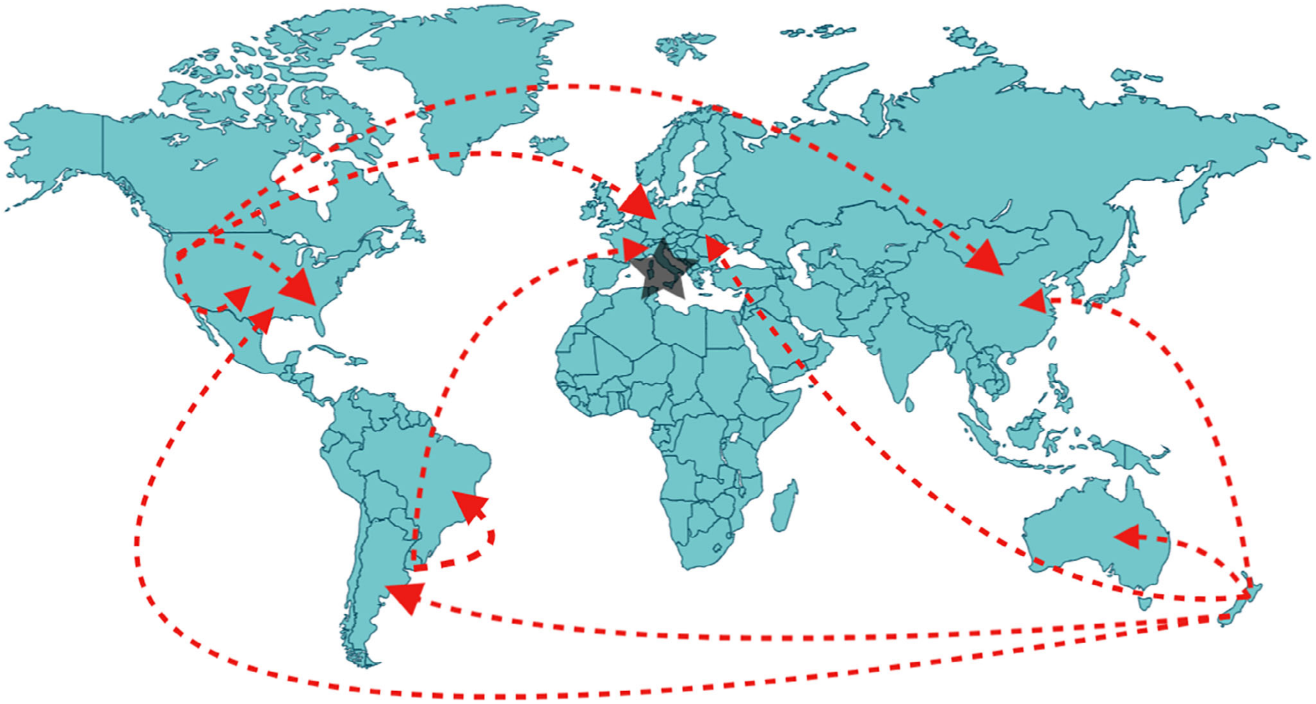

**Figure 2.** Current dispersion routes of alleles in *Lolium* spp. The star represents the center of origin of *Lolium* spp. *Lolium* spp. were initially grown for commercial reasons (mainly for pasture). At present, *Lolium* spp. seed production takes place in many countries around the world and are commercialized for pasture, turf, and as a cover crop and sold to many different markets (red dashed arrows). This human-mediated long-distance transport facilitates the introduction of new alleles to local populations around the world.[2]

summer drought, and leaf fungal diseases.[37] Therefore, crosses between members of these two genus may result in hybrids with superior performance as agricultural crops. However, given the high levels of sterility of intergeneric hybrids with *Festuca* spp. these are likely to contribute less to adaptation of *Lolium* populations as weeds of agricultural regions than hybrids among the *Lolium* spp. Further research is needed to fully understand this phenomenon.

### 3.1 Adaptation to new environments

Once a *Lolium* population experiences a new challenging environment (e.g. a new management practice or biotic/abiotic stress), elimination of most local individuals occurs, and it is expected that the genetic diversity (and effective population size) of the population will decrease. Unless new mutations arise in a timely manner, adaptation from standing genetic variation is the most common way that populations evolve. It is important to note, however, that because of the reduction in genetic diversity in the surviving individuals, the likelihood of adaptation to any additional environmental stress becomes low, simply because the required adaptive alleles might have become extinct from the population during the prior event. Therefore, additional immigration events may be the best way to supply new adaptive alleles in a timely manner. In this context, *Lolium* spp. are in a particularly advantageous position to recurrently adapt to new environments, as not only intra-specific (Fig. 3(A)), but also inter-specific (Fig. 3(B)) gene flow occurs from a number of close-related species. Gene flow via pollen is known to occur among *Lolium* spp.,

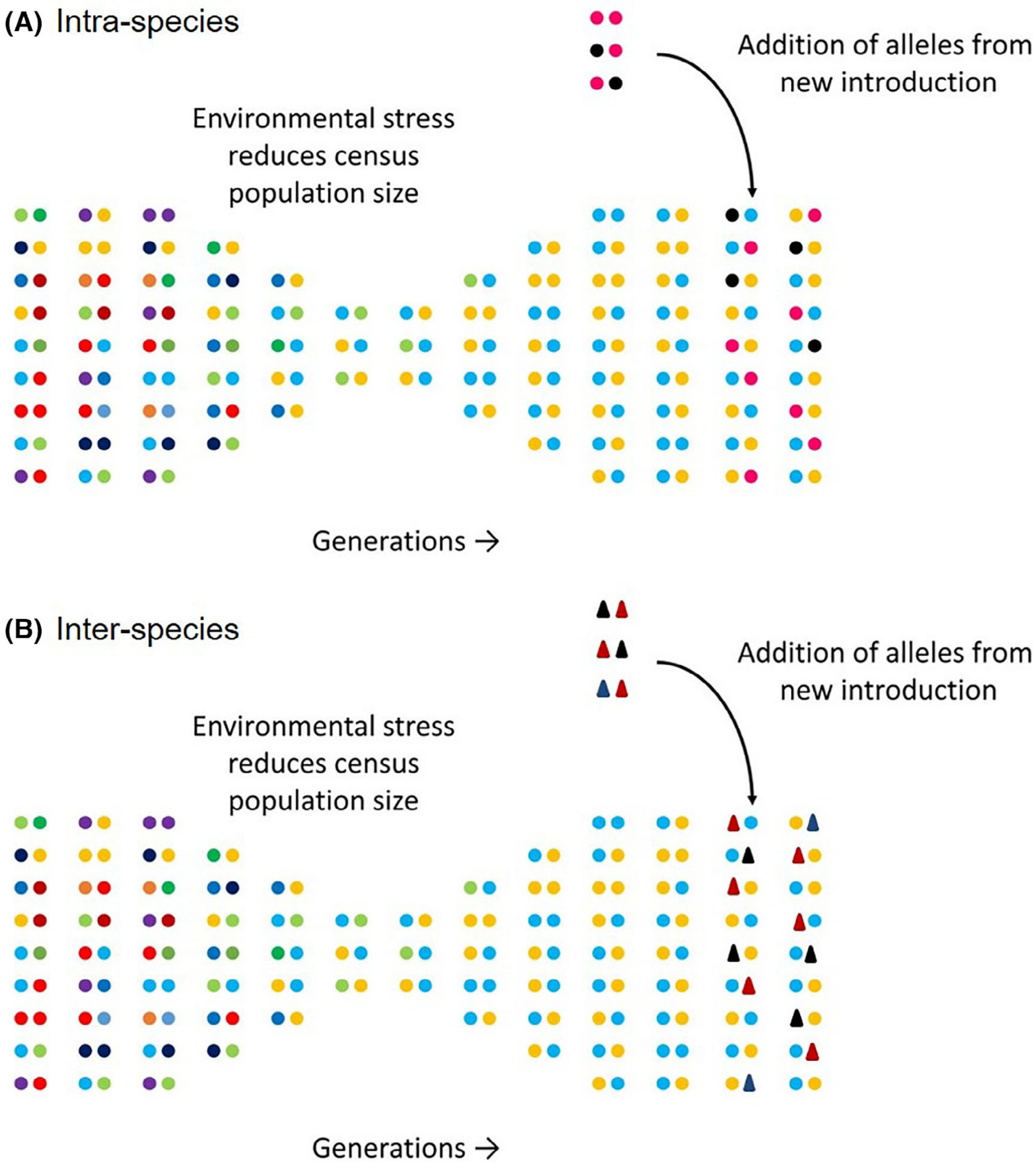

**Figure 3.** Following population bottlenecks, alleles that confer adaptation may arise in a population via immigration. This may be related to introduction of new alleles from new introduction of the same species (A) or via hybridization with other close-related species (B). These attributes may explain the variation in life history traits within a population and facilitate evolution to new agricultural environments.

as these species are inter-fertile. For instance, when *L. multiflorum* is grown in close proximity to *L. perenne*, the hybridization rate may be as high as 47.5%.[38] The ability to intermate among *Lolium* spp. is a tremendous advantage to populations that are colonizing new environments or are exposed to challenging selection pressures. There are many examples of inter-specific hybridization in the *Lolium* genus. Glyphosate-susceptible *L. multiflorum* and glyphosate-resistant *L. perenne* were crossed to study the inheritance pattern of glyphosate resistance.[39] Offspring of this cross showed moderate glyphosate-resistance and 75% of the plants survived the treatment with recommended field rate, suggesting glyphosate resistance was inherited through a single locus with incomplete dominance. Gene flow via pollen transfer between different *Lolium* spp. may facilitate adaptation under field conditions. Inter-specific hybrids may also be used in breeding systems to isolate genes of interest. Using crosses between the self-incompatible *L. multiflorum* and the self-fertile *L. temulentum*, a genetic linkage map was produced in order to study important agronomic traits in *Lolium* spp.[40] In regions where *Lolium* spp. are grown for seeds, as in Oregon, USA, and in regions of Canada and New Zealand, this may be an important source of contamination of feral traits to the seed crop, especially if highly undesirable traits, such as high dormancy levels, are present in the feral populations. The opposite is also likely to be true: gene flow from the crop may be an important source of new alleles in weedy populations. Alleles related to increased vigor and competition abilities at different developmental stages may be harnessed by weedy populations. This would allow weedy populations to 'exploit' the crop diversity and evolve enhanced growth characteristics, increasing their fitness and competitive abilities. Although both crop and weedy *Lolium* spp. are grown in close proximity in several countries, studies exploring the movement of crop productivity characteristics to weed populations are scarce.

### 3.2 Stress-induced adaptation

Weed management practices affect the overall plant performance, not only those traits involved in the survival in the new environment, but also to other unrelated traits. To illustrate this idea, ecological studies conducted to test the fitness cost of herbicide-sensitive and -resistant *Lolium* populations can show differences in life history traits between the populations.[41,42] However, since many of these studies compare populations with different genetic backgrounds, the differences observed may be attributed to the high variability within *Lolium* spp., associated with a genetic bottleneck after recurrent herbicide applications, and may not be directly linked to the herbicide resistance alleles.

Given the rapid evolution observed in *Lolium* populations, the relative contribution between genetic and epigenetic modifications in the adaptability to challenging environments remains unclear. The rate of epigenetic alterations have been reported to be far greater than that of genetic mutations.[43] More epigenetics research is needed to assess the importance of this mechanism in the evolution of *Lolium* populations as major weed species, as limited research has addressed this topic in weeds and other non-model plants in general. Epigenetic mechanisms might be particularly important in *Lolium* populations that do not carry the adapted standing alleles in environments that change continually. Furthermore, inter-specific hybridization not only recruits new alleles to plant populations, but may also alter the epigenome of the offspring.[44,45] DNA methylation has been shown in other plant species to be the main epigenetic mechanism that allows plant populations to respond to sudden environment changes,[46,47] as frequently as it happens in agricultural systems. Environmental clues, particularly non-lethal stresses, elicit epigenetic variations in plants that are inheritable and may be stable for as long as the stress continues. This mechanism may provide the phenotypic plasticity necessary to survive while waiting for additional introductions to occur or new mutations to arise. A common example of non-lethal stress to weeds is the use of low herbicide rates, or herbicide applications on older weeds. For example, a *L. rigidum* population from Australia was exposed to sub-lethal rates of pyroxasulfone, a very-long chain fatty acid inhibitor, for three generations, resulting in an offspring that exhibited increased levels of resistance to this herbicide.[48] In another *L. rigidum* population from Australia, recurrent selection for three subsequent generations with reduced rates of diclofop-methyl, resulted in resistance to the commercial field rate of diclofop-methyl. Increased resistance to other acetyl-coenzyme A carboxylase inhibitors (fluazifop-P-butyl, haloxyfop-R-methyl and clethodim) and to acetolactate synthase inhibiting herbicides (imazethapyr) was also observed.[49] Three subsequent selections have led to reduced sensitivity to glufosinate in a *L. multiflorum* population from California, USA, however, in this case, no cross resistance to other herbicides from different modes of action was recorded.[50] Although the presence of epigenetic modifications have not been tested in low-dose selection studies, the observed increase in survival frequency may be associated with modifications in the epigenome.

## 4 ADAPTATION TO NEW AGRICULTURAL PRACTICES AND PREDICTED CLIMATE CHANGE SCENARIOS

### 4.1 Phenological adaptation to weed management practices

Resistance to herbicides is not the only adaptation by *Lolium* spp. to agricultural practices. In southern Australia, *Lolium* spp. was widely planted as a pasture species in provide valuable fodder for sheep production that dominated agricultural activity until the late 1980s. Following a decline in wool prices, more land was devoted to growing grain crops. Early studies with *Lolium* spp. seed germination in the 1970s and early 1980s showed low persistence of seed in the soil seed bank with less than 10% persisting between years.[51] By the 2000s, 20% of the seed remained dormant between years.[52] This change in seed dormancy is likely driven by continuous crop production systems where early germinating weeds were controlled by herbicides. In Western Australia, a correlation was observed between the extent of herbicide resistance in populations and increased seed dormancy.[22] Populations of *Lolium* spp. from continuously cropped fields have also changed with more of the population emerging later to avoid early season controls.[53] Similar patterns of phenotypic shifts have been identified in the germination rate, dormancy, and seed aging, as well as response to herbicide treatment, after *L. multiflorum* was allowed to grow in isolation or in mixture with the close-related species *F. rubra* over a 5-year period.[53] The shifts in germination response were likely due to the survival of late emerging individuals that carry alleles for increased dormancy. Because sublethal doses of herbicides were used, it is possible that epigenetic alterations may be involved in the shifts observed.

Early emerging weed populations are less likely to be exposed to post-emergence wheat herbicides, therefore, selection pressure from herbicides is more pronounced on weeds that germinate after the wheat sowing operations are concluded. The presence of sub-populations within *Lolium* populations is a clear example of the genetic diversity in these species, and the likelihood of adaptation to new management practices. Late germinating sub-populations of *L. multiflorum* evolved diclofop resistance, whereas the early germinating remained susceptible.[54] The differences in germination timing could be attributed to different temperature requirements of the sub-populations, an example of within population variability that allows *Lolium* spp. to thrive not only in the presence of herbicide (because there are resistant and susceptible sub-populations), but also to tillage (because of the non-uniform germination pattern). Early germinating seeds are exposed to tillage operations as part of the soil preparation in the wheat crop, whereas late germinating seeds will develop plants that are exposed solely to the herbicide.

### 4.2 Adaptation to altered environmental conditions

There has been adaptation to changing environments in *Lolium* spp. For example, time to flowering in *Lolium* spp. collected from Western Australian fields was correlated with the extent of growing season.[55] Flowering occurred earlier in collections from lower latitudes, where the growing season is shorter. Flowering occurred later in collections from higher latitudes (southern locations in the wheatbelt of Western Australia) with a 25 day difference in time to 50% spike emergence over 6° of latitude. There has also been range expansion of *Lolium* spp. in Australia. Despite being a weed of Mediterranean climate systems, *Lolium* spp. is now present in the summer rainfall dominant northern grain growing region of Australia where it was traditionally absent.[8] There has also been seasonal expansion where recently *Lolium* spp. has become a serious weed of cotton production systems in Australia, even though *Lolium* spp. is usually a winter weed and cotton is a summer crop.[56] *Lolium* populations expanding to new environments may be negatively affected by inbreeding depression and low genetic variation (Fig. 4). These effects may be reduced by an inter-specific hybridization event, serving as an additional source of variation, allowing them to restore the genetic diversity and counter inbreeding depression. Hybridization may also produce new phenotypes that were absent in the parental populations as reviewed elsewhere.[57] The role of heterosis in weed populations and its importance in adaptation in *Lolium* populations remain unclear.

### 4.3 Genetic diversity hastens the adaptation of *Lolium* spp. as an invasive species

The preceding examples show how *Lolium* spp. can adapt to changing agricultural practices due to the high genetic diversity present within populations,[18,19] potential involvement in epigenetics in the rapid adaptation, and migration. The movement of *Lolium* populations to agricultural landscapes provides an opportunity for exchange of genetic material to create new combination of traits that enable adaptation. Australia is a good example where there have been multiple introductions of *Lolium* spp. overlaid by the widespread distribution of 'Wimmera' cultivar (*L. rigidum*) as a pasture species.[58] The introduction of new genotypes may be sufficient to drive populations that are currently of little concern to expand their range and become more troublesome. *Lolium* spp., as a result of its forage value, have multiple avenues for distribution: through deliberately sown seed; as a contaminant in hay or grain; inside animals shipped between countries; and on vehicles. Because of the recruitment of new allele sources associated with the movement of *Lolium* spp., the expansion of these species to new agricultural and non-agricultural environments, as well as adaptation to new management practices, are inevitable.

An obvious example of how *Lolium* spp. adapt to management practices is the evolution of herbicide resistance. Initially, resistant individuals are at extremely low frequencies in a population. However, repeated use of herbicides will increase the frequency of resistant individuals, shifting the mean response to a herbicide that is repeatedly utilized. Although other examples of agricultural adaptation in *Lolium* spp. are scarce, one could hypothesize a number of ways populations could adapt to management. For example, no-till farming systems result in earlier sowing of crops. Where post-emergent herbicides are unavailable, due to

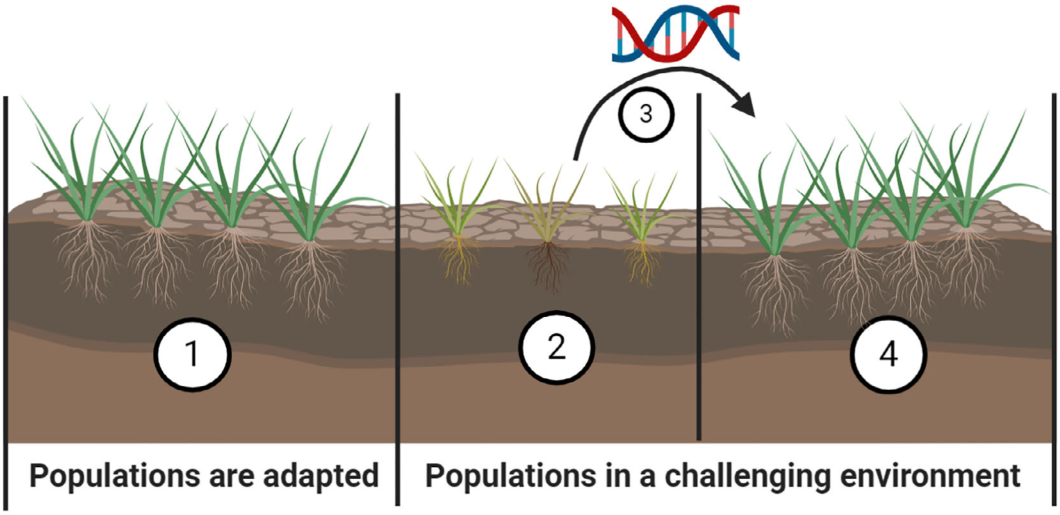

**Figure 4.** Constraints for range expansion of *Lolium* populations. A population adapted to local conditions will not expand to new environments unless beneficial alleles are introduced (1). If individuals manage to establish, plant development will be compromised because of inbreeding depression and lack of adapted alleles (2). Once new alleles are introduced to the population (3), colonization of new environments is now possible and genetic diversity is partially restored (4).

resistance, evolution of increased seed dormancy could be an advantage in these systems allowing individuals to avoid pre-emergent herbicide controls. There is some limited evidence of this occurring in *L. rigidum* in Australia.[21] Likewise, *Lolium* spp. could adapt to the use of crop residue used as mulch by delaying its germination. This is because seed cohorts that germinate early in the season cannot push through the crop residue, but late-germination cohorts may be successful after partial degradation of the crop residue.

There is no doubt that *Lolium* spp. will continue to evolve as agricultural practices change. The extent of herbicide resistance in *Lolium* spp. in Australia has resulted in the adoption of a range of harvest weed seed control practices to reduce the amount of weed seed returning to the weed seed bank.[59] Differences in the timing of seed shedding in *Lolium* populations exist[60,61] and could be easily selected. Early data suggests no significant increase in early seed shattering,[22] but the introduction of new genotypes with higher shattering may provide the trigger for change. Harvest weed seed control techniques challenge for the adoption of these techniques in wheat production regions in the Pacific Northwest in the United States.

The climate of much of the world's agricultural production areas is changing. The climate is predicted to become warmer in most locations, wetter in some, drier in others, and rainfall is predicted to become more erratic. *Lolium* spp. has the ability to become a more problematic weed with climate change, due to its ability to rapidly adapt to new environments. The examples earlier from around the world show the ability of *Lolium* populations to adapt to different climatic conditions.

## 5 PERSPECTIVES AND CHALLENGES FOR FUTURE RESEARCH

Although *Lolium* spp. have been studied for decades, many issues regarding weed biology, ecology, and evolution remain unanswered. Specifically, topics on (i) rapid distinction among *Lolium* spp., (ii) better understanding of trait dominance in hybrids, (iii) drivers of evolution to different management practices, and (iv) the use of basic knowledge to drive management decisions, require further attention from the scientific community.

### 5.1 Distinction among *Lolium* spp.

It is inarguable that it is difficult to tell apart *Lolium* spp., particularly in geographic locations where their distributions overlap. However, it is clear that, although these species are very close phylogenetically, their responses to the environment and management practices are distinct. Therefore, their correct identification is crucial before future studies on their biology are conducted. For instance, although *L. perenne* and *L. multiflorum* co-occur in the Pacific Northwest in the United States, they have distinct biology and responses to the environment. *L. multiflorum* is more adapted to flooding conditions, whereas *L. perenne* is not. *L. perenne* requires a lengthy vernalization period in order to flower, whereas *L. multiflorum* does not exhibit such a requirement. More strikingly is the fact that herbicide resistance is more common in *L. multiflorum* than in *L. perenne*. These distinctions demand a more accurate identification of the *Lolium* spp. Even more problematic is that the inter-fertility of the three species leads to introgression between these species, resulting in numerous hybrids.[62] However, pure populations still exist and even in a hybrid population the dominance of one species over the other in the genetic admixture may be pronounced. The correct identification of the genetic material being investigated is important, particularly in studies comparing the biology and ecology of *Lolium* populations. Molecular markers have been used in the past for this purpose and are relatively easy to perform.[34,63] Several of these approaches have been used to distinguish between *Lolium* spp., aimed at improving the effectiveness of breeding. Although hybrid cultivar plants of *L. multiflorum* × *L. perenne* were successfully detected using simple sequence repeat (SSR) markers,[34] more data and development of new molecular techniques are needed for faster identification of hybrids between *Lolium* spp. and of weed field populations. Here, we have suggested that hybridization between *Lolium* spp. has aided the adaptation and spread of weedy *Lolium* spp. types. However, there is currently little genetic evidence available that addresses this issue. It will be important to unravel the role of hybridization in the invasiveness of these weed species to better understand the risks for future range expansion.

### 5.2 Drivers of evolution to new management practices and environmental conditions

Throughout this article, we argued that the high levels of population heterozygosity maintained by the mating system in *Lolium* spp., along with the long-distance gene flow, and potential involvement of epigenetics, are keys to the rapid adaptability to challenging environments. An example of range expansion may be the colonization of temperate and humid areas that are prone to flooding. However, limited research has focused on the phenotypic traits of hybrids and how trait dominance is expressed. The admixture proportions from each species should be examined, as it may affect the overall genetic diversity within the studied samples and likely affect the results.

*Lolium* spp. have exhibited an astonishing ability to adapt to new environments. However, much of the underlying adaptability processes remain unknown, especially because of the difficulties associated with correlating the genotypes with the phenotypes and the limited genomic resources available for this species. Some tools that could be used to investigate the genetic basis of evolution in *Lolium* spp. are, therefore, the various *-omics*. A chromosome level genome assembly could facilitate the identification of candidate genes involved in the various phenotypes observed in *Lolium* spp. There are currently several research groups interested in providing this resource to the scientific community.

### 5.3 The use of basic knowledge to drive management decisions

Given that *Lolium* spp. range expansion to new environments is unavoidable, predictive tools must be developed to diminish the impact of these new introductions. In particular, climate change predictions could be paired with other predictive models to anticipate where *Lolium* spp. have the potential to colonize and establish.[64] As previously noted, shifts in germination patterns and seed longevity have direct impact on the infestation potential of weeds, and knowing when seeds are more prone to germinate may assist land managers in the decision-making process. An important approach that has recently emerged as a useful tool in weed management is harvest weed seed control.[22] This Australian innovation has been developed to target weed seed retained at crop maturity and thereby reduce the soil seed bank for next year. This tool was developed mainly for the harvest of grass weed seeds such as *L. rigidum*, a major weed in commercial wheat growing in several places around the world. Although no evidence for adaptation has yet been observed,[22] increased use of this tool could lead to the evolution of early flowering and seed shattering *L. rigidum* populations. As we have seen for many weed management practices (e.g. herbicides, tillage, etc.) the evolutionary response will not take long, as early flowering and seed shattering alleles probably exist in the genetic pool of these populations. *Lolium* spp. have colonized all types of agricultural and non-agricultural environments, and have shown a tremendous potential to adapt to new environments. It is crucial that more basic knowledge is obtained to reduce the impact of *Lolium* spp. on agricultural areas, reduce their interference, and maintain the economic sustainability of production systems.

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