Evol Biol (2017) 44:411–426
DOI 10.1007/s11692-017-9419-6

SYNTHESIS PAPER

Does the Cost of Adaptation to Extremely Stressful Environments Diminish Over Time? A Literature Synthesis on How Plants Adapt to Heavy Metals and Pesticides

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Received: 19 May 2016 / Accepted: 27 April 2017 / Published online: 13 May 2017

Abstract Populations adapted to locally stressful environmental conditions are predicted to carry costs in performance and fitness, particularly when compared to non-stress adapted populations in the absence of stress. However, empirical observations found fitness costs incurred by stress-resistant genotypes are often ambiguous or absent. Compensatory evolution may purge genotypes with relatively high costs over time, resulting in the recovery of fitness in a stress-resistant population. We assessed the magnitude of adaptation costs over time to test for a reduction in negative genetic effects by compiling published data on measures of fitness from plant populations inhabiting mine tailings and populations adapted to herbicides. Heavy metal contaminated sites represent a stress that is immediate and unchanging; herbicides represent a stress that changes over time with dosage or the type of herbicide as treated populations become more resistant. To quantify costs, for each comparison we recorded the performance of plants from stress and non-stress environments grown under benign conditions. Time since the initiation of the stress was determined to test whether costs change over time. Costs were overall constant through time. The magnitude of cost were consistent with trade-offs for heavy metal resistance and certain herbicide mechanisms (triazine and resistance via P450 enzyme), but not for other herbicides where costs were inconsistent and appear to be low if not absent. Superior stress-resistant populations with higher performance than non-stress populations were found from both herbicide and metal stress, with some extreme cases early from time since initiation. There was an increasing benefit to cost ratio over time for herbicide resistant populations. We found that adaptation to stressful environments is generally costly except in herbicide resistance, and that costs are not diminished over time. Stress-resistant populations without costs also arise infrequently, though these populations may often be restricted from spreading.

Keywords Meta-regression · Negative pleiotropic effects · Local adaptation · Herbicide resistance · Degree of tolerance · Time since adaptation · Compensatory evolution

Introduction

A fundamental goal of evolutionary ecology is to understand how organisms adapt to changing environments and to identify the long-term evolutionary consequences of adaptation. Understanding evolutionary mechanisms and limits to adaptation may allow for predicting changes to species ranges with future environmental change, as well as aid species to shift their ranges (Holt 2003). Adaptation to new or novel environments is an ongoing process in populations under a range of ecological scenarios (Linhart and Grant 1996); including range expansion into new environments, adaptation to climate change (Jump and Peñuelas 2005), adaptation to new parasites and pathogens (Kaltz and Shykoff 1998), and anthropogenic stresses such as air pollution, pesticides and heavy metals (Hutchinson 1984). Invasive and agricultural plant species often evolve resistance to lethal herbicides and pesticides (Jasieniuk et al.

Electronic supplementary material The online version of this article (doi:10.1007/s11692-017-9419-6) contains supplementary material, which is available to authorized users.

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1996). Similarly, many plant species can also evolve metal toxicity resistance where their susceptible counterparts are most likely killed in these environments (Antonovics et al. 1971).

Of interest are two sometimes opposing fundamental forces occurring within populations, which can determine the ability of genotypes to adapt to new or changing environments through natural selection: First, trade-offs may prevent simultaneous increases to two traits or functions even where there is selection for both traits (Kawecki and Ebert 2004). Second, selection for alleles which track traits closer toward environmental optima occurs where alleles that contribute less to fitness are repeatedly purged through natural selection (Orr 1998; Stanton et al. 2000). This should result in genotypes better adapted to the environment. Adaptation to novel environments involves ongoing optimisation; while at the same time adaptation likely incurs costs and trade-offs in performance and fitness (Bergelson and Purrington 1996).

Costs arising from adapting to stressful environments may prevent any one genotype or species from spreading into all environments. However, many comparisons between stress-resistant and non-stress genotypes under benign conditions show minimal or no differences in performance, and stress-resistant populations appear to have adapted to stressful habitats without costs (Andersson and Levin 1999; reviewed in; Bergelson and Purrington 1996; Dechamps et al. 2007) or with low costs (Harper et al. 1997). In some cases, the stress-resistant genotype seems to outperform non-stress genotypes in the absence of the stressor (e.g. Mateos-Naranjo et al. 2011). However, stress-resistant genotypes tend to be restricted to their stressful habitats (Antonovics and Bradshaw 1970). This suggests costs may be absent, or may be difficult to detect. For instance, trade-offs may still be obscured if treatment conditions are not at physiological limits, and any trade-off that is expressed may not be interpreted meaningfully without exploring the physiological limits for genotypes (Grubb 2016). Changes in adaptation costs over time may be fundamental to understanding of how species adapt to environmental change, and how populations evolve over time.

Distinguishing different mechanisms contributing to adaptation can be useful in explaining the variation in the manifestation of costs across stress-resistant genotypes. Two distinct sources of costs are identifiable from the literature: Allocation costs and negative genetic effects (synonymous with ‘genetic cost’, ‘pleiotropic cost’, ‘genetic trade-off’- Posthuma and Van Straalen 1993; Vila-Aiub et al. 2009) can account for the consequences on fitness of stress-resistant genotypes. Allocation costs (i.e. trade-offs) involve shifts in the distribution of resources as a result of selection within a new environment toward a new trait optimum, however resulting in a cost to fitness in other environments with different optima (Meharg 1994). That is, selection pulls a fitness trait towards the optimum value of the new environment, which also moves the trait value away from the optimum of the previous environment and reducing fitness there. Trade-offs can also occur among traits (Grubb 2016). Selection for an increase in trait A (e.g. leaf longevity) must also result in reduction of trait B (e.g. growth rate) (Grubb 2016). On a genetic level, these are characterised by an overexpression of existing genes rather than the evolution of novel genes (Singh et al. 2015), and adaptation via mutations of small effect size on phenotype (i.e. small effects) (Dittmar et al. 2016). This can occur through selection for traits that reduce performance, i.e. smaller size, slower growth, or the conservation of resources under stressful environments (Chapin et al. 1993). Populations adapting to a steeper environmental cline were found to incur greater trade-offs relative to populations adapting to shallower clines (Collins and de Meaux 2009). For heavy metal and herbicide resistance, allocation costs are the main source of cost involved with the production of metabolic enzymes (non-target site resistance—e.g. cytochrome P450) which break down herbicides before they can have an effect (Vila-Aiub et al. 2005, 2009), as well as the production of overexpressed transporter proteins in metal hyperaccumulators (Singh et al. 2015). These metabolic activities require resources that could otherwise be used for growth and other performance traits.

Negative genetic effects (NGE) consist of linkage of deleterious alleles with stress adaptation genes, i.e. antagonistic pleiotropy or epistasis which results in a decrease in performance in other environments (Remold 2012; Dittmar et al. 2016), or through a negative effect on additive variance (Goodnight 1988) where effective genetic variance may be reduced by epistatic effects (Draghi et al. 2011). Adaptation to heavy metal and herbicide stress often involves few mutations with large effects, with some genetic modifiers contributing to resistance (Macnair 1983, 1993; Patra et al. 2004), but not in all cases (Gartside and McNeilly 1974). NGE are believed to be associated with mutations of large effects, such as those involved in adaptation heavy metal and herbicide stress (genetic mechanisms discussed in more detail below). Importantly, NGE such as antagonistic pleiotropic effects may have long-term evolutionary consequences through imposing restrictions on adaptation (Fisher 1930; Dittmar et al. 2016), whereas mutations of small effects that are linked to allocation costs are not believed to be associated with strong NGE (Dittmar et al. 2016).

Other than these two main sources of cost, ecological costs are another source of cost that is less distinct, environment-dependent, and may stem from allocation costs or NGE. Ecological costs are expressed in the presence of competition or natural enemies, and not necessarily only

under the absence of stress (Vila-Aiub et al. 2009). For example stress-resistant genotypes may be inferior competitors due to slower growth rates from allocation of resources to stress resistance mechanisms; and in the case of NGE manifesting as an ecological cost—lower fitness and performance stemming from detrimental genetic effects can likewise result in inferior competitors. A decrease in the body size (e.g. Cable et al. 2007) due to adaptation costs in host animal species could increase mortality from pathogens.

In practice, even the two main sources of costs have potential overlap in manifestation, leading to some ambiguity that is difficult to disentangle without explicit investigation into the adaptive mechanisms and evolutionary constraints (Duncan et al. 2011). For instance, say a NGE has a direct deleterious effect of reducing and restricting the expression of trait A. This results in extra resources which may naturally be allocated to trait B, which could have unpredictable ecological effects. Moreover, the same NGE may not necessarily manifest as fitness cost if their effects on fitness are environment-dependent (e.g. the genotype coincidentally finds itself in a habitat where trait B is favored. The overall benefits of having extra resources towards trait B overcome the cost of the reduction in trait A; or in habitats where reduced trait A happens to be favoured).

Understanding the costs associated with certain mechanisms of resistance is important in separating NGE from allocation costs. During an adaptive walk towards some optimum, mutations of larger effects contributing to adaptation are predicted to be exponentially rare towards increasing effect sizes (for adaptation without immigration), compared to alleles of small effects which are predicted to be more common (Orr 1998), thus a large proportion of the phenotypic change can be attributed to a few alleles with large effects where they are present, though alleles with small effects should be more common. However, with ongoing migration, many alleles with small effects are predicted to be replaced by alleles with large effects due to stabilising selection for alleles with greater persistence—a "genetic clustering" effect (Yeaman and Whitlock 2011). This is backed by the empirical observations of few major mutations with a few modifier genes granting heavy metal resistance (Macnair 1991; Patra et al. 2004), but whether this occurs requires further study. For adaptation to moving optimums such as herbicides; depending on the speed of movement, alleles with larger effect sizes are predicted to be favoured, with an increasing degree of pleiotropy (Dittmar et al. 2016). It could be predicted that a moving herbicide stress may have a higher probability of incurring NGE whenever associated with mutations of large effects, and intuitively may have higher costs than heavy metals, which is a constant stress. However, this would strongly depend on the rate of optimum change, as alleles of small effects are associated with slow changes (Dittmar et al. 2016). This can account for variation in mechanisms across populations.

Empirical evidence is consistent with the notion that mutations of large effects (i.e. major mutations) are likely to be associated with NGE (Vila-Aiub et al. 2009; Dittmar et al. 2016). Phenotypically these manifest as shifts in traits which may be detrimental to fitness (e.g. Weis and Weis 1989; Vila-Aiub et al. 2009). For instance, ALS resistant *Amaranthus powellii* have shifts in performance traits that are associated with NGE (Tardif et al. 2006). NGE are believed to be associated with resistance via target site resistance which are associated with amino acid substitutions. Target site resistance involve alteration of the binding site structure of enzymes such that the herbicide cannot bind to the enzyme (Vila-Aiub et al. 2009); and with heavy metal resistance (Macnair 1991), of which involve mutant alleles of large effects. In contrast, costs from non-target site resistance—which involve metabolism of the poison, or transport of the poison away from the site of action (e.g. to vacuoles), are thought to be associated with allocation costs (Vila-Aiub et al. 2009).

For heavy metal resistance, physiological mechanisms of resistance include the restriction of transport of metal to shoots and detoxification of the heavy metal. Hyperaccumulator species (i.e. metallophytes) translocate metals to the shoots via heavy metal transporting proteins (Singh et al. 2015), where it is accumulated at high concentrations without toxic effects on the plant. The genetics of resistance for the majority of heavy metals is via a mutant major gene with some minor gene mutations, which are associated with peptides that bind to heavy metals (Macnair 1993). In contrast, the genetics of resistance for hyperaccumulators is not due to the presence of a novel gene, but is from the greater expression of existing genes (i.e. upregulation—Singh et al. 2015). Thus, costs from hyperaccumulators is predicted to come from allocation costs, rather than gene mutations that may be more associated with pleiotropic effects (Vila-Aiub et al. 2009). Resistance to SO_2 may be given by many alleles with small effects from standing genetic variation (Taylor 1978).

Strictly speaking, NGE are only truly a cost if it reduces fitness for all other environments encountered by the cost-carrying genotype. However, in practice, the effects of NGE under various environments are not known, as genetic effects are environment-dependent and may have neutral or positive effects in some particular environments (Remold 2012; Szamecz et al. 2014), making it difficult to fully understand the effects of genetic effects. Compensatory evolution is a process where genotypes with deleterious mutations and alleles are under selection pressure to mitigate the alleles' negative effects on fitness; where beneficial mutations are accumulated or linked to counter the effect

of costs (e.g. alleles that restore lost function), and genetic linkages with costly alleles are broken over time (Szamecz et al. 2014). Alternatively, novel cost-free genotypes may arise to replace cost-carrying stress-resistant genotypes (Peck and Welch 2004; e.g. introduced populations becoming invasive following enemy release—Colautti et al. 2004). Compensation of costs may be detectable as a recovery of fitness and performance over time in stress-resistant genotypes. This phenomenon is frequently observed in microorganisms and fungi (Grether 2005; Andersson and Hughes 2010), but largely unexplored in plants and animals (but see Labbé et al. 2007 for an example on mosquitoes).

Detecting compensatory evolution would be considered indicative of the presence of NGE (Qian et al. 2012). Importantly, it could indicate that evolutionary restraints incurred are recoverable, which can potentially result in stress-resistant genotypes that may spread outside its habitat. It can also provide a time-scale for the recovery of fitness, and the magnitude of the effects of NGE over allocation costs may be quantifiable. However, NGE may remain genetically linked to the adaptation alleles over time (e.g. genetic hitchhiking). For instance, very low linkage distances (i.e. centimorgan; cM) between the mutant adaptation allele and costly allele may take a considerably longer time to be separated. Compensatory evolution can potentially result in polymorphic populations of stress-resistant and non-stress individuals, and may even replace the surrounding non-stress genotype (Fisher 1930; Levins 1962; Gomulkiewicz and Holt 1999). This may occur more readily where costs of adapting to specific stressors are relatively low and costs are related to other abiotic factors associated with the stress (Che-Castaldo and Inouye 2015). Observations of stress-resistance without the presence of costs may also be explained by prior compensatory evolution.

Metal toxicity stress acts on many different facets in the physiology of the plant, including altering enzyme and hormone activities, water relations, and root growth (Barcelò and Poschenrieder 1990). In addition to the heavy metal stress, mine soils are often associated with other abiotic stresses (Che-Castaldo and Inouye 2015). Heavy metal stress typically represents one increment or 'step' in environmental difference compared to prior conditions—the absence of stress before mining operations. New and different heavy metals are not typically added to a mine site, and re-colonisation of the mine site (or colonisation of contaminated spoils) occurs after the beginning of mining activity. In contrast, herbicide stress represents a stress impacting on the plant physiology in only specific pathways—generally pesticides directly act on photosynthesis through inhibition of or parts of photosystems I and II, including Rubisco activity (Powles and Yu 2010). Herbicide dosage is typically increased as populations become resistant and lower doses become ineffective (Vila-Aiub and Ghersa 2005). Alternatively another type of herbicide may be used, at times resulting in the evolution of multi-herbicide resistant genotypes (Powles and Yu 2010). Thus herbicide stress can change and increase over time.

Certain mechanisms of herbicide resistance incur costs of adaptation consistently. Target site resistance to triazine class herbicides and some non-target resistance mechanisms (e.g. P450 metabolic enzyme) tend to consistently be associated with costs (Vila-Aiub et al. 2009). However resistance to other herbicides may or may not involve costs (e.g. target site resistance to ACCase inhibitors—Vila-Aiub et al. 2005). Costs related to target site resistance to other herbicides are less consistent (e.g. ALS and ACCase inhibitors: Purba et al. 1996; Vila-Aiub et al. 2005). The mode of resistance to some herbicide groups is dependent on the population or species (Purba et al. 1996). Interestingly, Vila-Aiub et al. (2005) found that the non-target P450 resistance in SLR 31 *Lolium rigidum* was costly, while target site resistance to ACCase had no costs. Different populations of the same species may utilise a different mechanism to deal with the same stressor for some herbicides (Vila-Aiub et al. 2009). In those cases it is important to consider each case individually. Reduction and elimination of costs over time by compensatory evolution since adaptation can explain the inconsistency in finding adaptation costs. We predict that compensatory evolution will reduce costs over time.

Here, we synthesise published data comparing performance of stress genotypes from novel stress habitats with non-stress genotypes to assess whether the presence and magnitude of cost change over time. We examined the costs of adaptation over time in plant populations found in heavy metal and herbicide stress. Mechanisms of resistance across herbicides are more variable, and populations within a species may evolve different mechanisms to a particular herbicide (Purba et al. 1996; Vila-Aiub et al. 2009). Both types of stress are lethal to susceptible individuals and provide strong selection pressures. We assessed the magnitude of costs in heavy metal and herbicide stress-resistant populations over time and whether it differs with the type of stress and life history of plants. In so doing, we attempt to distinguish NGE from the overall costs by testing for compensatory evolution (Grether 2005; Andersson and Hughes 2010), while allocation costs may not (stress-resistant populations may become better at tolerating the stress, but the fundamental allocation cost will remain over time). We tested for a significant decreasing relationship between the magnitude of cost, and time since the initiation of the stress—which would indicate compensatory evolution. In contrast, costs not changing over time would indicate unavoidable allocation costs.

Past studies suggest with increased resistance to stress, there should also be an increase in costs (Agrawal et al. 2004; Sletvold et al. 2010). We assessed the efficiency of adaptation (degree of resistance relative to costs) for each stress population. We predict that the cost of adaptation will diminish over time (and the efficiency of adaptation will increase over time), and the magnitude of costs will be maintained for herbicide stress as the stress constantly changes compared to metal stress which is a constant stress. We also predict costs will be reduced faster in annual species relative to perennials due to a shorter lifespan and generation time, which may allow molecular evolution to occur faster (Smith and Donoghue 2008).

Methods

Compilation of Data

We searched JSTOR (http://www.jstor.org), and ISI Web of Science (Thomson Reuters) for studies using a combination of the terms 'metal', 'salt', 'SO_2 toleran* (or resistan*)', 'adapt*', 'plant' and 'herbicide toleran*' and 'populations' as well as specific heavy metals and herbicides. Other related terms were also used, such as 'genotype', 'biotype' and 'susceptible'. We searched for within-species comparisons of performance. SO_2 and salt stresses were grouped with heavy metals, as they may not change with time as herbicide application. We only included studies that raised plants from seed or clones sourced from wild populations—so that only wild field populations were included. Additional studies were found from the reference sections of relevant studies and reviews. We limited our search to plant populations and identified studies where the performance of stress and non-stress genotypes was measured under benign conditions in the absence of the stressor (e.g. glasshouse studies and common garden experiments). A few studies comparing the performance of genotypes under the presence of intra- and interspecific competition were also included. Data from the same species from different studies (or different populations from the same study) were used as independent data points. Where plant performance was measured under a mixture of stress and non-stress plants grown together, the performance measurements for both stress and non-stress plants at 1:1 density ratio was chosen for the data point.

We included studies where time at the beginning of stress application was reported to determine the time since the initiation of stress (i.e. from the time at the start of herbicide application or mining, to the time the study was carried out). The time since start of mining corresponds with the time when mine spoil and metal-contaminated water was exposed to the surrounding population, and should be closer to the time since actual adaptation had occurred. Where the time was not reported in the study, online searches for the specific sites given were carried out to determine the time. The time since initiation of herbicides within an area was also accessed from other sources, such as the International Survey of Herbicide Resistant Weeds (Heap 2014—http://www.weedscience.org). If no date of stress initiation could be obtained, the study was excluded. Studies using herbicide-resistant agricultural species and genetically engineered resistant strains were also excluded. Studies including a few different stress populations within a species (with or without different time since initiation) were included as separate data points. Where there was data from multiple populations within a local area with the same source of stress (e.g. one herbicide application program applied to several populations), population data were pooled to one data entry.

Data Recorded

For each study we recorded: (1) a measure of performance from the stress and non-stress populations under the same benign conditions as well as the standard deviations. Performance measures most commonly reported include dry weight, seed mass, and leaf number. Where more than one measure of performance was reported in a study, we recorded the primary response variable from the study or the variable better reflecting individual fitness (e.g. reproductive mass or seed production was chosen over vegetative mass; Vila-Aiub et al. 2009), (2) number of replicates for the study group, (3) time since initiation of stress: the time since the mining or herbicide application had begun up to the time of the study; (4) the life history of each species (i.e. annual or perennial); and (5) the type of stress (heavy metal or herbicide). We also recorded the heavy metals and herbicides involved in each case. If life history was not given in the study, then life history was accessed using online floras databases. Short lived biennials were grouped with the annuals. We used the program 'Datathief' (developed by B. Tummers, 1999; accessible http://www.nikhef.nl/~keeshu/datathief) to extract data from figures where the values were not reported in the text.

Effect size Calculation

To make the performance data comparable across experiments, Log-Response Ratio (PerfLRR) was used to standardise performance differences across studies. PerfLRR was calculated using the following equation:

$$PerfLRR = \ln\left(F_s/F_{ns}\right) \quad (1)$$

Where F_{ns} is the performance of non-stress individuals, and F_s is the performance of resistant individuals. PerfLRR has

a neutral point of zero and standardises performance differences in traits across species. Negative values indicate a cost in the stress-resistant genotype, and positive values indicate stress-resistant genotypes with higher fitness than non-stress genotypes. We set performance values to 0.01 in the case where the performance is 0.

Where performance was measured across intervals of time, then the measurement at the final growth stage for each population (e.g. largest size attained) was used. For heavy metal stress, the time since the initiation of stress varies by several orders of magnitude compared to herbicide stress thus time was log-transformed (log-10).

Resistance Mechanisms

Adaptation occurs via mutations of varying (i.e. large and small) effects on phenotype. Heavy metals characteristically involve adaptation by a small number of mutant genes with major effects (i.e. major genes; Macnair 1993; Patra et al. 2004). Stress that may be conferred by polygenic additive genetic variation (many loci of small effects) had only four cases (i.e. SO_2, Taylor 1978; and zinc; Gartside and McNeilly 1974), and were excluded to control for mechanisms of resistance. Salt resistance accounts for one study in our search (Online Resource Table S1), and was considered to be given by major gene mutation as suggested by results from studies on salt resistant mutants of *Arabidopsis* (Zhu 2001), and other populations from other plant species (Munns 2005).

For herbicide stress, a large portion of studies consisted of triazine (and the related atrazine) resistance. Triazine resistance is known to incur costs consistently across studies (Vila-Aiub et al. 2009). To account for herbicide mechanisms with consistent costs, triazine and atrazine were analysed separately to the other herbicides. The P450 mode of resistance also tends to have consistent costs—it was therefore grouped with triazine (Vila-Aiub et al. 2005). Unfortunately, the majority of the studies did not report the mode of resistance by the specific populations (i.e. target versus non-target site mechanisms). Although some mechanisms of resistance could be deduced by the herbicide (e.g. resistance to paraquat—a PSI system electron diverter; is generally given by non-target site resistance (Purba et al. 1996); and resistance to propanil seems to be given by target site resistance; Carey et al. 1997), others cannot be determined by the herbicide alone (e.g. resistance to ACCase inhibitor can be given by both target and non-target site—Purba et al. 1996; Vila-Aiub et al. 2009). There was insufficient data for grouping by target versus non-target site resistance. Altogether, we analysed three groups of stress resistance separately: heavy metals, herbicide mechanisms with consistent costs, and herbicide mechanisms without consistent costs.

Fitness Benefits of Stress Adaptation Relative to Costs

We used data from studies examining performance under high stress and no stress to examine the efficiency of adaptation (i.e. performance benefits under the stressor relative to costs) over time. For studies measuring performance of both stress and non-stress genotypes over a stress gradient, a point along the stress gradient was chosen where the resistance was most pronounced—the measurements at the level of stress where the stress-resistance has the most advantage. Studies were included only if the stress treatment used was relevant to the environment of the stress genotype. That is, the performances of both genotypes under the specific mine soil or the herbicide. The performance trait measured was the same one used for quantifying the costs within each study. For instance, if dry weight was used for quantifying costs in the absence of stress, then dry weight must also be used in the presence of the stress for consistency. For these comparisons, a Cost index was used (in place of the LRR) to standardise the magnitude of costs across studies. Within stress treatments, a Benefit index (B) was calculated for each study. These are given as follows:

$$C = \left(F_s - F_{ns}\right)/F_s \tag{2a}$$

$$B = \left(F_{s,t} - F_{ns,t}\right)/F_{s,t} \tag{2b}$$

The parameters in Eq. 2a are the same as that in Eq. 1. $F_{s,t}$ is the performance of stress-resistant individuals under stress treatment, and $F_{ns,t}$ is the performance of non-stress individuals under stress treatment. This Benefit index (B) is analogous to the Cost index (C); unlike the Cost index which gauges the cost growing under lack of stress (from the perspective of the benign environment), this index gauges the adverse effects endured by the non-stress genotype in the presence of the stress.

To assess the difference between costs and benefits bestowed by resistance, from B and C, a Net Benefit index (NB) was calculated to quantify the degree of stress adaptation relative to the magnitude of cost for each case. NB quantifies the efficiency of adaptation—it standardises differences between stress resistance and cost across studies. NB is calculated as:

$$NB = B - C \tag{3}$$

Within Population Variation Over Time

In addition, we assessed whether within population trait variation decreased over time. A reduction in genetic variation may also constitute a cost. This was calculated from standard deviations of both stress and non-stress populations:

$$PopVar = \ln\left(\frac{stdev_s}{mean_s} \Big/ \frac{stdev_{ns}}{mean_{ns}}\right) \tag{4}$$

where $stdev_s$ and $stdev_{ns}$ are the standard deviations; $mean_s$ and $mean_{ns}$ are the means of the trait for the stress-resistant and the non-stress populations respectively.

Data Analysis

Overall magnitude of costs (PerfLRR) was analysed using meta-analysis. Random effects meta-regressions were used to quantify the relationship between the effect size PerfLRR and Time (time since initiation of stress), with Family as a covariate for heavy metal stress, herbicides with consistent costs, and herbicides without consistent costs. Family was analysed as a random effect. We use a random effects model using restricted maximum likelihood estimate (REML). Data for PerfLRR were also analysed against Time independently for heavy metals with major gene mutations, herbicides with known consistent costs, and other herbicides. For studies testing the degree of resistance under stress treatment, we tested the relationship between *NB, B*, and *C* versus Time using Bayesian linear regressions. Shapiro–Wilk normality test shows the distributions of residuals are not significantly different from normal—except for PopVar for heavy metals. For this we used quantile regression to test whether PopVar changes over time for heavy metals. Multivariate ANOVA was used to analyse the effects of stressor type, life history and plant family on PerfLRR and Time since initiation of stress. Herbicide time data was also log-transformed (as the heavy metal time data) for running the MANOVA. Levene's test was used to test for heterogeneity in the variances. Meta-analyses were carried out using OpenMEE software (Dietz et al. 2016—available at http://www.cebm.brown.edu/open_mee). All other analyses were carried out using R (ver. 3.3.2). The "quantreg" package was used for quantile regression.

Results

The search yielded data for adaptation costs over time in 29 species from 12 families for metal stress from mine sites (data from 27 studies), and 19 species from 4 families for herbicide stress (data from 31 studies). Data sources, effect size and variance, study species, and references are provided in the supplementary material (Online Resource Table S1). Accounting for studies assessing multiple populations with different times since initiation of stress, or populations from different regions gives a total of 78 measures of cost over time (34 for metal stress, 43 for herbicides—of which there are 21 cases for consistent herbicides and 22 for other herbicides). Within each comparison, the stress-resistant population should be relatively closely related to non-stress populations, if not directly derived from the non-stress population as the populations were generally in close proximity.

Resistance was overall costly for heavy metal and herbicides with known consistent costs (PerfLRR -0.28 ± 0.50 SD, $P < 0.001$; and -0.27 ± 0.34 SD, $P < 0.001$ respectively). Interestingly, costs were overall absent for the other herbicides (-0.01 ± 0.65 SD, $P = 0.84$). The effect size (PerfLRR) ranges from -1.61 to 0.61 for heavy metals, -0.93–0.06 for herbicides with consistent costs, and -1.82–1.46 for herbicides with inconsistent costs. Out of the 38 cases for heavy metal resistance, around 6 of the stress genotypes had equal or superior performance than the genotype from the non-stress environment. Similarly, for herbicide resistance there was equal or superior performance in 12 out of 40 comparisons.

Costs seem to be present very early on in both types of stresses, as well as superior stress-resistant populations (Fig. 1). Time for heavy metal stress ranged from 6 to 3000 years (mean 409.74 ± 834.46 SD). A few sites began mining operations in prehistoric times as early as the Bronze Age. Time since initiation of herbicide stress ranged from 0 to 39 years (mean 12.35 ± 10.1 SD). There was no significant relationship between PerfLRR and Time for heavy metal (Fig. 1), consistent-herbicides (Fig. 2), and other herbicides (Tables 1, 2, 3; Fig. 3). Time × Family interaction effects were significant for heavy metal and consistent herbicides (Tables 1, 2). Family effect was significant for consistent herbicides (Table 2), but not for other herbicides (Table 3). Population variance did not differ significantly over time for heavy metal and herbicide groups (Online Resource Table S2). There was significant residual heterogeneity for heavy metal stress ($\tau^2 = 0.12 \pm 0.05$, $P < 0.001$), consistent-herbicides ($\tau^2 = 0.05 \pm 0.02$, $P < 0.001$) and other herbicides ($\tau^2 = 0.41 \pm 0.17$, $P < 0.001$) suggesting there are other unaccounted for covariates influencing costs.

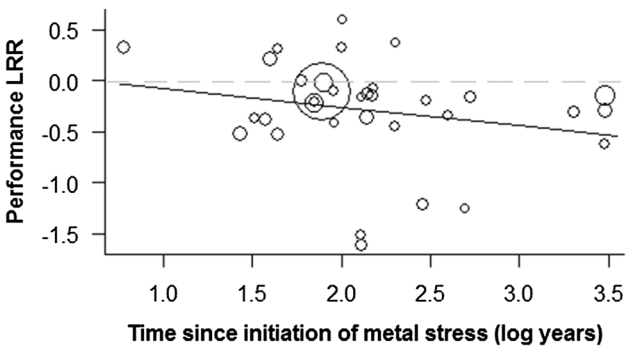

Fig. 1 Effect size (PerfLRR) versus Time (time since stress initiation in log years) for heavy metal stress, with each *circle* representing an individual comparison (n = 34). The *dashed boundary* is where the stress-resistant and non-stress genotype have equal performance

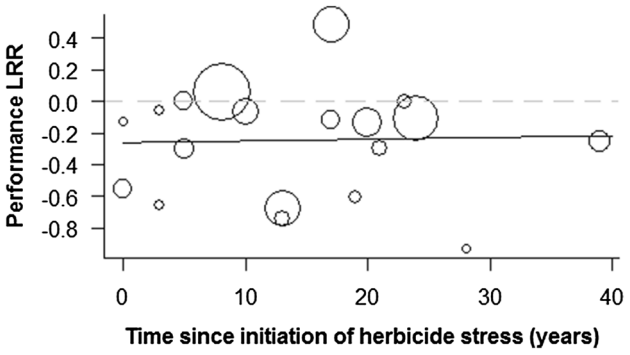

Fig. 2 Effect size (PerfLRR) versus Time for triazine, atrazine resistance, and resistance via P450 mechanism (n = 21)

For benefits to costs comparison, the majority of studies that accessed performance under the stressor reported the same trait under stress and non-stress treatments. A 'super' outlier genotype which had very high performance (relative to other stress-resistant genotypes present at the same site, and to the respective non-stress genotype) under both benign and stress treatments (from Davis et al. 2009) was excluded from this analysis. The relationship between *B* and Time, and *C* and Time were not significant for both stresses (Table 4a, b). The relationship between *NB* and Time for heavy metal resistance was not significant (Table 4c, Fig. 4a), but was significant for herbicide resistance (Fig. 4b).

Multivariate ANOVA results report that stress type, life history, and plant family have no significant effects on PerfLRR and Time. Interaction effects were also not significant (Table 5). Levene's test show the variances for PerfLRR and Time were homogenous (PerfLRR: F = 1.15, sig. 0.33; Time: F = 0.81, sig. 0.69).

Discussion

Plants can frequently and sometimes extremely rapidly evolve resistance to extreme stresses such as heavy metals (Macnair 1993) and pesticides (Bergelson and Purrington 1996). We found that adapting to lethal stresses

Table 1 Meta-regression results for heavy metal resistance

Source	Q	df	P	
(a)				
Time	3.10	1	0.08	
Family	18.90	11	0.06	
Time × Family	23.20	5	**<0.001**	
	Estimate (±SE)	CI (lower)	CI (upper)	P
(b)				
Intercept	0.43 (0.38)	−0.33	1.18	0.27
Time	−0.35 (0.21)	−0.76	0.05	0.09
Betulaceae	−12.84 (9.42)	−31.31	5.63	0.17
Caryophyllaceae	−0.73 (0.46)	−1.62	0.17	0.11
Fabaceae	−0.16 (0.97)	−2.05	1.73	0.87
Lamiaceae	0.21 (0.37)	−0.51	0.93	0.57
Linderniaceae	−0.15 (0.40)	−0.93	0.63	0.71
Phrymaceae	−0.02 (0.37)	−0.75	0.70	0.96
Plantaginaceae	−48.29 (15.69)	−79.04	−17.54	**<0.01**
Plumbaginaceae	−0.29 (0.91)	−2.07	1.50	0.75
Polygonaceae	−3.94 (1.07)	−6.03	−1.85	**<0.001**
Salicaceae	0.25 (0.40)	−0.53	1.03	0.53
Sapindaceae	0.60 (0.38)	−0.15	1.35	0.12
Time × Betulacae	6.86 (4.86)	−2.67	16.40	0.16
Time × Fabaceae	0.10 (0.38)	−0.64	0.84	0.79
Time × Plantaginaceae	22.27 (7.32)	7.93	36.62	**<0.01**
Time × Plumbaginaceae	0.23 (0.39)	−0.53	0.98	0.56
Time × Polygonaceae	1.30 (0.38)	0.56	2.04	**<0.001**

(a) Omnibus results for the effects of time since initiation of stress, family and interaction effect

(b) Coefficients and confidence intervals of moderators with Poaceae as the reference level. Bold P-values indicate significant effects (P < 0.05)

Table 2 Meta-regression results for triazine and atrazine resistance, and P450 enzyme mechanism

Source	Q	df	P	
(a)				
Time	0.16	1	0.69	
Family	13.18	3	**<0.01**	
Time × Family	11.17	3	**0.01**	
	Estimate (±SE)	CI(lower)	CI (upper)	P
(b)				
Intercept	0.07 (0.19)	−0.31	0.45	0.71
Time	−0.01 (0.01)	−0.03	0.01	0.38
Amaranthaceae	−0.24 (0.25)	−0.73	0.26	0.35
Asteraceae	−0.69 (0.29)	−1.25	−0.12	**0.02**
Solanaceae	−0.97 (0.42)	−1.79	−0.16	**0.02**
Time × Amaranthaceae	0.00 (0.01)	−0.03	0.03	0.98
Time × Asteraceae	0.01 (0.02)	−0.02	0.05	0.39
Time × Solanaceae	0.09 (0.03)	0.04	0.14	**<0.01**

(a) Omnibus results for the effects of time since initiation of stress, family and interaction effect

(b) Coefficients and confidence intervals of moderators with Poaceae as the reference level. Bold P-values indicate significant effects ($P < 0.05$)

Table 3 Meta-regression results for resistance to other herbicides

Source	Q	df	P	
(a)				
Time	0.02	1	0.90	
Family	0.73	2	0.69	
Time × Family	0.28	2	0.87	
	Estimate (±SE)	CI(lower)	CI (upper)	P
(b)				
Intercept	0.11 (0.34)	−0.56	0.78	0.75
Time	−0.01 (0.02)	−0.05	0.04	0.76
Amaranthaceae	0.00 (0.61)	−1.20	1.20	1.00
Asteraceae	−0.51 (0.58)	−1.65	0.63	0.38
Time × Amaranthaceae	0.00 (0.07)	−0.13	0.14	0.95
Time × Asteraceae	0.02 (0.03)	−0.05	0.09	0.60

(a) Omnibus results for the effects of time since initiation of stress, family and interaction effect

(b) Coefficients and confidence intervals of moderators with Poaceae as the reference level

overall incurs costs to plants, although in some instances there were no costs and in few cases the stress-resistant population performed better than the non-stress population. Compensatory evolution predicts costs should be reduced over time due to selection against costs (Andersson and Hughes 2010; Qian et al. 2012). Our results suggest that costs appear generally consistently among stress-resistant genotypes in plants, and costs do not reduce over time. The overall appearance of costs agrees with available empirical evidence (reviewed in Bergelson and Purrington 1996), with the exception of herbicide resistance (i.e. resistance to ACCase and PSI photosystem inhibitors, paraquat, etc.) which can explain previous findings of low or absent costs. The results suggest compensatory evolution may not be a key mechanism for cost-free and low cost adaptation in plants; at least for most cases. Among the herbicide groups, we found the manifestation of costs agrees with anecdotal observations where the triazine herbicide family and the P450 mechanism demonstrate costs consistently (Fig. 2), but other herbicides do not (Fig. 3, Purba et al. 1996; Vila-Aiub et al. 2009). There was a trend for increasing costs in heavy metal stress over a very long period of time (over

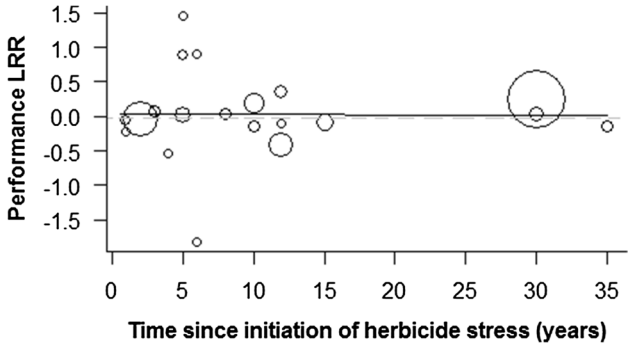

Fig. 3 Effect size (PerfLRR) versus Time for other herbicides (n = 22)

centuries), suggesting there may be increasing specialisation or an accumulation of NGE over very long periods of time—however, this effect is modest. Plant family had significant interaction effects for triazine resistance / P450 mechanisms. In addition, costs for heavy metal resistance demonstrate similar patterns. These suggest some plant families may incur costs differently to others. However this study only involves relatively few families (and some families may be over-represented—e.g. Poaceae).

The maintenance of the magnitude of cost suggests two possible possibilities: First, NGE are absent or are purged extremely soon after adaptation (e.g. less than ten years after introduction of stress) and the remaining costs consist of allocation costs for most cases. Very high migration rates from other areas around the region can result in the adaptation given by many alleles of small effects due to genetic drift and intermixing, which can replace mutations of large effects and their associated NGE extremely quickly, even if initially present (Yeaman and Whitlock 2011). Second, NGE do contribute to a significant proportion of the cost, but remain closely linked to the stress adaptation alleles. As long as there is no traction for selection (i.e. there is no genetic variation for stress genotypes for lower costs—Bridle and Vines 2007; Carter and Nguyen 2011), resistance genes may remain linked to NGE (Weis and Weis 1989). Antagonistic pleiotropic effects have been shown to persist over time even where the beneficial effect of the gene is only minor, suggesting costs may be linked strongly to specific genes and may appear time and time again. For instance, genetic defects in humans (e.g. sickle cell disease) with only modest benefits (i.e. higher resistance to malaria; Carter and Nguyen 2011) may be examples of NGE associated with the alleles for disease resistance. An alternate

Table 4 Results from Bayesian linear regression

	Estimate (SE)	CI (lower)	CI(upper)	t	P	R^2
(a)						
Heavy metal						
Intercept	0.35 (0.17)	0.00	0.69	2.07	**<0.05**	
Time	0.07 (0.08)	−0.09	0.22	0.83	0.41	−0.01
Herbicides						
Intercept	0.66 (0.11)	0.42	0.91	6.02	**<0.001**	
Time	0.01 (0.01)	−0.01	0.02	1.3	0.24	0.05
(b)						
Heavy metal						
Intercept	0.00 (0.17)	−0.34	0.34	0.02	0.98	
Time	0.05 (0.08)	−0.10	0.21	0.69	0.49	−0.01
Herbicides						
Intercept	0.26 (0.15)	−0.09	0.60	1.71	0.11	
Time	−0.01 (0.01)	−0.03	0.01	−1.63	0.13	0.13
(c)						
Heavy metal						
Intercept	0.35 (0.25)	−0.16	0.84	1.40	0.17	
Time	0.01 (0.11)	−0.22	0.25	0.11	0.91	−0.03
Herbicides						
Intercept	0.40 (0.12)	0.13	0.66	3.31	**<0.01**	
Time	0.02 (0.01)	0.01	0.04	3.28	**<0.01**	0.47

Bold P-values indicate significant differences (P < 0.05)

(a) Benefit

(b) Cost

(c) Net benefit indices versus time since initiation of stress

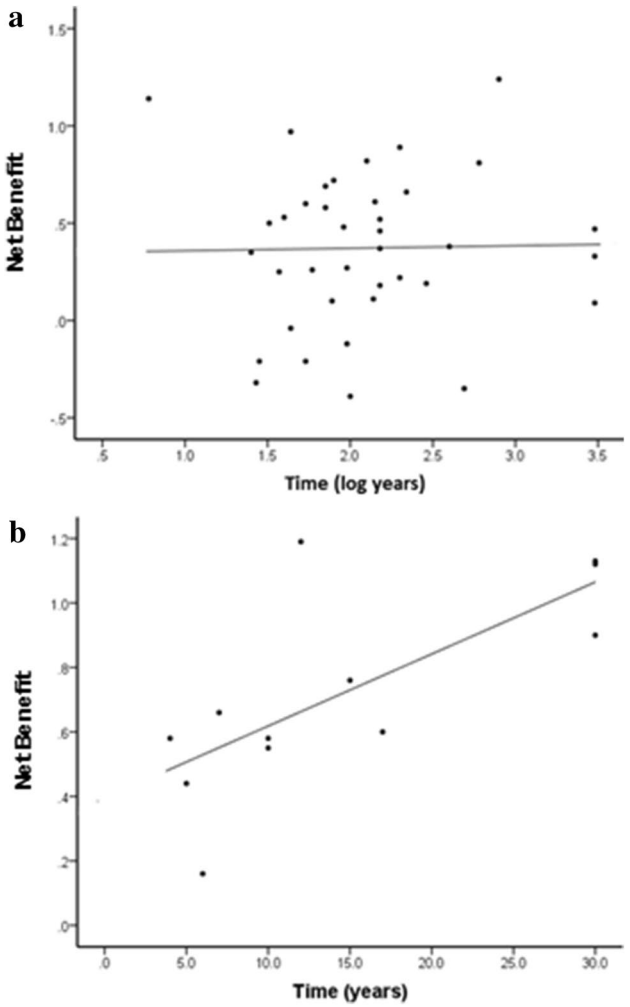

Fig. 4 Net benefit (*NB*) indices versus Time for: **a** heavy metal (n = 38) and **b** herbicide (n = 12) resistance

Table 5 Results from multivariate ANOVA (MANOVA) for the interaction effects of stress type, life history, and plant family on PerfLRR and time since initiation of stress

Effect	F	df	P
Stress type	0.34	2	0.72
Life history	0.76	2	0.47
Plant family	1.23	14	0.28
Stress type × Life history	0.00	2	1.00
Stress type × Family	0.00	2	1.00
Life history × Plant family	1.55	3	0.21
Stress type × Life history × Plant family	0.00	2	1.00

P-values from Roy's largest root

possibility for these results is that for cases where the initiation of stress was a long time ago, populations outside of the area of stress may have already changed through time with natural selection (e.g. to environmental changes that are independent of the stressful area). These comparisons may be between populations that are very different in genetic structure. Similarly, if costs were not detected easily in studies, a much larger sample size may be required in order to find patterns (Grubb 2016). However, changes in costs could potentially become much less biologically meaningful if they can only be detected when sample sizes are very large.

From these studies, costs seem to be present very soon after the initiation of the stress (Figs. 1, 2, 3). In contrast if adaptation to the stress had not yet occurred it is predicted that comparison of stress and non-stress populations should show little difference in performance (PerfLRR around 0), as the comparison would be among susceptible (or of similar) genotypes. This observation suggests adaptation may occur very quickly, where selection from stress is so strong that only few genotypes survive, and that initial adaptation occurs from standing genetic variation (Wu et al. 1975; Macnair 1993). Adaptation via a single major mutation is considered unlikely (Barrett and Schluter 2008). There are two scenarios that can explain the finding of major mutations in stress-resistant genotypes, if initial adaptation is predicted to arise from alleles of small effects.

First, initial adaptation occurs through selection on standing genetic variation (mutations of small effects), and then mutations with large effects arise due to genetic clustering effects (Yeaman and Whitlock 2011). Intriguingly, the clustering effect suggests a switch in genetic mechanism over time is possible (e.g. from non-target to target site ACCase resistance). From the Yeaman and Whitlock (2011) model results, clustering occurs more than 50 generations after adaptation for intermediate migration rates, and almost immediately after under very low migration. While it is expected that finding major mutations is more likely for heavy metal stress, because sampling occurs decades after mining; herbicide target site resistance suggests major mutations can already arise quickly within a decade (Délye et al. 2013; e.g. triazine resistance). The Yeaman and Whitlock (2011) model predicts this can occur at exceedingly low migration rates, which could explain finding major mutations early in herbicide resistance. Second, some major mutations may not be costly and may not be associated with strong NGE (e.g. target site resistance to non-triazine herbicides: Vila-Aiub et al. 2005; Délye et al. 2013) which can explain finding major mutations soon after adaptation because they may exist as standing genetic variation. Some mutations may even be associated with beneficial genetic effects (also discussed below; Délye et al. 2013). A high mutation rate is not necessarily required for selection for resistance (Jasieniuk et al. 1996). Some mine site populations may arise in a similar way. For instance, Hayes et al. (2003) found stress-resistance was also present

in plants surrounding a mine site. In addition, pseudometallophyte species can inhabit both mine and non-mine areas easily (McNaughton et al. 1974; Dechamps et al. 2007). Therefore it is probable that in some cases, the lack of costs could facilitate the spread of stress-resistance, even if spread is limited.

Are Higher Performing Stress Genotypes Superior?

Interestingly, there were a few populations with superior performances soon after the initiation of the stress. These indicate cases where stress-resistant populations performed better than the non-stress populations under benign conditions. Many more have only modest costs of adaptation. These values are more typical to differences in adaptation among non-extreme environments or local adaptation (reviewed in Hereford 2009). There is a strong possibility that the evolution of cost-free genotypes may be more commonplace than commonly perceived (Hayes et al. 2003; Andersson and Hughes 2010; Remold 2012). In addition, this finding lends support to the idea that evolution to extreme stressors is not necessarily costly, but rather that costs may be associated with other abiotic environmental variables (Che-Castaldo and Inouye 2015), or ecological factors. Pleiotropic effects associated with adaptation may sometimes have positive effects on fitness, even under the absence of stress (reviewed in Vila-Aiub et al. 2009). In a remarkable example, a genotype from Davis et al. (2009) demonstrated superior performance compared to other stress-resistant genotypes—both under benign conditions and under the stressors. There is so far little evidence that cost-free genotypes tend to spread into areas outside; otherwise superior stress genotypes could quickly invade the low stress habitats and replace the non-stress genotypes (Peck and Welch 2004). For instance, the superior ile-1781-Leu mutant for ACCase resistance only had limited spread, though much more than the other genetic mutants in the area (Délye et al. 2013). Alternatively, these may represent the case where the adaptive loci have conditional neutrality (such as epistasis) instead of antagonistic pleiotropic effects (Draghi et al. 2011), in that case a cost may not be apparent. The appearance of these genotypes is nonetheless very intriguing, and future study into their population genetic mechanisms and ecological factors surrounding these genotypes is warranted.

Higher performance trait values expressed by stress-resistant populations may in fact be costly if they are detrimental to fitness (i.e. a maladaptive trait shift). If the trait shift is away from optimal values, the new trait value may not be beneficial in either stress or non-stress environments (He et al. 2010; Paulander and Hughes 2010; Schuler and Orrock 2012). For instance, growing large could be a cost under stressful environments (He et al. 2010). The reduction in the incidence of these 'superior' stress-resistant populations after longer periods of exposure in heavy metal habitats (and also a few extreme cases from herbicide stress) suggests genotypes with high performance, and particularly those with extremely high performance found inside stressful areas may have been purged over time. However, these populations with extremely low values are few. Compensatory selection may be acting against these cost carrying 'large and unfit' genotypes. From this perspective, adaptation costs may not necessarily be associated with decreasing performance trait values (e.g. decrease in size, number of flowers). A shift towards a larger size or higher performance values may be maladaptive as well (such as the number of seeds produced by an individual; Vila-Aiub et al. 2009); other unaccounted factors (e.g. ecological costs) could explain why better performing stress-resistant genotypes are yet unable to spread into other areas. Likewise, some NGE may have the greatest effects only under certain environmental or ecological settings, and the NGE were not captured by studies.

Alternatively, higher performance is not a cost and has fitness benefits over non-stress genotypes, but demographic or genetic constraints may be limiting these superior 'master of all' genotypes (Lenormand 2002; Richards et al. 2006) where they may have otherwise invaded and settled into areas occupied by non-stress individuals (Remold 2012). For example, density dependence effects may inhibit migrants from these populations from successfully spreading, especially where dispersal of the superior genotype into surrounding areas is too low. If the fitness of hybrids (non-stress × resistant genotype) is low, the resistance genotype can also be prevented from spreading (Gomulkiewicz and Holt 1999). Plants grown under nutrient stress (such as at a mine site) may also grow fast and capitalise during less stressful times and reproduce quickly (Weiner et al. 1997; Bonser 2013). Future studies may test whether these higher performing stress genotypes are incurring costs in the environment. Overall, adaptation costs can explain decreasing population herbicide resistance after discontinuation of the stress (Bourdot et al. 1996).

Independent of costs; the fast growing genotypes represent genotypes with a fast life history (Franco and Silvertown 1996; Phillips 2009) that have initially survived selection in a harsh environment, as they could reproduce and survive before the toxicity of metal or herbicides kill them. Studies that assessed their resistance to the stress found that these genotypes still grow faster under the stress (Online Resource Table S1). These fast and very fast growing genotypes may have been eventually replaced by true stress-resistant genotypes that are more optimised to the stress environment—i.e. genotypes with slower growth and stress resistance mechanisms. Future studies may assess these high performance genotypes occurring in stressful

environments. Future studies may assess when and where cost-free adaptation may evolve and spread.

Stress-Resistance versus Costs

We found the difference between resistance and costs increased over time. This effect may be due to increased dosage of herbicides in attempts to kill resistant plants leading to increased resistance consequently increasing resistance. In contrast, resistance in mine sites is a constant stress and the efficiency did not change over time. Overall costs did not increase with increasing herbicide resistance. Likewise, we found no significant relationship between Benefit index and Cost index for herbicide and metal stress. This observation is contrary to the expectation of higher costs with increasing resistance (Sletvold et al. 2010; Cipollini et al. 2014). This suggests costs may not come only from the allocation of resources (i.e. allocation costs). Genetic factors may contribute to differences in the magnitude of costs (Macnair 1983). The link between different mechanisms of resistance with genetic costs warrants further study. The genetic mechanisms of resistance need to be established in order to assess patterns of costs across different types of stress.

Costs in Genotypes and Environment

The high variability in costs through time for both heavy metal and herbicide stress suggests that costs are dependent on the nature of the environmental conditions and the evolutionary potential of a genotype. The most appropriate comparison of course would be between a stress genotype and its associated ancestral genotype(s); however including this criterion would limit the search to only a few studies.

It is very important to consider gene flow, migration, and population genetic structure of the populations because migration affects the distribution of alleles of different size of effects, where lower levels of immigration would favor a higher number of loci with alleles of large effects, and higher levels of migration favours loci with small effects (Yeaman and Whitlock 2011). Firstly, it is predicted that alleles of large effects would increase over time under low migration rates due to a genetic clustering effect where genetic architecture becomes more complex over time (Yeaman and Whitlock 2011). A greater incidence of NGE is therefore predicted in isolated stress-adapted populations with relatively low migration. Secondly, adaptation to a moving optimum (e.g. herbicides) may more likely to involve major gene changes relative to a non-moving optimum (Dittmar et al. 2016). Herbicide resistance appears to involve both alleles of large and small effects (target site and non-target site resistance, respectively), and these distinctions do not appear to be good predictors for costs—herbicides with consistent and inconsistent costs involve both mechanisms. The specific genes or physiological mechanisms involved for each stressor may be more important. However, here we were not able to distinguish herbicides by target site and non-target site resistance, or to determine which herbicide-resistant populations had greater herbicide application over time.

Our results suggest a complex dynamic between specific genetic mechanisms to groups of stressors and migration rate. In the case of heavy metal resistance, a lower migration rate (e.g. due to the constant nature of the stress and selection pressure from other abiotic stresses at mine sites) could be contributing to a gradual accumulation of NGE as major alleles (and modifiers) are accumulated (in the sense of Yeaman and Whitlock 2011). Future studies can examine the presence of costs and genetic composition in stress-resistant genotypes among different phylogenetic groups, under varying migration rates and gene flow. Exploring additional factors that have strong influence on NGE, such as effective population size, mutation and recombination rates should be exciting and worthwhile next steps in studying the adaptive evolution of populations.

There are only a handful of studies reporting costs over time for herbivores and pathogens in stress and non-stress populations, and none reported a time since introduction of the pathogen-herbivore. This highlights a gap in understanding of adaptation costs to biotic stress where there is co-evolution between the host and the pathogen or herbivore. Studies may examine whether continuous cost alleviation and incursion, and co-evolutionary responses in both host and antagonist may contribute to cycles of adaptation and counter-adaptation (Parker and Gilbert 2004; Brown and Tellier 2011; Carter and Nguyen 2011). Future study on the role of costs of adaptation to stressful environments on evolution would likely yield interesting insights.

Conclusions

The persistence of costs over time in stress genotypes suggests adaptation to stressful environments may often render stress genotypes disadvantaged outside of their environments. In most cases, allocation costs may be responsible for the costs in performance and seem to be preventing stress genotypes spreading. The lack of relationship between the degree of stress resistance and the magnitude of costs indicate negative genetic effects (NGE) may be responsible for some of the cost. Stress resistant genotypes with higher performance from both types of stresses suggest NGE may manifest as a response that is potentially maladaptive. Alternatively, fast growth genotypes may be under selection initially in the stressful environment and then are replaced by slower growing, truly stress-resistant

genotypes. In addition, the overall inconsistency and lack of costs in herbicide groups suggest there may be unquantified costs or factors that are limiting the spread of stress genotypes, and that mechanisms involved in adaptation to some groups of stress are not very costly.

Acknowledgements The authors thank S. Rutherford for helpful discussions on population genetics. J. S.H. Wan and C.K. Pang were supported by Australian Post Graduate Scholarships.

Compliance with Ethical Standards

Ethical Standards We declare that the experiments comply with the current laws of the country which they were performed.

Conflict of interest All authors have been named in the manuscript. The authors declare that there is no conflict of interest.

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